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5°C and the mixture was then stirred for 1 hour at ambient temperature. Water was added and the volatiles were removed by evaporation. The solid residue was dissolved in methylene chloride/methanol and a 5M solution of hydrogen chloride in isopropanol (0.3ml) was added. The solvent was removed by evaporation, the solid residue was resuspended in ether.

5 collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((1-methylimidazol-2-yl)methoxy)quinazoline hydrochloride** (99mg, 63%).

¹H NMR Spectrum: (DMSO-d₆) 3.93(s, 3H); 4.01(s, 3H); 5.67(s, 2H); 7.16(d, 1H); 7.52(d, 1H); 7.58(s, 1H); 7.70(s, 1H); 7.78(s, 1H); 8.31(s, 1H); 8.8(s, 1H); 10.58(s, 1H); 11.35(br s, 1H)

MS - ESI: 430 [M+H]⁺

Elemental analysis:	Found	C 45.8	H 4.3	N 12.9
C ₂₀ H ₁₇ N ₃ O ₃ ClF 1.4H ₂ O 2HCl	Requires	C 45.5	H 4.2	N 13.3%

15 The starting material was prepared as follows:

Diethyl azodicarboxylate (219μl, 1.4mmol) was added dropwise to a solution of 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (400mg, 0.7mmol), (prepared as described for the starting material in Example 33), 2-hydroxymethyl-1-methylimidazole (82mg, 0.83mmol), (J. Chem. Soc. 1927, 3128-3136), and triphenylphosphine (365mg, 1.4mmol) in methylene chloride (12ml) cooled at 0°C. The mixture was stirred for 1 hour at ambient temperature and further 2-hydroxymethyl-1-methylimidazole (68mg, 0.69mmol), triphenylphosphine (91mg, 0.34mmol) and diethyl azodicarboxylate (54μl, 0.34mmol) were added. The mixture was stirred for 1 hour at ambient temperature and the solvent was removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (94/6) to give 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-6-methoxy-7-((1-methylimidazol-2-yl)methoxy)quinazoline (116mg, 25%).

¹H NMR Spectrum: (CDCl₃) 1.16(s, 9H); 3.75(s, 3H); 3.93(s, 3H); 5.28(s, 2H); 6.84(s, 1H); 6.91(s, 1H); 7.02(s, 1H); 7.17(d, 1H); 7.32-7.48(m, 8H); 7.78(2d, 4H); 8.08(s, 1H); 8.18(d, 1H)

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Example 74

A mixture of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxyquinazoline hydrochloride (400mg, 0.98mmol), 2-chloromethyl-1-methylimidazole hydrochloride (210mg, 1.25mmol), potassium carbonate (580mg, 4.2mmol) and potassium iodide (17mg, 0.1mmol) in DMF (20ml) was stirred at 65°C for 4.5 hours followed by 17 hours at ambient temperature. The solvent was removed by evaporation and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water, brine, dried (MgSO₄) and the solvent removed by evaporation. The solid residue was purified by column chromatography eluting with methylene chloride/methanol (97/3) to give a yellow solid (258mg). This solid was dissolved in methanol (5ml) and a 1M aqueous sodium hydroxide solution (660μl, 0.66mmol) was added. The mixture was stirred for 15 minutes, then water was added and the mixture adjusted to pH7 with concentrated hydrochloric acid. The aqueous mixture was extracted with ethyl acetate and the combined organic extract was washed with water, brine, dried (MgSO₄) and the solvent removed by evaporation. The residue was purified by flash chromatography eluting with methylene chloride/methanol (95/5). The purified solid product was dissolved in methanol and methanolic hydrogen chloride (1.5ml of a 7.5M solution) was added. The volatiles were removed by evaporation, the solid residue was suspended in pentane, collected by filtration, washed with pentane and dried under vacuum to give 4-(2-fluoro-5-hydroxy-4-methylanilino)-7-((1-methylimidazol-2-yl)methoxy)quinazoline hydrochloride (105mg, 44%).

¹H NMR Spectrum: (DMSO-d₆) 2.16(s, 3H); 3.92(s, 3H); 5.71(s, 2H); 6.90(d, 1H); 7.1(d, 1H); 7.52(d, 1H); 7.64(d, 1H); 7.71(s, 1H); 7.78(s, 1H); 8.77(d, 1H); 8.82(s, 1H); 9.7(br s, 1H); 11.45(br s, 1H)

MS - ESI: 380 [MH]⁺

Elemental analysis:	Found	C 52.2	H 5.0	N 15.1
C ₂₀ H ₁₈ N ₃ O ₂ F 0.9H ₂ O 1.8HCl	Requires	C 52.1	H 4.7	N 15.2%

The starting material was prepared as follows :-

Sodium (368mg, 16mmol) was added to benzyl alcohol (10ml, 96mmol) and the mixture was heated at 148°C for 30 minutes. 7-fluoro-3,4-dihydroquinazolin-4-one (656mg,

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4mmol), (J. Chem. Soc. section B 1967, 449), was added and the mixture maintained at 148°C for 24 hours. The reaction mixture was allowed to cool, the solution was poured on to water (170ml) and the aqueous mixture adjusted to pH3 with concentrated hydrochloric acid. The precipitate was collected by filtration, washed with water, then ether and dried under vacuum to give 7-benzyloxy-3,4-dihydroquinazolin-4-one (890mg, 89%) as a white solid.

m.p. 267-269°C

¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 5.32(s, 2H); 7.25(d, 1H); 7.32-7.52(m, 6H); 8.12(d, 1H); 8.99(s, 1H)

MS - ESI: 252 [MH]⁺

10	Elemental analysis:	Found	C 71.4	H 4.9	N 10.7
	C ₁₅ H ₁₂ N ₂ O ₂ 0.04H ₂ O	Requires	C 71.2	H 4.8	N 11.1%

A mixture of 7-benzyloxy-3,4-dihydroquinazolin-4-one (800mg, 3.17mmol) and DMF (100μl) in thionyl chloride (20ml, 0.27mmol) was heated at reflux for 3 hours. Excess thionyl chloride was removed by evaporation and the residue azeotroped with toluene and dried under vacuum to give 7-benzyloxy-4-chloroquinazoline hydrochloride (835mg, 86%) as a cream solid.

m.p. 131-132°C

¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 5.32(s, 2H); 7.29(d, 1H); 7.34-7.52(m, 6H); 8.12(d, 1H); 9.03(s, 1H)

20 MS - ESI: 270 [MH]⁺

2-Fluoro-5-methoxycarbonyloxy-4-methylaniline (883mg, 4.4mmol), (prepared as described for the starting material in Example 12), was added to a solution of 7-benzyloxy-4-chloroquinazoline hydrochloride (1g, 3.7mmol) in 2-pentanol (15ml) at 120°C and the mixture was then heated at reflux for 4 hours. The mixture was allowed to cool and the precipitate was collected by filtration, washed with isopropanol followed by ether and dried under vacuum to give 7-benzyloxy-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline hydrochloride (1.65g, 97%) as a cream solid.

m.p. 219-220°C

¹H NMR Spectrum: (DMSO-d₆) 2.22(s, 3H); 3.86(s, 3H); 5.37(s, 2H); 7.30-7.60(m, 9H); 8.60(d, 1H); 8.80(s, 1H); 11.2(s, 1H)

30

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MS - ESI: 434 [MH]⁺

Elemental analysis:

Found C 60.1 H 4.9 N 8.5

C₂₄H₂₀N₃O₄F 1HCl 0.5H₂O

Requires C 60.2 H 4.6 N 8.8%

- 7-Benzyloxy-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline
 5 hydrochloride (1.53g, 3.25mmol) and 10% palladium-on-charcoal catalyst (180mg) in a mixture of methanol (75ml), DMF (6ml) and trichloromethane (30ml) was stirred under hydrogen at 1.5 atmospheres pressure for 45 minutes. The catalyst was removed by filtration through diatomaceous earth and the solvent removed from the filtrate by evaporation. The residue was triturated with ether, the resulting solid was collected by filtration and dried under
 10 vacuum to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxyquinazoline hydrochloride (1.23g, 84%) as an orange solid.
 m.p. 205-210°C

¹H NMR Spectrum: (DMSO-d₆) 2.22(s, 3H); 3.85(s, 3H); 7.24(d, 1H); 7.35(dd, 1H); 7.42(d, 1H); 7.45(d, 1H); 8.58(d, 1H); 8.81(s, 1H); 11.40(s, 1H); 11.76(s, 1H)

- 15 MS - ESI: 344 [MH]⁺

Example 75

- Diethyl azodicarboxylate (244mg, 1.4mmol) was added dropwise to a suspension of
 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline
 20 hydrochloride (261mg, 0.7mmol), (prepared as described for the starting material in Example 22), triphenylphosphine (367mg, 1.4mmol) and 2-(1,2,4-triazol-1-yl)ethanol (95mg, 0.84mmol), (Ann. Pharm. Fr. 1977, 35, 503-508), in methylene chloride (5ml). The mixture was stirred for 1 hour at ambient temperature and further triphenylphosphine (184mg, 0.7mmol), 2-(1,2,4-triazol-1-yl)ethanol (63mg, 0.56mmol) and diethyl azodicarboxylate
 25 (122mg, 0.7mmol) were added. The mixture was stirred for a further 2.5 hours and the solvent was removed by evaporation. The residue was dissolved in methanol (5ml) and 2M aqueous sodium hydroxide solution (2ml) was added. The mixture was stirred for 20 minutes and the mixture was partitioned between ether and water. The aqueous layer was acidified to pH7 with 2M hydrochloric acid and the resulting precipitate was collected by filtration.
 30 washed with water, and dried under vacuum. The resulting solid was dissolved in methylene chloride/methanol and a 5M solution of hydrogen chloride in isopropanol (0.5ml) was added.

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The volatiles were removed by evaporation, the solid was resuspended in ether, collected by filtration, washed with ether and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-(1,2,4-triazol-1-yl)ethoxy)quinazoline** hydrochloride (180mg, 56%).

¹H NMR Spectrum: (DMSO-d₆) 2.16(s, 3H); 3.97(s, 3H); 4.59(t, 2H); 4.74(t, 2H); 6.9(d, 1H); 7.10(d, 1H); 7.37(s, 1H); 8.03(s, 1H); 8.23(s, 1H); 8.62(s, 1H); 8.79(s, 1H); 9.7(br s, 1H); 11.4(s, 1H)

MS - ESI: 411 [MH]⁺

Elemental analysis:	Found	C 53.2	H 4.8	N 18.4
C ₂₀ H ₁₉ N ₆ O ₃ F 0.1H ₂ O 1.2HCl	Requires	C 52.7	H 4.5	N 18.4%

Example 76

Tetrabutylammonium fluoride (608μl of a 1M solution in THF, 0.67mmol) was added to a solution of 4-(4-chloro-5-diphenyl-t-butyloxy-2-fluoroanilino)-6-methoxy-7-((3-thienyl)methoxy)quinazoline (224mg, 0.33mmol) in THF (5ml) cooled at 5°C. After stirring for 1 hour at ambient temperature, water was added. The THF was removed by evaporation. The precipitate was collected by filtration and dried by azeotroping with ethanol. The solid was dissolved in methylene chloride/methanol and a solution of 5M hydrochloric acid in isopropanol was added. The volatiles were removed by evaporation. The residue was suspended in ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((3-thienyl)methoxy)quinazoline** hydrochloride (132mg, 85%).

m.p. 277-281°C

¹H NMR Spectrum: (DMSO-d₆) 3.99(s, 3H); 5.34(s, 2H); 7.15(d, 1H); 7.26(d, 1H); 7.49(s, 1H); 7.53(d, 1H); 7.61(m, 1H); 7.75(s, 1H); 8.22(s, 1H); 8.8(s, 1H); 10.59(s, 1H); 11.38(br s, 1H)

MS - ESI: 432 [MH]⁺

Elemental analysis:	Found	C 51.0	H 3.5	N 8.9
C ₂₀ H ₁₅ N ₃ O ₃ ClFS 0.1H ₂ O 1HCl	Requires	C 51.1	H 3.5	N 8.9%

The starting material was prepared as follows:

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Diethyl azodicarboxylate (274 μ l, 1.7mmol) was added dropwise to a solution of 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (400mg, 0.7mmol), (prepared as described for the starting material in Example 33), 3-thiophenemethanol (119mg, 1mmol) triphenylphosphine (456mg, 1.7mmol) in methylene chloride (12ml) cooled at 0°C. The mixture was stirred for 2 hours at ambient temperature, the solvent was removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/ether (95/5). The purified product was triturated with petroleum ether/ethyl acetate (8/2) and the solid product was collected by filtration, washed with ether and dried under vacuum to give 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-6-methoxy-7-((3-thienyl)methoxy)quinazoline (223mg, 47%).
¹H NMR Spectrum: (DMSO-d₆) 1.09(s, 9H); 3.85(s, 3H); 5.23(s, 2H); 7.04(d, 1H); 7.21(d, 1H); 7.25(s, 1H); 7.4-7.5(m, 6H); 7.58(m, 2H); 7.62-7.75(m, 6H); 8.1(s, 1H); 9.22(br s, 1H)

Example 77

Diethyl azodicarboxylate (274 μ l, 1.7mmol) was added dropwise to a solution of 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (400mg, 0.7mmol), (prepared as described for the starting material in Example 33), triphenylphosphine (456mg, 1.7mmol), 2-(4-pyridyl)ethanol (128mg, 1mmol), (Zhur. Obshech. Khim. 1958, 28, 103-110), in methylene chloride (12ml) cooled at 0°C. The mixture was stirred for 2 hours at ambient temperature and the solvent was removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (97/3) to give a white solid (416mg). A portion of this solid (390mg) was dissolved in THF (6ml), the solution was cooled to 0°C and tetrabutylammonium fluoride (1.1ml of a 1M solution in THF, 1.1 mmol) was added and the mixture was stirred for 2 hours at ambient temperature. Water was added, the organic solvent was removed by evaporation and the resulting precipitate was collected by filtration. The solid was dissolved in methylene chloride/methanol and a 5M solution of hydrogen chloride in isopropanol (0.5ml) was added. The volatiles were removed by evaporation and the solid was resuspended in isopropanol and collected by filtration, washed with isopropanol and ether and dried under vacuum to give 4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline hydrochloride (123mg, 42%).

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¹H NMR Spectrum: (DMSO-d₆; CD₃COOD) 3.49(t, 2H); 3.99(s, 3H); 4.6(t, 2H); 7.16(d, 1H); 7.41(s, 1H); 7.51(d, 1H); 8.05(br s, 2H); 8.19(s, 1H); 8.84(s, 1H); 8.86(br s, 2H)

MS - ESI: 441 [MH]⁺

Elemental analysis: Found C 50.4 H 4.7 N 10.0

5 C₂₂H₁₈N₄O₃ClF 1.1H₂O 1.8HCl Requires C 50.5 H 4.5 N 10.4%
0.23isopropanol

Example 78

Using an analogous procedure to that described in Example 77, 4-(4-chloro-5-diphenyl-t-butyisilyloxy-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (300mg, 0.52mmol), (prepared as described for the starting material in Example 33), was treated with 4-hydroxymethyl-2-methylthiazole (100mg, 0.87mmol) to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((2-methylthiazol-4-yl)methoxy)quinazoline** hydrochloride (132mg, 52%).

15 ¹H NMR Spectrum: (DMSO-d₆) 2.68(s, 3H); 4.00(s, 3H); 5.35(s, 2H); 7.17(d, 1H); 7.52(d, 1H); 7.56(s, 1H); 7.72(s, 1H); 8.29(s, 1H); 8.83(s, 1H); 10.63(br s, 1H); 11.58(s, 1H)

MS - ESI: 447 [MH]⁺

Elemental analysis: Found C 48.2 H 3.7 N 11.2

C₂₀H₁₆N₄O₄ClFS 0.6H₂O 1.2HCl Requires C 47.9 H 3.7 N 11.2%

20

The starting material was prepared as follows :-

A solution of 4-chloromethyl-2-methylthiazole (1.84g, 10mmol) in water (9ml) and concentrated hydrochloric acid (2ml) was heated at reflux for 20 hours. The mixture was allowed to cool and was adjusted to pH5 with 2M aqueous sodium hydroxide solution and the mixture was extracted with ethyl acetate. The organic extract was washed with water and brine, dried (MgSO₄) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (97/3) to give 4-hydroxymethyl-2-methylthiazole (800mg, 54%).

25 ¹H NMR Spectrum (CDCl₃) 2.72(s, 3H); 2.92(br s, 1H); 4.73(s, 2H); 7.03(s, 1H)

30

Example 79

Diethyl azodicarboxylate (197µl, 1.2mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.6mmol), (prepared as described for the starting material in Example 24), 3-thiophenemethanol (107mg, 0.93mmol) and triphenylphosphine (328mg, 1.2mmol) in methylene chloride (6ml) cooled at 0°C. The mixture was stirred for 2 hours at ambient temperature and further triphenylphosphine (157mg, 0.57mmol), 3-thiophenemethanol (107mg, 0.93mmol) and diethyl azodicarboxylate (98.5µl, 0.59mmol) were added. The mixture was stirred for 2 hours at ambient temperature and the solvent was removed by evaporation. The residue was dissolved in ethyl acetate and the solution was washed with water and brine, dried (MgSO₄) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/ethyl acetate (4/6). The resulting oil was dissolved in ether and a 5M solution of hydrogen chloride in isopropanol (1ml) was added. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((3-thienyl)methoxy)quinazoline** hydrochloride (59mg, 20%).

¹H NMR Spectrum: (DMSO-d₆) 3.99(s, 3H); 5.34(s, 2H); 7.25(d, 1H); 7.43(d, 1H); 7.45(s, 1H); 7.58-7.63(m, 2H); 7.7(dd, 1H); 7.72(dd, 1H); 8.17(s, 1H); 8.78(s, 1H)

MS - ESI: 416 [MH]⁺

Elemental analysis:	Found	C 53.5	H 3.7	N 9.0
C ₂₀ H ₁₅ N ₃ O ₂ ClFS 0.95HCl	Requires	C 53.3	H 3.6	N 9.3%

Example 80

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 24), 2-acetamido-4-chloromethylthiazole (164mg, 0.86mmol) and potassium carbonate (216mg, 1.5mmol) in DMF (5ml) was stirred at 40°C for 7 hours. The mixture was partitioned between ethyl acetate and water and the aqueous layer was adjusted to pH7 with 2M hydrochloric acid. The organic phase was washed with water, brine, dried (MgSO₄) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (95/5). The purified solid was dissolved in a mixture of methylene chloride and methanol and a 5M solution of hydrogen chloride in isopropanol (1.0ml) was added. The

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volatiles were removed by evaporation to give a solid, which was triturated with ether, collected by filtration and dried under vacuum to give 7-((2-acetamidothiazol-4-yl)methoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (96mg, 24%).

5 m.p. 194-202°C

¹H NMR Spectrum: (DMSO-d₆) 2.14(s, 3H); 4.0(s, 3H); 5.31(s, 2H); 7.34(s, 1H); 7.45(dd, 1H); 7.52(s, 1H); 7.60(t, 1H); 7.68(dd, 1H); 8.30(s, 1H); 8.81(s, 1H)

MS - ESI: 474 [MH]⁺

Elemental analysis: Found C 46.9 H 3.8 N 13.2

10 C₂₁H₁₇N₅O₇ClF 1.1H₂O 1.1HCl Requires C 47.3 H 3.8 N 13.1%

Example 81

Diethyl azodicarboxylate (295μl, 1.8mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (300mg, 0.93mmol), (prepared as described for the starting material in Example 24), 2-(1,2,4-triazol-1-yl)ethanol (159mg, 1.4mmol), (Ann. Pharm. Fr. 1977, 35, 503-508), and triphenylphosphine (492mg, 1.8mmol) in methylene chloride (10ml). The mixture was stirred for 2 hours at ambient temperature and further triphenylphosphine (246mg, 0.9mmol) and diethyl azodicarboxylate (147μl, 0.9mmol) were added. The mixture was stirred for 1 hour at ambient temperature and the resulting precipitate was collected by filtration, washed with methylene chloride and ether and dried under vacuum. This solid was suspended in methylene chloride/methanol and a 5M solution of hydrogen chloride in isopropanol (1.0ml) was added. The volatiles were removed by evaporation, the residue was triturated with ether. The resulting solid was collected by filtration, washed with ether and dried under vacuum to give 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1,2,4-triazol-1-yl)ethoxy)quinazoline hydrochloride (219mg, 52%).

25 m.p. 169-174°C

¹H NMR Spectrum: (DMSO-d₆) 3.99(s, 3H); 4.60(t, 2H); 4.74(t, 2H); 7.43(d, 1H); 7.45(s, 1H); 7.59(t, 1H); 7.67(dd, 1H); 8.06(s, 1H); 8.41(s, 1H); 8.68(s, 1H); 8.83(s, 1H)

MS - ESI: 415 [MH]⁺

30 Elemental analysis: Found C 47.0 H 4.3 N 16.5

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$C_{19}H_{16}N_6O_2ClF \cdot 1.6 H_2O \cdot 1HCl$ Requires C 47.0 H 4.4 N 16.4%
0.35 isopropanol

Example 82

5 Diethyl azodicarboxylate (295 μ l, 1.8 mmol) was added dropwise to a solution of 1-(3-hydroxypropyl)-[1,2,4]-triazole (119 mg, 0.93 mmol), (EP0060696 A1), 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200 mg, 0.62 mmol), (prepared as described for the starting material in Example 24), and triphenylphosphine (492 g, 1.8 mmol) in methylene chloride (4 ml) and the mixture stirred for 3 hours at ambient temperature. The
10 mixture was purified by column chromatography eluting with methylene chloride/acetonitrile/methanol (60/32/8). The purified product was triturated with a mixture of pentane and ether, collected by filtration and dried under vacuum to give a white solid. This solid was dissolved in methylene chloride/methanol and ethereal hydrogen chloride (1 ml of a 5 M solution) was added. The volatiles were removed by evaporation. The solid residue
15 was suspended in ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(1,2,4-triazol-1-yl)propoxy)quinazoline hydrochloride** (121 mg, 39%).

^1H NMR Spectrum: (DMSO- d_6 ; CF_3COOD) 2.44(t, 2H); 4.0(s, 3H); 4.3(t, 2H); 4.5(t, 2H); 7.32(s, 1H); 7.47(dd, 1H); 7.62(t, 1H); 7.70(dd, 1H); 8.08(s, 1H); 8.41(s, 1H); 8.87(s, 1H);
20 9.10(s, 1H)

MS - ESI: 429 [MH] $^+$

Elemental analysis: Found C 47.8 H 4.2 N 16.6

$C_{20}H_{18}N_6O_2ClF \cdot 0.2H_2O \cdot 2HCl$ Requires C 47.5 H 4.1 N 16.6%

25 **Example 83**

Diethyl azodicarboxylate (209 mg, 1.2 mmol) was added dropwise to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (128 mg, 0.4 mmol), (prepared as described for the starting material in Example 24), triphenylphosphine (314 mg, 1.2 mmol) and 2-(N-methyl-N-(pyridazin-4-yl)amino)ethanol (80 mg, 0.52 mmol) in methylene chloride
30 (5 ml) and the mixture stirred for 2 hours at ambient temperature. The solvent was removed by evaporation, the residue was triturated with ether and the resulting solid was collected by

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filtration. The solid was purified by column chromatography eluting with methylene chloride/methanol (9/1 followed by 8/2) to give a white solid. This solid was dissolved in methylene chloride/methanol and ethereal hydrogen chloride (0.5ml of a 4M solution) was added. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-methyl-N-(pyridazin-4-yl)amino)ethoxy)quinazoline hydrochloride** (110mg, 60%).

¹H NMR Spectrum: (DMSO-d₆) 3.11(s, 3H); 3.89(s, 3H); 3.94(t, 2H); 4.37(t, 2H); 6.85(dd, 1H); 7.21(s, 1H); 7.35(dd, 1H); 7.55(dd, 1H); 7.59(t, 1H); 7.8(s, 1H); 8.36(s, 1H); 8.59(d, 1H); 8.90(d, 1H); 9.57(s, 1H)

Elemental analysis:	Found	C 47.2	H 4.6	N 14.7
C ₂₂ H ₂₀ N ₆ O ₂ ClF 1.5H ₂ O 2.15HCl	Requires	C 47.2	H 4.5	N 15.0%

The starting material was prepared as follows:

A solution of 4-bromo-3,6-dichloro-pyridazine (1.11g, 5mmol), (J.Chem. Soc., Perkin Trans I, 1974, 696), and 2-(methylamino)ethanol (0.75g, 10mmol) in isopropanol (10ml) was heated at reflux for 30 minutes. The solvent was removed by evaporation, the residue was partitioned between methylene chloride and water and the aqueous layer was adjusted to pH9 with solid potassium carbonate. The organic layer was separated, washed with brine, dried (MgSO₄) and the solvent removed by evaporation. The residue was triturated with ether, collected by filtration and dried under vacuum to give 2-(N-(3,6-dichloropyridazin-4-yl)-N-methylamino)ethanol (1g, 90%).

¹H NMR Spectrum: (CDCl₃) 2.1(br s, 1H); 3.09(s, 3H); 3.71(t, 2H); 3.93(t, 2H); 6.8(s, 1H)
MS - ESI: 221 [MH]⁺

A mixture of 2-(N-(3,6-dichloropyridazin-4-yl)-N-methylamino)ethanol (444mg, 2mmol) and 10% palladium-on-charcoal catalyst (150mg) in ethanol (15ml), methanol (5ml) and aqueous ammonia (15ml) was stirred under hydrogen at 3 atmospheres pressure for 4 hours. The catalyst was removed by filtration and the solvent removed from the filtrate by evaporation. The residue was dissolved in methylene chloride, the insoluble material was removed by filtration and the solvent was removed from the filtrate by evaporation. The residue was purified by column chromatography on neutral aluminum oxide eluting with

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methylene chloride/methanol (95/5 followed by 90/10). The purified product was triturated with petroleum ether, the solid product was collected by filtration and dried under vacuum to give 2-(N-methyl-N-(pyridazin-4-yl)amino)ethanol (275mg, 91%).

¹H NMR Spectrum: (CDCl₃) 3.06(s, 3H); 3.57(t, 2H); 3.89(t, 2H); 6.52(dd, 1H); 8.48(d, 1H);
 5 8.54 (d, 1H)

MS - ESI: 153 [MH]⁺

Example 84

2M Aqueous sodium hydroxide solution (560μl, 1.1mmol) was added to a solution
 10 of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-((4-pyridyl)carboxamido)quinazoline (250mg, 0.56mmol) in methanol (7ml) cooled at 0°C and the mixture then stirred for 1 hour at ambient temperature. The mixture was diluted with water and the mixture adjusted to pH6 with 2M hydrochloric acid. The resulting solid was collected by filtration, washed with water and dried under vacuum. The solid was dissolved
 15 in methylene chloride/methanol and isopropanolic hydrogen chloride (0.7ml of a 5M solution) was added. The volatiles were removed by evaporation, the solid residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-((4-pyridyl)carboxamido)quinazoline hydrochloride** (241mg, 93%).

20 MS - ESI: 390 [MH]⁺

¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 2.2(s, 3H); 6.94(d, 1H); 7.13(d, 1H); 8.18(d, 1H); 8.53(d, 2H); 8.68(s, 1H); 8.77(d, 1H); 8.94(s, 1H); 9.20(d, 2H)

Elemental analysis:	Found	C 52.0	H 4.3	N 14.3
C ₂₁ H ₁₆ N ₅ O ₂ F 1.2H ₂ O 1.95HCl	Requires	C 52.3	H 4.3	N 14.5%

25

The starting material was prepared as follows:

A mixture of 7-nitro-3,4-dihydroquinazolin-4-one (J. Chem. Soc. 1950, 1104-1111) (5g, 26mmol) in thionyl chloride (50ml) and DMF (1ml) was heated at reflux for 1.5 hours. Excess thionyl chloride was removed by evaporation and the residue azeotroped with toluene.
 30 The residue was suspended in ether, collected by filtration and dried under vacuum to give 4-chloro-7-nitroquinazoline hydrochloride (6.4g, 100%).

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¹H NMR Spectrum: (DMSO-d₆) 8.26(dd, 1H); 8.36(d, 1H); 8.40(s, 1H); 8.42(dd, 1H)

MS - ESI: 209 [MII]⁺

A solution of 4-chloro-7-nitroquinazoline hydrochloride (2.46g, 10mmol) and 2-fluoro-5-methoxycarbonyloxy-4-methylaniline (2.2g, 11mmol), (prepared as described for the starting material in Example 12), in isopropanol (25ml) was heated at 50°C for 1 hour. The mixture was allowed to cool, the precipitated solid was collected by filtration recrystallised from methylene chloride/methanol/isopropanol to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-nitroquinazoline hydrochloride (1.8g, 45%) as a yellow solid.

¹H NMR Spectrum: (DMSO-d₆) 2.21(s, 3H); 3.86(s, 3H); 7.40(d, 1H); 7.46(d, 1H); 8.49(dd, 1H); 8.63(s, 1H); 8.84(s, 1H); 8.89(d, 1H)

MS - ESI: 373 [MH]⁺

Elemental analysis:	Found	C 50.0	H 3.6	N 13.8
C ₁₇ H ₁₃ N ₄ O ₅ F 1HCl	Requires	C 50.0	H 3.5	N 13.7%

A mixture of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-nitroquinazoline hydrochloride (5.3g, 13mmol) and 10% palladium-on-charcoal catalyst (1g) in ethanol (100ml), ethanolic hydrogen chloride (1.8ml of a 7M solution) and methanol (20ml) was stirred under hydrogen at 1.7 atmospheres pressure for 75 minutes. The catalyst was removed by filtration through diatomaceous earth and the filter pad thoroughly washed with methylene chloride, methanol and ether and the solvent was removed from the filtrate by evaporation to give 7-amino-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline hydrochloride (4.8g, 97%) as a yellow solid.

¹H NMR Spectrum: (DMSO-d₆) 2.22(s, 3H); 3.87(s, 3H); 6.77(s, 1H); 7.08(dd, 1H); 7.15(m, 2H); 7.41(m, 2H); 8.35(d, 1H); 8.63(s, 1H); 11.03(s, 1H)

MS - ESI: 343 [MH]⁺

A solution of 7-amino-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline hydrochloride (0.45g, 1.2mmol) and isonicotinoyl chloride hydrochloride (296mg, 1.66mmol) in pyridine (15ml) was stirred for 2 hours at ambient temperature, followed by 1 hour at 40°C. Further isonicotinoyl chloride hydrochloride (84mg, 0.46mmol) was added and the mixture was stirred at 40°C for 2 hours. The volatiles were removed by evaporation, the mixture was diluted with water. The aqueous mixture was adjusted to pH7 and extracted with ethyl acetate. The combined extracts were washed with

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brine, dried (MgSO_4) and the solvent removed by evaporation. The residue was purified by flash chromatography eluting with methylene chloride/methanol (95/5 followed by 92/8). The purified solid was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-((4-

pyridyl)carboxamido)quinazoline (264mg, 49%).

^1H NMR Spectrum: (DMSO-d_6) 2.19(s, 3H); 3.86(s, 3H); 7.31(d, 1H); 7.45(d, 1H); 7.92(d, 2H); 7.98(d, 1H); 8.31(s, 1H); 8.43(d, 1H); 8.47(s, 1H); 8.83(d, 2H); 9.78(br s, 1H); 10.89(br s, 1H)

10 **Example 85**

4-Chloro-2-fluoroaniline (77mg, 0.53mmol) was added to a solution of 4-chloro-6-methoxy-7-(2-(N-methyl-N-(6-methylpyrimidin-4-yl)amino)ethoxy)quinazoline hydrochloride (140mg, 0.35mmol) in isopropanol (5ml) and the mixture heated at reflux for 1 hour. The solvent was removed by evaporation and the residue was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate solution. The organic layer was separated, washed with brine, dried (MgSO_4) and the solvent removed by evaporation. The residue was purified by flash chromatography eluting with methylene chloride/methanol (95/5). The purified solid was dissolved in methylene chloride/methanol and ethereal hydrogen chloride (1ml of a 5M solution) was added. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-methyl-N-(6-methylpyrimidin-4-yl)amino)ethoxy)quinazoline** hydrochloride (75mg, 39%).

^1H NMR Spectrum: (DMSO-d_6 ; CF_3COOD) 2.46 and 2.47(2s, 3H); 3.35 and 3.42(2s, 3H); 3.97 and 3.98 (2s, 3H); 4.2(br s, 1H); 4.3(br s, 1H); 4.5(br s, 2H); 7.05 and 7.3(2s, 1H); 7.4 and 7.5(m, 2H); 7.62(t, 1H); 7.7(d, 1H); 8.25(br s, 1H); 8.8 and 8.9(2s, 2H)

MS - ESI: 469 [MH] $^+$

The starting material was prepared as follows:

Sodium hydride (1.44g of a 60% suspension in mineral oil, 36mmol) was added in portions over 20 minutes to a solution of 7-benzyloxy-3,4-dihydroquinazolin-4-one (8.46g, 30mmol), (prepared as described for the starting material in Example 70), in DMF (70ml) and

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the mixture stirred for 1.5 hours. Chloromethyl pivalate (5.65g, 37.5mmol) was added dropwise and the mixture stirred 2 hours at ambient temperature. The mixture was diluted with ethyl acetate (100ml) and poured onto ice/water (400ml) and 2M hydrochloric acid (4ml). The organic layer was separated and the aqueous layer extracted with ethyl acetate, the combined extracts were washed with brine, dried (MgSO_4) and the solvent removed by evaporation. The residue was triturated with a mixture of ether and petroleum ether, the solid was collected by filtration and dried under vacuum to give 7-benzyloxy-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (10g, 84%).

^1H NMR Spectrum: (DMSO-d_6) 1.11(s, 9H); 3.89(s, 3H); 5.3(s, 2H); 5.9(s, 2H); 7.27(s, 1H); 7.35(m, 1H); 7.47(t, 2H); 7.49(d, 2H); 7.51(s, 1H); 8.34(s, 1H)

A mixture of 7-benzyloxy-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (7g, 17.7mmol) and 10% palladium-on-charcoal catalyst (700mg) in ethyl acetate (250ml), DMF (50ml), methanol (50ml) and acetic acid (0.7ml) was stirred under hydrogen at atmospheric pressure for 40 minutes. The catalyst was removed by filtration and the solvent removed from the filtrate by evaporation. The residue was triturated with ether, collected by filtration and dried under vacuum to give 7-hydroxy-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (4.36g, 80%).

^1H NMR Spectrum: (DMSO-d_6) 1.1(s, 9H); 3.89(s, 3H); 5.89(s, 2H); 7.0(s, 1H); 7.48(s, 1H); 8.5(s, 1H)

Diethyl azodicarboxylate (679mg, 3.9mmol) was added dropwise to a suspension of 7-hydroxy-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (918mg, 3mmol), triphenylphosphine (1g, 3.9mmol) and 2-(N-methyl-N-(t-butylcarbonyl)amino)ethanol (682mg, 3.9mmol), prepared as described below, in methylene chloride (20ml) and the mixture stirred for 1 hour at ambient temperature. Further 2-(N-methyl-N-(t-butylcarbonyl)amino)ethanol (105mg, 0.6mmol), triphenylphosphine (786mg, 3mmol) and diethyl azodicarboxylate (522mg, 3mmol) were added and the mixture stirred for 30 minutes at ambient temperature. The mixture was concentrated to half volume by evaporation and purified by column chromatography eluting with methylene chloride/ether (7/3 increasing to 1/1) to give 6-methoxy-7-(2-(N-methyl-N-(t-butylcarbonyl)amino)ethoxy)-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (1.3g, 98%).

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¹H NMR Spectrum: (CDCl₃) 1.2(s, 9H); 1.45(s, 9H); 3.05(br s, 3H); 3.72(br s, 2H); 3.98(s, 3H); 4.25(br s, 2H); 5.95(s, 2H); 7.1(br s, 1H); 7.6(s, 1H); 8.2(s, 1H)

A solution of 6-methoxy-7-(2-(N-methyl-N-(t-butylcarbonyl)amino)ethoxy)-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (1.39g, 3mmol) in methylene chloride (4ml) and TFA (4ml) was stirred at ambient temperature for 1 hour. Toluene was added, and the volatiles were removed by evaporation. The residue was triturated with ether and the resulting solid was collected by filtration. The solid was dissolved in water, sodium hydrogen carbonate was added and the aqueous mixture was extracted with methylene chloride. The organic extract was dried (MgSO₄) and the solvent removed by evaporation. The residue was triturated with ether and the solid was collected by filtration to give 6-methoxy-7-(2-(methylamino)ethoxy)-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (800mg, 73%).
¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 1.13(s, 9H); 2.72(s, 3H); 3.45(br s, 2H); 3.95(s, 3H); 4.5(t, 2H); 5.94(s, 2H); 7.31(s, 1H); 7.6(s, 1H); 8.47(s, 1H)
MS - ESI: 364 [MH]⁺

A solution of 6-methoxy-7-(2-(methylamino)ethoxy)-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (363mg, 1mmol) and 4-chloro-6-methylpyrimidine (257mg, 2mmol), (J. Het. Chem., 1969, 6, 879), in N,N-diisopropylethylamine (2ml) was heated at reflux for 30 minutes. The volatiles were removed by evaporation and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried (MgSO₄) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (95/5) to give 6-methoxy-7-(2-(N-methyl-N-(6-methylpyrimidin-4-yl)amino)ethoxy)-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (365mg, 80%).
¹H NMR Spectrum: (CDCl₃) 1.19(s, 9H); 2.36(s, 3H); 3.18(s, 3H); 3.95(s, 3H); 4.09(t, 2H); 4.34(t, 2H); 5.9(s, 2H); 6.3(s, 1H); 7.14(s, 1H); 7.63(s, 1H); 8.17(s, 1H); 8.5(s, 1H)
MS - ESI: 456 [MH]⁺

A solution of 6-methoxy-7-(2-(N-methyl-N-(6-methylpyrimidin-4-yl)amino)ethoxy)-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (365mg, 0.8mmol) in methanolic ammonia (30ml of a 3M solution) was stirred at ambient temperature for 16 hours. The volatiles were removed by evaporation, the residue was triturated with ether, the solid was collected by filtration, washed with ether and dried under vacuum to give 6-methoxy-7-

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(2-(N-methyl-N-(6-methylpyrimidin-4-yl)amino)ethoxy)-3,4-dihydroquinazolin-4-one (250mg, 92%).

¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 2.44(s, 3H); 3.32 and 3.39(2s, 3H); 3.86 and 3.87(2s, 3H); 4.12(t, 1H); 4.25(t, 1H); 4.42(m, 2H); 7.02 and 7.23(2s, 1H); 7.24(t, 1H); 7.50(s, 1H); 8.55 and 8.8(2m, 1H); 8.78 and 8.80(2s, 1H)
MS - ESI: 342 [MH]⁺

A mixture of 6-methoxy-7-(2-(N-methyl-N-(6-methylpyrimidin-4-yl)amino)ethoxy)-3,4-dihydroquinazolin-4-one (250mg, 0.73mmol) in thionyl chloride (5ml) and DMF (0.1ml) was heated at reflux for 1 hour. The mixture was diluted with toluene and the volatiles were removed by evaporation. The residue was triturated with methylene chloride/ether, the solid was collected by filtration and dried under vacuum to give 4-chloro-6-methoxy-7-(2-(N-methyl-N-(6-methylpyrimidin-4-yl)amino)ethoxy)quinazoline hydrochloride (260mg, 90%).

2-(N-methyl-N-(t-butylcarbonyl)amino)ethanol was prepared as follows :-

A solution of di-t-butylidicarbonate (4.52g, 20mmol) in THF (10ml) was added to a solution of 2-(N-methylamino)ethanol (1.5g, 20mmol) in a mixture of water (10ml) and THF (10ml). The mixture was stirred at ambient temperature for 18 hours, the THF was removed by evaporation and the aqueous residue was partitioned between ether and water. The organic layer was washed with water, brine, dried (MgSO₄) and evaporated to give 2-(N-methyl-N-(t-butylcarbonyl)amino)ethanol (3g, 85%).

¹H NMR Spectrum (CDCl₃) 1.46(s, 9H); 2.92(s, 3H); 3.39 (t, 2H); 3.75(t, 2H).
MS - ES: 176 [MH]⁺

Example 86

Diethyl azodicarboxylate (295μl, 1.8mmol) was added dropwise to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.62mmol), (prepared as described for the starting material in Example 24), 2-(3,5-dimethyl-[1,2,4]-triazol-4-yl)ethanol (114mg, 0.81mmol), (EP 0329357 A1), and triphenylphosphine (492mg, 1.8mmol) in methylene chloride (4ml) and the mixture stirred for 1 hour at ambient temperature. The precipitated solid was collected by filtration, washed with ether and dried under vacuum. The solid was dissolved in methylene chloride/methanol and ethereal hydrogen chloride (2ml of 4.5M solution) was added. The volatiles were removed by evaporation, the residue was

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suspended in ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(2-(3,5-dimethyl-[1,2,4]-triazol-4-yl)ethoxy)-6-methoxyquinazoline** hydrochloride (184mg, 54%).

¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 2.78(s, 6H); 4.03(s, 3H); 4.57(t, 2H); 4.75(t, 2H);
 5 7.37(s, 1H); 7.46(d, 1H); 7.64(t, 1H); 7.66(d, 1H); 8.31(s, 1H); 8.87(s, 1H)

MS - ESI: 443 [MH]⁺

Elemental Analysis:

Found	C	48.0	H	4.6	N	16.1
Requires	C	47.7	H	4.6	N	15.9%

C₂₁H₂₀N₆O₂ClF 1H₂O 1.85HCl

10 **Example 87**

Diethyl azodicarboxylate (295μl, 1.8mmol) was added dropwise to a solution of the 75/25 mixture of 2-(2,4-dimethylimidazol-1-yl)ethanol and 2-(2,5-dimethylimidazol-1-yl)ethanol (114mg, 0.81mmol), 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.62mmol), (prepared as described for the starting material in
 15 Example 24), and triphenylphosphine (492mg, 1.8mmol) in methylene chloride (4 ml) and the mixture stirred for 4 hours at ambient temperature. Further triphenylphosphine (49mg, 0.18mmol), mixture of imidazolylethanol (26mg, 0.18mmol) and diethyl azodicarboxylate (29μl, 0.18mmol) were added and the mixture stirred for 1 hour. The precipitated solid was collected by filtration, washed with methylene chloride, and dried under vacuum. The solid
 20 was dissolved in methylene chloride/methanol and ethereal hydrogen chloride (1.5ml of a 4.5M solution) was added. The volatiles were removed by evaporation, the solid residue was suspended in ether, collected by filtration, washed with ether and dried under vacuum to give a 75/25 mixture of **4-(4-chloro-2-fluoroanilino)-7-(2-(2,4-dimethylimidazol-1-yl)ethoxy)-6-methoxyquinazoline** hydrochloride and **4-(4-chloro-2-fluoroanilino)-7-(2-(2,5-**
 25 **dimethylimidazol-1-yl)ethoxy)-6-methoxyquinazoline** hydrochloride (159mg, 48%).

¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 2.23 and 2.43(2s, 3H); 2.73 and 2.76(2s, 3H); 4.02(s, 3H); 4.6(br s, 2H); 4.6 and 4.75(m, 2H); 7.3-7.5(m, 3H); 7.61(t, 1H); 7.68(d, 1H); 8.24(s, 1H); 8.88(s, 1H)

MS - ESI: 442 [MH]⁺

30 Elemental analysis:

Found	C	49.9	H	4.6	N	13.3
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$C_{22}H_{21}N_4O_2ClF \cdot 1.1H_2O \cdot 1.85HCl$ Requires C 50.1 H 4.8 N 13.3%

The starting material was prepared as follows:

2,4-Dimethylimidazole (1.5g, 15.6mmol) was added in portions to a suspension of
5 sodium hydride (936mg of a 60% suspension in mineral oil, 23mmol) in DMF (8ml) and the
mixture was stirred for 30 minutes at ambient temperature. 2-Bromoethanol (1.66ml,
23mmol) was added and the mixture stirred at 100°C for 16 hours. The solvent was removed
by evaporation and concentrated hydrochloric acid (1ml) was added to the residue. The
resulting solid was triturated with methylene chloride, collected by filtration and dried under
10 vacuum. The solid was purified by column chromatography on neutral alumina eluting with
methylene chloride/methanol (97/3) and then column chromatography eluting with methylene
chloride/methanol (93/7 followed by 90/10) to give a 75/25 mixture of 2-(2,4-
dimethylimidazol-1-yl)ethanol and 2-(2,5-dimethylimidazol-1-yl)ethanol (650mg, 29%).
MS - ESI: 140 [MH]⁺

15

Example 88

Diethyl azodicarboxylate (236μl, 1.5mmol) was added dropwise to a suspension of
4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), (prepared
as described for the starting material in Example 24), triphenylphosphine (393mg, 1.5mmol)
20 and 2-(3-pyridyl)ethanol (86mg, 0.7mmol), (J.Heterocycl. Chem. 1992, **29**, 1663), in
methylene chloride (6ml) and the mixture stirred for 4 hours at ambient temperature. The
mixture was poured directly on to a silica column and eluted with methylene
chloride/acetonitrile/methanol (60/35/5). The purified solid was dissolved in methylene
chloride/methanol and ethereal hydrogen chloride (1.5ml of a 4.5M solution) was added. The
25 volatiles were removed by evaporation, the solid residue was suspended in ether, collected by
filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-
methoxy-7-(2-(3-pyridyl)ethoxy)quinazoline hydrochloride** (154mg, 52%).
¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 3.45(t, 2H); 4.01(s, 3H); 4.56(t, 2H); 7.44(s, 1H);
7.46(d, 1H); 7.61(t, 1H); 7.67(d, 1H); 8.13(t, 1H); 8.19(s, 1H); 8.71(d, 1H); 8.88(s, 1H); 8.9(d,
30 1H); 9.01(s, 1H)

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MS - ESI: 425 [MH]⁺

Elemental analysis:

Found C 52.7 H 4.3

C₂₂H₁₈N₄O₂ClF 0.8H₂O 1.8HCl

Requires C 52.3 H 4.3%

5 **Example 89**

Diethyl azodicarboxylate (236μl, 1.5mmol) was added dropwise to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), (prepared as described for the starting material in Example 24), triphenylphosphine (393mg, 1.5mmol) and 2-(6-methyl-2-pyridyl)ethanol (96mg, 0.7mmol), (J. Chem. Soc. A, 1971, 388), in methylene chloride (6ml) and the mixture stirred for 16 hours at ambient temperature. The mixture was poured directly on to a silica column and eluted with methylene chloride/methanol (95/5). The purified solid was dissolved in methylene chloride/methanol and ethereal hydrogen chloride (1.5ml of a 4.5M solution) was added. The mixture was diluted with ether and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(6-methyl-2-pyridyl)ethoxy)quinazoline hydrochloride** (97mg, 34%).

¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 2.78(s, 3H); 3.64(t, 2H); 3.98(s, 3H); 4.67(t, 2H); 7.46(s, 1H); 7.48(br s, 1H); 7.62(t, 1H); 7.68(dd, 1H); 7.85(d, 1H); 7.94(d, 1H); 8.19(s, 1H); 8.48(t, 1H); 8.88(s, 1H)

20 MS - ESI: 439 [MH]⁺

Elemental Analysis:

Found C 52.7 H 4.5 N 10.7

C₂₃H₂₀N₄O₂ClF 1H₂O 1.8HCl Requires C 52.9 H 4.6 N 10.7%**Example 90**

25 A mixture of 4-chloro-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline (49mg, 0.16mmol) and 3-hydroxyaniline (21mg, 0.19mmol) in isopropanol (3ml) and isopropanolic hydrogen chloride (0.2ml of a 5M solution) was stirred at 80°C for 1 hour. The precipitated solid was collected by filtration, washed with isopropanol and ether and dried under vacuum to give **4-(3-hydroxyanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline** hydrochloride (56mg, 93%).

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¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 4.01(s, 3H); 4.64(t, 2H); 4.78(t, 2H); 6.71(d, 1H); 7.1(m, 2H); 7.28(t, 1H); 7.41(s, 1H); 7.74(s, 1H); 7.83(s, 1H); 8.21(s, 1H); 8.87(s, 1H); 9.22(s, 1H)

MS - ESI: 378 [MH]⁺

5	Elemental Analysis:	Found	C 52.7	H 4.9	N 15.1
	C ₂₀ H ₁₉ N ₅ O ₃ · 0.6H ₂ O · 1.85HCl	Requires	C 52.7	H 4.9	N 15.4%

The starting material was prepared as follows:

Diethyl azodicarboxylate (435mg, 2.5mmol) was added dropwise to a suspension of 7-hydroxy-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (612mg, 2mmol), (prepared as described for the starting material in Example 85), 2-(imidazol-1-yl)ethanol (280mg, 2.5mmol), (J. Med. Chem. 1993, **25** 4052-4060), and triphenylphosphine (655mg, 2.5mmol) in methylene chloride (10ml) at 5°C. The mixture was stirred for 10 minutes at 5°C and then 1 hour at ambient temperature. The mixture was poured directly on to a silica column and eluted with methylene chloride/methanol (95/5) to give 7-(2-(imidazol-1-yl)ethoxy)-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (640mg, 80%).

¹H NMR Spectrum: (CDCl₃) 1.19(s, 9H); 3.98(s, 3H); 4.34(m, 2H); 4.45(m, 2H); 5.94(s, 2H); 7.02(s, 1H); 7.07(s, 1H); 7.11(s, 1H); 7.64(s, 1H); 7.67(s, 1H); 8.17(s, 1H)

MS - ESI: 423 [MNa]⁺

20	Elemental Analysis:	Found	C 58.3	H 6.4	N 13.9
	C ₂₀ H ₂₄ N ₄ O ₅ · 0.7H ₂ O	Requires	C 58.2	H 6.2	N 13.6%

A solution of 7-(2-(imidazol-1-yl)ethoxy)-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (640mg, 1.6mmol) in saturated methanolic ammonia (10ml) was stirred for 15 hours at ambient temperature. The volatiles were removed by evaporation, the solid was triturated with ether, collected by filtration and dried under vacuum to give 7-(2-(imidazol-1-yl)ethoxy)-6-methoxy-3,4-dihydroquinazolin-4-one (412mg, 90%).

¹H NMR Spectrum: (DMSO-d₆) 3.89(s, 3H); 4.4-4.5(m, 4H); 6.9(s, 1H); 7.16(s, 1H); 7.28(s, 1H); 7.47(s, 1H); 7.7(s, 1H); 7.99(s, 1H)

MS - ESI: 287 [MH]⁺

30	Elemental Analysis:	Found	C 57.8	H 5.2	N 19.3
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$C_{14}H_{14}N_4O_3 \cdot 0.3H_2O$ Requires C 57.7 H 5.1 N 19.2%

A mixture of 7-(2-(imidazol-1-yl)ethoxy)-6-methoxy-3,4-dihydroquinazolin-4-one (412mg, 1.44mmol), thionyl chloride (5 ml) and DMF (0.2ml) was heated at reflux for 1 hour. The mixture was diluted with toluene and the volatiles were removed by evaporation. The residue was suspended in methylene chloride, cooled to 0°C and aqueous sodium hydrogen carbonate solution was added. The resulting precipitate was collected by filtration and dried under vacuum to give 4-chloro-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline (258mg, 59%).

¹H NMR Spectrum: (DMSO-d₆) 4.01(s, 3H); 4.47(m, 2H); 4.53(m, 2H); 6.89(s, 1H); 7.27(s, 1H); 7.41(s, 1H); 7.49(s, 1H); 7.70(s, 1H); 8.88(s, 1H)

MS - ESI: 327 [MNa]⁺

Example 91

Diethyl azodicarboxylate (220μl, 1.4mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (150mg, 0.47mmol), (prepared as described for the starting material in Example 24), 2-(1,2,4-triazol-4-yl)ethanol (64mg, 0.56mmol) and triphenylphosphine (369mg, 1.4mmol) in methylene chloride (5ml) and the mixture stirred for 30 minutes at ambient temperature. Further 2-(1,2,4-triazol-4-yl)ethanol (16mg, 0.14mmol), triphenylphosphine (37mg, 0.14mmol) and diethyl azodicarboxylate (22μl, 0.14mmol) was added and the mixture stirred for 1 hour at ambient temperature. The precipitated solid was collected by filtration, washed with methylene chloride and methanol and dried under vacuum. The solid was dissolved in methylene chloride/methanol and ethereal hydrogen chloride (1.5ml of a 2.2M solution) was added. The volatiles were removed by evaporation, the solid residue was suspended in ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1,2,4-triazol-4-yl)ethoxy)quinazoline** hydrochloride (93mg, 40%).

¹H NMR Spectrum: (DMSO-d₆; CF₃COOD) 4.02(s, 3H); 4.66(t, 2H); 4.85(t, 2H); 7.41(s, 1H); 7.46(dd, 1H); 7.62(t, 1H); 7.69(dd, 1H); 8.11(s, 1H); 8.89(s, 1H); 9.55 (s, 2H)

MS - ESI: 415 [MH]⁺

Elemental analysis: Found C 45.9 H 3.7 N 17.1

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$C_{19}H_{16}N_6O_2ClF \cdot 0.5H_2O \cdot 2HCl$ Requires C 45.9 H 3.9 N 16.9%

The starting material was prepared as follows:

- A solution of N,N-dimethylformamide azine (1g, 7mmol), (J. Chem. Soc. C, 1967, 1664), p-toluene sulphonic acid (45mg) and ethanolamine (4.3g, 70mmol) in benzene (15ml) was heated at reflux for 8 hours. The mixture was allowed to cool, the solvent was removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol (90/10 followed by 85/15) to give 2-(1,2,4-triazol-4-yl)ethanol (328mg, 41%).
- ¹H NMR Spectrum: (CDCl₃) 3.97(t, 2H); 4.11(t, 2H); 4.9(br s, 1H); 8.06(s, 2H)
- MS - ESI: 113 [MH]⁺

Example 92

- 1,1'-(azodicarbonyl)dipiperidine (480mg, 1.9mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.63mmol), 3-benzyloxypropanol (150μl, 0.95mmol) and tributylphosphine (459μl, 1.86mmol) in methylene chloride (20ml) at 5°C. The reaction was stirred for 1 hour at 5°C and then for 18 hours at ambient temperature. The mixture was diluted with ether and stirred for 15 minutes. The insolubles were removed by filtration and the volatiles were removed from the filtrate by evaporation. The residue was partitioned between ethyl acetate and water, and the organic layer was separated, dried (MgSO₄) and the solvent removed by evaporation. A 1M solution of ethereal hydrogen chloride was added to the residue, the resulting solution was reduced in volume by evaporation and the resulting precipitate was collected by filtration and dried to give **7-(3-benzyloxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride** (90mg, 31%).
- ¹H NMR Spectrum (CDCl₃) 2.12(t, 2H); 3.62(t, 2H); 4.00(t, 3H); 4.28(t, 2H); 4.45(s, 2H); 7.21-7.38(m, 6H); 7.42(d, 1H); 7.60(t, 1H); 7.64(dd, 1H); 8.22(s, 1H); 8.80(s, 1H)
- MS - ESI: 468 [MH]⁺

Example 93

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- 1,1'-(azodicarbonyl)dipiperidine (840mg, 3mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (315mg, 1mmol), ethyl 4-hydroxymethyl-2-pyridinecarboxylate (250mg, 1.4mmol), (J. Het. Chem. 1993, 30, 631-635) and tributylphosphine (800µl, 3mmol) in methylene chloride (50ml) at 0°C. The mixture was allowed to warm to ambient temperature over 2 hours, the insolubles were removed by filtration and the filtrate was washed with water and brine, dried (Na₂SO₄) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (10:0 increasing to 9:1). The purified product was recrystallised from methylene chloride/hexane to give **4-(4-chloro-2-fluoroanilino)-7-(2-ethoxycarbonylpyrid-4-yl)methoxy-6-methoxyquinazoline** (285mg, 60%).
- m.p. 212-214°C
- ¹H NMR Spectrum (DMSO-d₆) 1.30(t, 3H); 3.96(s, 3H); 4.35(q, 2H); 5.45(s, 2H); 7.14(s, 1H); 7.35(dd, 1H); 7.5-7.6(m, 2H); 7.85(s, 1H); 8.15(s, 1H); 8.35(s, 1H); 8.75(d, 1H); 9.55(s, 1H)
- Elemental analysis:
- | | | | | |
|----|---|----------|--------|---------------|
| | Found | C 58.9 | H 4.4 | N 12.0 |
| 15 | C ₂₄ H ₂₆ ClFN ₄ O ₄ 0.5 H ₂ O | Requires | C 58.7 | H 4.4 N 11.5% |

Example 94

- 1,1'-(azodicarbonyl)dipiperidine (1.68g, 6mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (640mg, 2mmol), 4-hydroxymethyl-2-(methylamino)pyridine (385mg, 2.8mmol) and tributylphosphine (1.6ml, 6mmol) in methylene chloride (50ml) at 0°C. The mixture was allowed to warm to ambient temperature over 2 hours, the insolubles were removed by filtration and the filtrate was washed with water and brine, dried (Na₂SO₄) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (10:0 increasing to 9:1). The purified product was dissolved in acetone/methanol and a 1M solution of ethereal hydrogen chloride was added. The resulting precipitate was collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-7-(2-(methylamino)pyrid-4-yl)methoxy-6-methoxyquinazoline hydrochloride** (395mg, 45%).
- ¹H NMR Spectrum (DMSO-d₆) 2.95(d, 3H); 4.05(s, 3H); 5.42(s, 2H); 6.90(d, 1H); 7.15(s, 1H); 7.40(d, 1H); 7.44(s, 1H); 7.58(t, 1H); 7.62(dd, 1H); 7.95(d, 1H); 8.46(s, 1H); 8.75(s, 1H); 9.06(br s, 1H); 11.83(br s, 1H)

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MS - ESI: 440 [MH]⁺

The starting material was prepared as follows :-

A mixture of 2-chloro-4-hydroxymethylpyridine (1.0g, 7mmol), (prepared as described for the starting material in Example 58), and methylamine (30ml of a 30% solution in ethanol) was heated in a Carius tube for 16 hours at 200°C. The mixture was allowed to cool and the mixture partitioned between saturated aqueous sodium hydrogen carbonate solution and ethyl acetate. The organic layer was separated, dried (MgSO₄) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with ethyl acetate to give 4-hydroxymethyl-2-(methylamino)pyridine (440mg, 46%) as a yellow oil.

¹H NMR Spectrum (DMSO-d₆) 2.72(d, 3H); 4.35(d, 2H); 5.15(t, 1H); 6.30(br d, 1H); 6.35(d, 1H); 6.38(s, 1H); 7.85(d, 1H)

Example 95

1,1'-(azodicarbonyl)dipiperidine (1.68g, 6mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (640mg, 2mmol), 4-hydroxymethyl-2-(dimethylamino)pyridine (426mg, 2.8mmol) and tributylphosphine (1.6ml, 6mmol) in methylene chloride (50ml) at 0°C. The mixture was allowed to warm to ambient temperature over 2 hours, the insolubles were removed by filtration and the filtrate was washed with water and brine, dried (Na₂SO₄) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (100:0 increasing to 95:5). The purified product was dissolved in acetone/methanol and a 1M solution of ethereal hydrogen chloride was added. The resulting precipitate was collected by filtration and dried to give 4-(4-chloro-2-fluoroanilino)-7-(2-(dimethylamino)pyrid-4-yl)methoxy-6-methoxyquinazoline hydrochloride (305mg, 30%).

m.p. 290°C

¹H NMR Spectrum (DMSO-d₆) 3.05(s, 6H); 4.05(s, 3H); 5.45(s, 2H); 6.95(d, 1H); 7.35(s, 1H); 7.42(dd, 1H); 7.56(t, 2H); 7.62(dd, 1H); 8.00(d, 1H); 8.55(s, 1H); 9.80(s, 1H); 11.95(br s, 1H)

MS - ESI: 454 [MH]⁺

Elemental analysis:

Found C 47.2 H 4.9 N 12.1

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$C_{23}H_{23}ClFN_4O_2 \cdot 3 HCl \cdot H_2O$ Requires C 47.6 H 4.5 N 12.1%

The starting material was prepared as follows :-

A mixture of 2-chloro-4-hydroxymethylpyridine (1.0g, 7mmol), (prepared as described for the starting material in Example 58), and dimethylamine (30ml of a 30% solution in ethanol) was heated in a Carius tube for 16 hours at 200°C. The mixture was allowed to cool and the mixture partitioned between saturated aqueous sodium bicarbonate solution and ethyl acetate. The organic layer was separated, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with ethyl acetate to give 4-hydroxymethyl-2-(dimethylamino)pyridine (1g, 94%) as a yellow oil.

^1H NMR Spectrum ($DMSO-d_6$) 3.00(s, 6H); 4.40(d, 2H); 5.20(t, 1H); 6.45(d, 1H); 6.55(s, 1H); 7.96(d, 1H)

MS - ESI: 153 [MH]⁺

15 **Example 96**

A mixture of 4-(3-hydroxyprop-2-en-1-yl)pyridine (180mg, 1.3mmol) and thionyl chloride (0.3ml) in toluene (10ml) was stirred at room temperature for 2 hours. The volatiles were removed by evaporation to give crude 4-(3-chloroprop-2-en-1-yl)pyridine hydrochloride (180mg, 0.94mmol). This product was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (500mg, 1.6mmol) and potassium carbonate (500mg, 4.9mmol) in DMF (20 ml) and the mixture stirred at 100°C for 1 hour. The reaction mixture was allowed to cool and partitioned between ethyl acetate and water. The organic layer was separated, washed with water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (100/0 increasing to 95/5) and then by reverse phase (C18) HPLC eluting with methanol/water (30/70 increasing to 50/50) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(pyrid-4-yl)prop-2-en-1-yloxy)quinazoline** (15mg, 4%).

^1H NMR Spectrum ($DMSO-d_6$) 4.00(s, 3H); 5.05(d, 2H); 6.93(d, 1H); 7.11(dt, 1H); 7.40(s, 1H); 7.40-7.43(m, 2H); 7.60(t, 1H); 7.65(d, 1H); 7.80(m, 2H); 8.05(s, 1H); 8.70(br s, 2H)

MS - ESI: 437 [MH]⁺

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The starting material was prepared as follows :-

- n-Butyllithium (25ml of a 1.6M solution in hexane, 40mmol) was added dropwise to a stirred suspension of 2-hydroxyethyltriphenylphosphonium bromide (7.74g, 20mmol) in THF (50ml) at -70°C and the mixture allowed to warm to -30°C and stirred for 2 hours. 4-Pyridinecarboxaldehyde (2.16g, 20mmol) was added to the resulting red solution, and the mixture stirred for 1 hour at -30°C and then cooled to -70°C. n-Butyllithium (12.5ml of a 1.6M solution in hexane, 20mmol) was added and the reaction mixture stirred at -70°C for 1 hour. The mixture was quenched with isopropanol and allowed to warm to ambient temperature. Saturated aqueous ammonium chloride solution was added, the organic layer separated and the aqueous layer extracted with ethyl acetate (3x50ml). The combined extracts were washed with brine and dried (MgSO₄) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with ethyl acetate to give 4-(3-hydroxyprop-2-en-1-yl)pyridine
- MS - ESI: 136 [MH]⁺

Example 97

- A suspension of 4-chloro-7-(2-(1,2,4-triazol-1-yl)ethoxy)-6-methoxyquinazoline (214mg, 0.7mmol), 4-bromo-2-fluoroaniline (160mg, 0.84mmol) in isopropanolic hydrogen chloride (1ml of a 5M solution) and isopropanol (5ml) was heated at 80°C for 1 hour. The mixture was allowed to cool, the precipitate was collected by filtration, washed with isopropanol and then ether and dried under vacuum at 70°C to give **4-(4-bromo-2-fluoroanilino)-7-(2-(1,2,4-triazol-1-yl)ethoxy)-6-methoxyquinazoline hydrochloride** (55mg, 15%).
- ¹H NMR Spectrum (DMSO-d₆) 3.99(s, 3H); 4.62(t, 2H); 4.75(t, 2H); 7.37(s, 1H); 7.5-7.7(m, 2H); 7.81(d, 1H); 8.04(s, 1H); 8.24(s, 1H); 8.63(s, 1H); 8.84(s, 1H); 11.52(s, 1H)
- MS - ESI: 459 [MH]⁺
- | | | | | |
|---|----------|--------|-------|---------|
| Elemental analysis | Found | C 41.8 | H 3.4 | N 15.6 |
| C ₁₉ H ₁₆ BrFN ₆ O ₂ 0.8 H ₂ O 1.9 HCl | Requires | C 42.0 | H 3.6 | N 15.5% |

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The starting material was prepared as follows :-

Diethyl azodicarboxylate (1.1ml, 7mmol) was added dropwise to a solution of 7-hydroxy-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (1.7 g, 5.55mmol), 2-(1,2,4-triazol-1-yl)ethanol (791mg, 7mmol), (Ann. Pharm. Fr. 1977,35,503-508) and triphenylphosphine (1.8g, 7mmol) cooled at 5°C. The mixture was allowed to warm to ambient temperature and stirred for 1 hour. The mixture was poured directly on to a column of silica and eluted with methylene chloride/methanol (95/5) to give 6-methoxy-3-((pivaloyloxy)methyl)-7-(2-(1,2,4-triazol-1-yl)ethoxy)-3,4-dihydroquinazolin-4-one (1.64g, 74%).

¹H NMR Spectrum (DMSO-d₆, CF₃COOD) 1.12(s, 9H); 3.87(s, 3H); 4.57(t, 2H); 4.74(t, 2H); 5.92(s, 2H); 7.24(s, 1H); 7.51(s, 1H); 8.36(d, 1H); 8.41(s, 1H); 9.02(d, 1H)

MS - ESI: 424 [MNa]⁺

Elemental analysis	Found	C 56.5	H 6.0	N 17.6
C ₁₉ H ₂₃ N ₃ O ₅	Requires	C 56.9	H 5.8	N 17.6

A solution of 6-methoxy-3-((pivaloyloxy)methyl)-7-(2-(1,2,4-triazol-1-yl)ethoxy)-3,4-dihydroquinazolin-4-one (1.6g, 4mmol) in saturated methanolic ammonia (25ml) was stirred at ambient temperature for 2 days. The volatiles were removed by evaporation, the solid residue was triturated with ether, collected by filtration and dried under vacuum to give 6-methoxy-7-(2-(1,2,4-triazol-1-yl)ethoxy)-3,4-dihydroquinazolin-4-one (1.11g, 98%)

¹H NMR Spectrum (DMSO d₆) 3.84(s, 3H); 4.51(t, 2H); 4.65(t, 2H); 7.16(s, 1H); 7.44(s, 1H); 7.89(s, 1H); 7.99(s, 1H); 8.55(s, 1H)

MS - EI: 287 [M]⁺

Elemental analysis	Found	C 53.9	H 4.6	N 24.6
C ₁₃ H ₁₃ N ₃ O ₃	Requires	C 54.4	H 4.6	N 24.4

A solution of 6-methoxy-7-(2-(1,2,4-triazol-1-yl)ethoxy)-3,4-dihydroquinazolin-4-one (1.11g, 3.86mmol) and DMF (0.6 ml) in thionyl chloride (15ml) was heated at reflux for 1 hour. The mixture was allowed to cool, toluene was added and the volatiles were removed by evaporation. The residue was partitioned between methylene chloride and water and the aqueous layer was adjusted to pH8.5 with saturated aqueous sodium hydrogen carbonate solution. The organic layer was separated, washed with brine, dried (MgSO₄), and the solvent removed by evaporation. The residue was purified by column chromatography eluting with

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methylene chloride/methanol (95/5). The purified solid was triturated with ether, collected by filtration, washed with water and then ether, and dried under vacuum to give 4-chloro-6-methoxy-7-(2-(1,2,4-triazol-1-yl)ethoxy)quinazoline (756mg, 65%).

¹H NMR Spectrum (DMSO d₆) 3.97(s, 3H); 4.65(dd, 2H); 4.70(dd, 2H); 7.39(s, 1H); 7.52(s,

5 1H); 7.99(s, 1H); 8.57(s, 1H); 8.89(s, 1H)

MS - ESI: 306 [MH]⁺

Example 98

The following illustrate representative pharmaceutical dosage forms containing the compound of formula I, or a pharmaceutically acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

	(a)	<u>Tablet I</u>	<u>mg/tablet</u>
		Compound X	100
15		Lactose Ph.Eur.....	182.75
		Croscarmellose sodium	12.0
		Maize starch paste (5% w/v paste)	2.25
		Magnesium stearate	3.0
20	(b)	<u>Tablet II</u>	<u>mg/tablet</u>
		Compound X	50
		Lactose Ph.Eur.....	223.75
		Croscarmellose sodium	6.0
		Maize starch.....	15.0
25		Polyvinylpyrrolidone (5% w/v paste).....	2.25
		Magnesium stearate	3.0
	(c)	<u>Tablet III</u>	<u>mg/tablet</u>
		Compound X	1.0
30		Lactose Ph.Eur.....	93.25

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		Croscarmellose sodium	4.0
		Maize starch paste (5% w/v paste)	0.75
		Magnesium stearate	1.0
5	(d)	<u>Capsule</u>	<u>mg/capsule</u>
		Compound X	10
		Lactose Ph.Eur.....	488.5
		Magnesium stearate	1.5
10	(e)	<u>Injection I</u>	<u>(50 mg/ml)</u>
		Compound X	5.0% w/v
		1N Sodium hydroxide solution.....	15.0% v/v
		0.1N Hydrochloric acid (to adjust pH to 7.6)	
15		Polyethylene glycol 400	4.5% w/v
		Water for injection to 100%	
	(f)	<u>Injection II</u>	<u>(10 mg/ml)</u>
		Compound X	1.0% w/v
20		Sodium phosphate BP	3.6% w/v
		0.1N Sodium hydroxide solution.....	15.0% v/v
		Water for injection to 100%	
	(g)	<u>Injection III</u>	<u>(1mg/ml.buffered to pH6)</u>
25		Compound X	0.1% w/v
		Sodium phosphate BP	2.26% w/v
		Citric acid	0.38% w/v
		Polyethylene glycol 400	3.5% w/v
		Water for injection to 100%	

30

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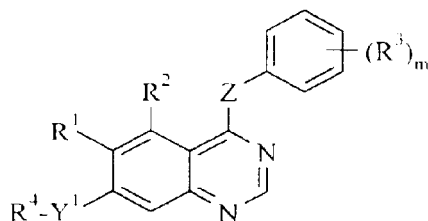
Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

5

CLAIMS

1. A quinazoline derivative of the formula I:



(I)

[wherein:

Y¹ represents -O-, -S-, -CH₂-, -SO-, -SO₂-, -NR⁵CO-, -CONR⁶-, -SO₂NR⁷-, -NR⁸SO₂- or -NR⁹-
 10 (wherein R⁵, R⁶, R⁷, R⁸ and R⁹ each independently represents hydrogen, C₁-₄alkyl or C₁-₄alkoxyC₂-₃alkyl);

R¹ represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, C₁-₄alkyl, C₁-₄alkoxy, C₁-₄alkylthio, or NR¹⁰R¹¹ (wherein R¹⁰ and R¹¹, which may be the same or different, each represents hydrogen or C₁-₄alkyl);

15 R² represents hydrogen, hydroxy, halogeno, C₁-₄alkyl, C₁-₄alkoxy, trifluoromethyl, cyano, amino or nitro;

m is an integer from 1 to 5;

R³ represents hydroxy, halogeno, C₁-₄alkyl, C₁-₄alkoxy, C₁-₄alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

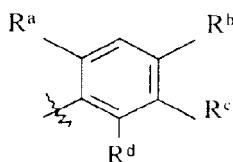
20 R⁴ is selected from one of the following eight groups:

1) X¹ (wherein X¹ represents a pyridone group, a phenyl group or a 5 or 6-membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, which pyridone, phenyl or heterocyclic group may carry up to 5 substituents selected from halogeno, amino, C₁-₄alkyl, C₁-₄alkoxy, C₁-₄hydroxyalkyl, C₁-₄aminoalkyl, C₁-₄alkylamino, C₁-₄hydroxyalkoxy, carboxy,
 25 cyano, -CONR¹²R¹³ and -NR¹⁴COR¹⁵ (wherein R¹², R¹³, R¹⁴ and R¹⁵, which may be the same or different, each represents hydrogen, C₁-₄alkyl or C₁-₄alkoxyC₂-₃alkyl));

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- 2) $C_{1-4}alkylX^1$ (wherein X^1 is as defined hereinbefore);
- 3) $C_{2-5}alkenylX^1$ (wherein X^1 is as defined hereinbefore);
- 4) $C_{2-5}alkynylX^1$ (wherein X^1 is as defined hereinbefore);
- 5) $C_{1-4}alkylY^2X^1$ (wherein Y^2 represents -O-, -S-, -SO-, -SO₂-, -OCO-, -NR¹⁶CO-, -CONR¹⁷-, -SO₂NR¹⁸-, -NR¹⁹SO₂- or -NR²⁰- (wherein R¹⁶, R¹⁷, R¹⁸, R¹⁹ and R²⁰ each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and X^1 is as defined hereinbefore)
- 6) $C_{2-5}alkenylY^3X^1$ (wherein Y^3 represents -O-, -S-, -SO-, -SO₂-, -OCO-, -NR²¹CO-, -CONR²²-, -SO₂NR²³-, -NR²⁴SO₂- or -NR²⁵- (wherein R²¹, R²², R²³, R²⁴ and R²⁵ each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and X^1 is as defined hereinbefore);
- 7) $C_{2-5}alkynylY^4X^1$ (wherein Y^4 represents -O-, -S-, -SO-, -SO₂-, -OCO-, -NR²⁶CO-, -CONR²⁷-, -SO₂NR²⁸-, -NR²⁹SO₂- or -NR³⁰- (wherein R²⁶, R²⁷, R²⁸, R²⁹ and R³⁰ each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and X^1 is as defined hereinbefore); and
- 8) $C_{1-4}alkylY^5C_{1-4}alkylX^1$ (wherein Y^5 represents -O-, -S-, -SO-, -SO₂-, -NR³¹CO-, -CONR³²-, -SO₂NR³³-, -NR³⁴SO₂- or -NR³⁵- (wherein R³¹, R³², R³³, R³⁴ and R³⁵ each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and X^1 is as defined hereinbefore); Z represents -NH-, -O-, -S-, or -CH₂-; with the proviso that where R⁴ is selected from one of the groups 1), 2), and 5) above and X^1 is unsubstituted phenyl or substituted phenyl with 1 to 2 substituents selected from halogeno, C₁₋₄alkyl and C₁₋₄alkoxy, then m is an integer from 3 to 5 and/or Z is -O-, -S-, or -CH₂-; and salts thereof.
2. A quinazoline derivative as claimed in claim 1 wherein R¹ represents hydrogen, hydroxy, methyl, ethyl, methoxy or ethoxy.
3. A quinazoline derivative as claimed in claim 1 or claim 2 wherein R² is hydrogen.
4. A quinazoline derivative as claimed in any one of the preceding claims wherein the phenyl group bearing (R³)_m is of the formula:-

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(IIa)

5 (wherein:

R^a represents hydrogen, methyl, fluoro or chloro;

R^b represents hydrogen, methyl, methoxy, bromo, fluoro or chloro;

R^c represents hydrogen or hydroxy; and

R^d represents hydrogen, fluoro or chloro.

10

5. A quinazoline derivative as claimed in any one of the preceding claims wherein Z is NH.

6. A quinazoline derivative as claimed in any one of the preceding claims wherein Y^1

15 represents -O-, -S-, -CH₂-, -NR⁵CO-, NR⁸SO₂- or -NH- (wherein R⁵ and R⁸ each independently represent hydrogen, C₁₋₂ alkyl or C₁₋₂ alkoxyethyl).

7. A quinazoline derivative as claimed in any one of the preceding claims wherein the moiety X¹ in the group R⁴ represents a pyridone group or a 5 or 6- membered aromatic

20 heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, which pyridone or heterocyclic group may, if desired, be substituted as defined in claim 1.

8. A quinazoline derivative as claimed in claim 7 wherein the moiety X¹ represents a

25 pyridone, pyridyl, imidazolyl, thiazolyl, thienyl, triazolyl or pyridazinyl group which may, if desired, be substituted as defined in claim 1.

9. A quinazoline derivative as claimed in any one of the preceding claims wherein R⁴

represents the group X¹-Y⁶-(CH₂)_n- in which Y⁶ is a direct bond -O-, -S- or -NH-, n is an integer from 1 to 3 and X¹ is as defined in any one of claims 1, 7 and 8.

10. A quinazoline derivative as claimed in claim 1 selected from :-
 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridyloxy)ethoxy)quinazoline
 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-[2-(4-oxo-1,4-dihydro-1-
 5 pyridyl)ethoxy]quinazoline
 4-(4-chloro-2-fluoroanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline
 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-pyridyl)propoxy)quinazoline
 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline
 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((4-pyridyl)methoxy)quinazoline
 10 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-methylimidazol-1-yl)ethoxy)quinazoline
 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1-methylimidazol-2-ylthio)ethoxy)quinazoline
 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1,2,4-triazol-1-yl)ethoxy)quinazoline
 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-(4-pyridyl)amino)ethoxy)quinazoline
 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1-methylimidazol-2-yl)ethoxy)quinazoline
 15 4-(4-chloro-2-fluoroanilino)-7-((2-cyano-4-pyridyl)methoxy)-6-methoxyquinazoline
 and salts thereof.
11. A quinazoline derivative as claimed in claim 1 selected from:-
 4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylmethoxy)quinazoline
 20 4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-pyridylmethoxy)quinazoline
 4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(1-methylimidazol-2-ylmethoxy)quinazoline
 4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-methylthiazol-4-ylmethoxy)quinazoline
 7-(2-acetamidothiazol-4-ylmethoxy)-4-(3-hydroxy-4-methylanilino)-6-methoxyquinazoline
 4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylpropoxy)quinazoline
 25 4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylpropoxy)quinazoline
 4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylmethoxy)quinazoline
 7-benzyloxy-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline
 7-benzyloxy-4-(2-fluoro-5-hydroxy-4-methylphenoxy)-6-methoxyquinazoline
 4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-((2-methylthiazol-4-
 30 yl)methoxy)quinazoline
 4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(4-pyridylmethoxy)quinazoline

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- 4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-((1-methylimidazol-2-yl)methoxy)quinazoline
 7-((2-acetamidothiazol-4-yl)methoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline
- 5 7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxyquinazoline
 4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(3-(4-pyridyl)propoxy)quinazoline
 4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline
 4-(4-chloro-2-fluoro-5-hydroxyanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline
 4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline
- 10 4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline
 4-(3-hydroxy-4-methylanilino)-6-methoxy-7-((1-methylbenzimidazol-2-yl)methoxy)quinazoline
 7-((2-chloro-6-methyl-4-pyridyl)methoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline
- 15 4-(4-chloro-2-fluorophenoxy)-6-methoxy-7-(4-pyridyl)methoxyquinazoline
 4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(4-pyridyl)methoxyquinazoline
 7-((2-chloro-4-pyridyl)methoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline
 7-(3,4-difluorobenzyloxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline
- 20 4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((1-methylimidazol-2-yl)methoxy)quinazoline
 4-(2-fluoro-5-hydroxy-4-methylanilino)-7-((1-methylimidazol-2-yl)methoxy)quinazoline
 4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-(1,2,4-triazol-1-yl)ethoxy)quinazoline
 4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(3-thienyl)methoxyquinazoline
- 25 4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline
 4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(4-pyridyl)carboxamidoquinazoline
 and salts thereof.

12. A quinazoline derivative as claimed in claim 1 selected from:-

- 30 4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(3-pyridylmethoxy)quinazoline
 4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline

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- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-pyridyloxy)ethoxy)quinazoline
- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-[N-methyl-N-(4-pyridyl)]aminoethoxy)quinazoline
- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-[2-(2-oxo-1,2-dihydro-1-pyridyl)ethoxy]quinazoline
- 5 7-(4 cyanobenzyloxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline
- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-methylimidazol-1-yl)propoxy)quinazoline
- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((2-methyl-4-pyridyl)methoxy)quinazoline
- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-oxo-1,2-dihydro-1-pyridyl)propoxy)quinazoline
- 10 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(1-methylimidazol-2-ylthio)propoxy)quinazoline
- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-pyridyloxy)propoxy)quinazoline
- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridylthio)ethoxy)quinazoline
- 15 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(3-pyridyloxy)ethoxy)quinazoline
- 7-benzyloxy-4-(2-fluoro-5-hydroxy-4-methylanilino)quinazoline
- 7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)quinazoline
- 4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((2-methylthiazol-4-yl)methoxy)quinazoline
- 20 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((3-thienyl)methoxy)quinazoline
- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-methyl-N-(pyridazin-4-yl)amino)ethoxy)quinazoline
- 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-methyl-N-(6-methylpyrimidin-4-yl)amino)ethoxy)quinazoline
- 25 4-(4-chloro-2-fluoroanilino)-7-(2-(3,5-dimethyl-1,2,4-triazol-4-yl)ethoxy)-6-methoxyquinazoline
- 4-(4-chloro-2-fluoroanilino)-7-(2-(2,4-dimethylimidazol-1-yl)ethoxy)-6-methoxyquinazoline
- 4-(4-chloro-2-fluoroanilino)-7-(2-(2,5-dimethylimidazol-1-yl)ethoxy)-6-methoxyquinazoline
- 4-(3-hydroxyanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline
- 30 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1,2,4-triazol-4-yl)ethoxy)quinazoline
- 4-(4-bromo-2-fluoroanilino)-7-(2-(1,2,4-triazol-1-yl)ethoxy)-6-methoxyquinazoline

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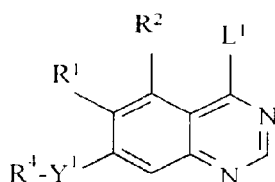
and salts thereof.

13. A quinazoline derivative as claimed in any one of the preceding claims in the form of a pharmaceutically acceptable salt.

5

14. A process for the preparation of a quinazoline derivative of formula I or salt thereof (as defined in claim 1) which comprises:-

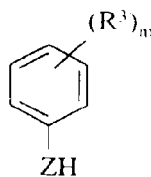
(a) the reaction of a compound of the formula III:



10

(III)

(wherein R¹, R², R³ and Y¹ are as defined in claim 1 and L¹ is a displaceable group), with a
15 compound of the formula IV:

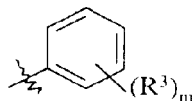


(IV)

20 (wherein Z, R³ and m are as defined in claim 1) whereby to obtain compounds of the formula I and salts thereof;

(b) for the preparation of compounds of formula I and salts thereof in which the group of formula IIb:-

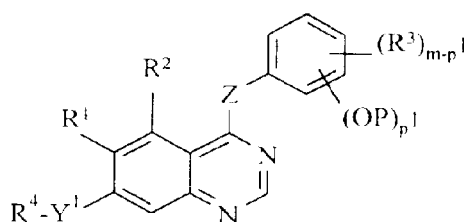
- 153 -



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(IIb)

(wherein R^3 and m are as defined in claim 1) represents a phenyl group carrying one or more hydroxy groups, the deprotection of a compound of formula V:



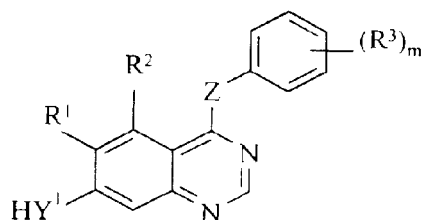
10

(V)

(wherein Y^1 , m , R^1 , R^2 , R^3 , R^4 and Z are as defined in claim 1. P represents a phenolic hydroxy protecting group and p^1 is an integer from 1 to 5 equal to the number of protected hydroxy groups and such that $m-p^1$ is equal to the number of R^1 substituents which are not protected hydroxy).

(c) for the preparation of those compounds of formula I and salts thereof wherein the substituent Y^1 is -O-, -S- or -NR^q-, the reaction, of a compound of the formula VI:

20



(VI)

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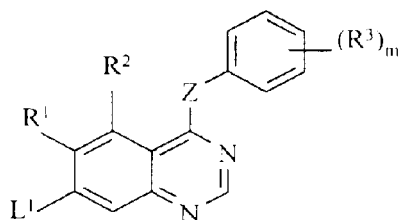
(wherein m, Y¹, R¹, R², R³ and Z are as defined in claim 1) with a compound of formula VII:



5

(wherein R⁴ is as defined in claim 1 and L¹ is as herein defined):

(d) the reaction of a compound of the formula VIII:



10

(VIII)

(wherein R¹, R², R³, Z and m are as defined in claim 1 and L¹ is as herein defined) with a compound of the formula IX:

15



(wherein R⁴ and Y¹ are as defined in claim 1);

(e) for the preparation of compounds of formula I and salts thereof wherein R⁴ is C₁-

20 ₃alkylX², [wherein X² is selected from one of the following three groups:

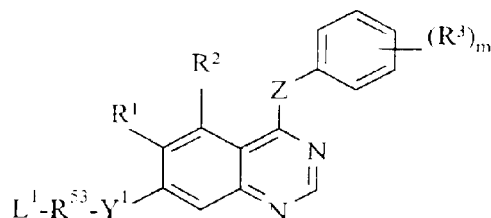
1) X¹ (wherein X¹ is as defined in claim 1);

2) Y⁷X¹ (wherein Y⁷ represents -O-, -S-, -SO₂-, -NR⁴⁷CO-, -NR⁴⁸SO₂- or -NR⁴⁹- (wherein R⁴⁷, R⁴⁸ and R⁴⁹ each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and X¹ is as defined in claim 1); and

25 3) Y⁸C₁₋₃alkylY⁵X¹ (wherein Y⁸ represents -O-, -S-, -SO₂-, -NR⁵⁰CO-, -NR⁵¹SO₂- or -NR⁵²- (wherein R⁵⁰, R⁵¹ and R⁵² each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and Y⁵ and X¹ are as defined in claim 1);]

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the reaction of a compound of the formula X:



(X)

5

(wherein Y^1 , R^1 , R^2 , R^3 , Z and m are as defined in claim 1, L^1 is as defined herein and R^{53} is C_{1-6} alkyl) with a compound of the formula XI:

 X^2-H

(XI)

10

(wherein X^2 is as defined herein) to give a compound of the formula I;

(f) for the preparation of those compounds of formula I and salts thereof wherein the substituent R^1 is represented by $NR^{10}R^{11}$, where one or both of R^{10} and R^{11} are C_{1-6} alkyl, the reaction of compounds of formula I wherein the substituent R^1 is an amino group with an

15 alkylating agent:

(g) for the preparation of those compounds of formula I and salts thereof wherein one or more of the substituents R^1 , R^2 or R^3 is an amino group, the reduction of a corresponding compound of formula I wherein the substituent(s) at the corresponding position(s) of the quinazoline and/or phenyl ring is/are a nitro group(s); and when a pharmaceutically acceptable

20 salt of a quinazoline derivative of formula I is required, reaction of the compound obtained with an acid or base whereby to obtain the desired pharmaceutically acceptable salt.

15. A pharmaceutical composition which comprises as active ingredient a quinazoline derivative of formula I as defined in claim 1 or a pharmaceutically acceptable salt thereof in

25 association with a pharmaceutically acceptable excipient or carrier.

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16. A method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined in claim 1.

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INTERNATIONAL SEARCH REPORT

In national Application No
PCT/GB 96/03075

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D239/94 C07D239/88 C07D401/12 C07D403/12 C07D409/12
C07D413/12 C07D417/12 A61K31/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 566 226 A (ZENECA LTD.) 20 October 1993 cited in the application see claims 1,11 ---	1,5,15
A	EP 0 635 498 A (ZENECA LTD.) 25 January 1995 cited in the application see claims 1,11 ---	1,5,15
A	EP 0 520 722 A (I.C.I. PLC) 30 December 1992 cited in the application see claims 1,2 ---	1,5,15
A,P	WO 96 30347 A (PFIZER INC.) 3 October 1996 see claims 1,24; examples 38,39,56 --- -/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- * "A" document defining the general state of the art which is not considered to be of particular relevance
- * "E" earlier document but published on or after the international filing date
- * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * "O" document referring to an oral disclosure, use, exhibition or other means
- * "P" document published prior to the international filing date but later than the priority date claimed

- * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- * "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- * "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * "&" document member of the same patent family

Date of the actual completion of the international search

1 April 1997

Date of mailing of the international search report

11.04.97

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Authorized officer

Hass, C

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/GB 96/03075

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	WO 96 15118 A (ZENECA LTD.) 23 May 1996 see claims 1,11 ---	1,5,15
A,P	WO 96 09294 A (THE WELLCOME FOUNDATION LTD.) 28 March 1996 see claims 1,7,9,20 ---	1,5,15
A	WO 95 21613 A (SUGEN INC. ET AL.) 17 August 1995 see abstract; claims 2,8 ---	1,5,15
A	EP 0 326 307 A (KYOWA HAKKO KOGYO CO., LTD.) 2 August 1989 see claims 1,5; page 23, compound 45 (example 48) -----	1,5,15

INTERNATIONAL SEARCH REPORT

national application No.

PCT/GB 96/ 03075

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 16
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/GB 96/03075

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 566226 A	20-10-93	AT 130000 T AU 661533 B AU 3101093 A CA 2086968 A DE 69300754 D DE 69300754 T ES 2078798 T HU 9500185 A NZ 245662 A SK 1693 A US 5457105 A ZA 9300015 A JP 6073025 A	15-11-95 27-07-95 22-07-93 21-07-93 14-12-95 28-03-96 16-12-95 28-07-95 26-09-95 09-09-93 10-10-95 20-07-93 15-03-94
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No
PCT/GB 96/03075

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9521613 A	17-08-95	AU 1842395 A CA 2182949 A EP 0748219 A	29-08-95 17-08-95 18-12-96
EP 326307 A	02-08-89	AT 110071 T DE 68917485 D DE 68917485 T JP 2022274 A US 5063227 A	15-09-94 22-09-94 09-02-95 25-01-90 05-11-91

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
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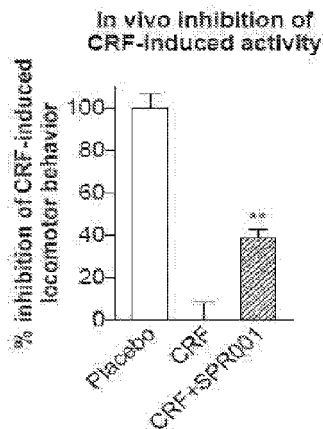


FIG. 11B

(57) Abstract: Provided herein are compounds and pharmaceutical compositions for the prevention and treatment of testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART).

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METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/663,951, filed April 27, 2018, and U.S. Provisional Patent Application No. 62/822,815, filed March 23, 2019, which are entirely incorporated herein by reference.

BACKGROUND

[0002] Corticotropes are basophilic cells in the anterior pituitary that produce melanocyte-stimulating hormone, adrenocorticotrophic hormone (ACTH) and lipotropin. The cells produce pro-opiomelanocortin (POMC) which undergoes cleavage to ACTH and β -lipotropin (β -LPH). These cells respond to corticotropin-releasing factor (CRF) and make up about 20% of the cells in the anterior pituitary. CRF is a releasing hormone that belongs to the corticotropin-releasing factor family and is the primary physiological regulator of proopiomelanocortin (POMC).

[0003] CRF is produced by parvocellular neuroendocrine cells within the paraventricular nucleus of the hypothalamus and is released at the median eminence from neurosecretory terminals of these neurons into the primary capillary plexus of the hypothalamo-hypophyseal portal system. The portal system carries the CRF to the anterior lobe of the pituitary, where it stimulates corticotropes to secrete ACTH and other biologically-active substances. ACTH, in turn, stimulates the synthesis of cortisol, glucocorticoids, mineralocorticoids and DHEA.

[0004] Testicular adrenal rest tumors (TART) and ovarian adrenal rest tumors (OART) are ACTH-responsive lesions of the testes and ovaries derived from adrenal tissue interchelated within these organs during embryogenesis. In response to high ACTH, hyperplasia of this tissue occurs, resulting in single or multiple lesions that may cause pain and infertility. The identification of compounds that modulate CRF function and downstream processes is an ongoing challenge. Currently, there are no non-steroidal, non-surgical solutions to control, shrink, or reduce TART. Disclosed herein, *inter alia*, are solutions to these and other problems in the art.

SUMMARY

[0005] Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is a serious genetic disorder characterized by impaired adrenal synthesis of cortisol and consequent overproduction of adrenocorticotrophic hormone (ACTH), 17-hydroxyprogesterone (17OHP), and adrenal androgens such as androstenedione (A4). CAH is an orphan condition with no FDA-approved therapy. To suppress androgen production, patients are often prescribed long-term

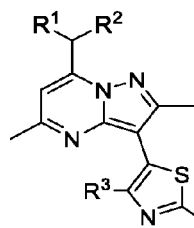
supraphysiologic doses of glucocorticoid (GC), which has adverse effects and fail to suppress 17OHP and A4 in the majority of patients.

[0006] Compound 1 may be a potent, selective, nonsteroidal, oral corticotropin-releasing factor type-1 (CRF1) receptor antagonist that reduces excess ACTH, 17OHP, and A4 to improve clinical and biochemical sequelae of CAH. This may obviate the need for high dose steroids.

[0007] One aspect provided herein is a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF₁) antagonist or a pharmaceutically acceptable salt thereof.

[0008] In some embodiments, the subject has congenital adrenal hyperplasia (CAH). In some embodiments, the size and/or number of the tumors is decreased or reduced.

[0009] One aspect provided herein is a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a compound of structural Formula (I):



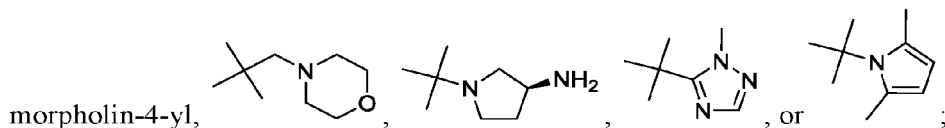
(I), or a pharmaceutically acceptable salt thereof,

wherein:

R¹ and R² are independently ethyl or n-propyl;

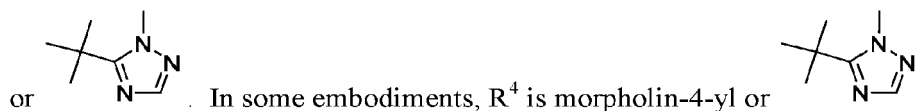
R³ is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R⁴ is hydrogen, Br, R^aR^bN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl,



R^a and R^b are independently hydrogen, C₁-C₃alkyl, H₂NCH₂CH₂-, (CH₃)₃COC(O)NHCH₂CH₂-, or CH₃CH₂CH₂NHCH₂CH₂-.

[0010] In some embodiments, R³ is Cl, Br, methyl, or trifluoromethyl. In some embodiments, R³ is Cl, Br, or methyl. In some embodiments, R⁴ is Br, R^aR^bN-, pyridin-4-yl, morpholin-4-yl,



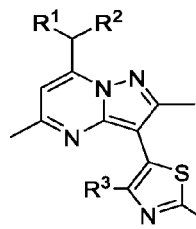
or . In some embodiments, R⁴ is morpholin-4-yl or

administered to the subject. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose from about 5 mg/day to about 25 mg/day is administered to the subject. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose from about 10 mg/day to about 400 mg/day is administered to the subject. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 100 mg/day to about 600 mg/day is administered to the subject.

[0017] In some embodiments, the compound is administered orally. In some embodiments, the compound is formulated as a capsule or tablet. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fed state. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fasted state.

[0018] In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at least once per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at bedtime. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 4 hours before sleep. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 2 hours before sleep. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 30 minutes before sleep. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the evening. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at about 10 pm at night.

[0019] One aspect described herein is a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a pharmaceutical composition, comprising a compound of structural Formula (I) and a pharmaceutically acceptable excipient:



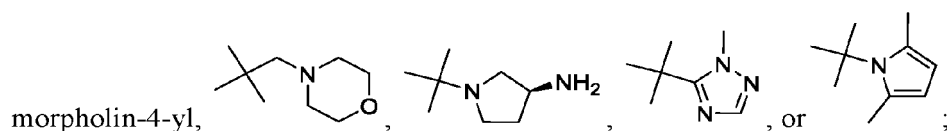
(I), or a pharmaceutically acceptable salt thereof,

wherein:

R¹ and R² are independently ethyl or n-propyl;

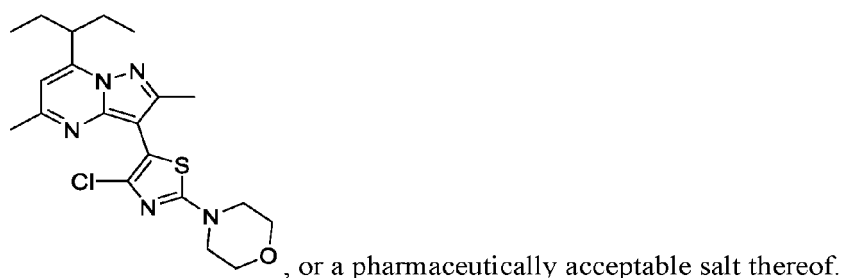
R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R^4 is hydrogen, Br, $R^a R^b N-$, methoxymethyl, n-butyl, acetamido, pyridin-4-yl,



R^a and R^b are independently hydrogen, C_1 - C_3 alkyl, $H_2NCH_2CH_2-$, $(CH_3)_3COC(O)NHCH_2CH_2-$, or $CH_3CH_2CH_2NHCH_2CH_2-$.

[0020] In some embodiments, the compound of Formula (I) has the structure:



[0021] In some embodiments, the pharmaceutical composition comprises from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0022] In some embodiments, the pharmaceutical composition comprises about 5 mg, about 10 mg, about 15 mg, about 20 mg, 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg about 500 mg, or about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

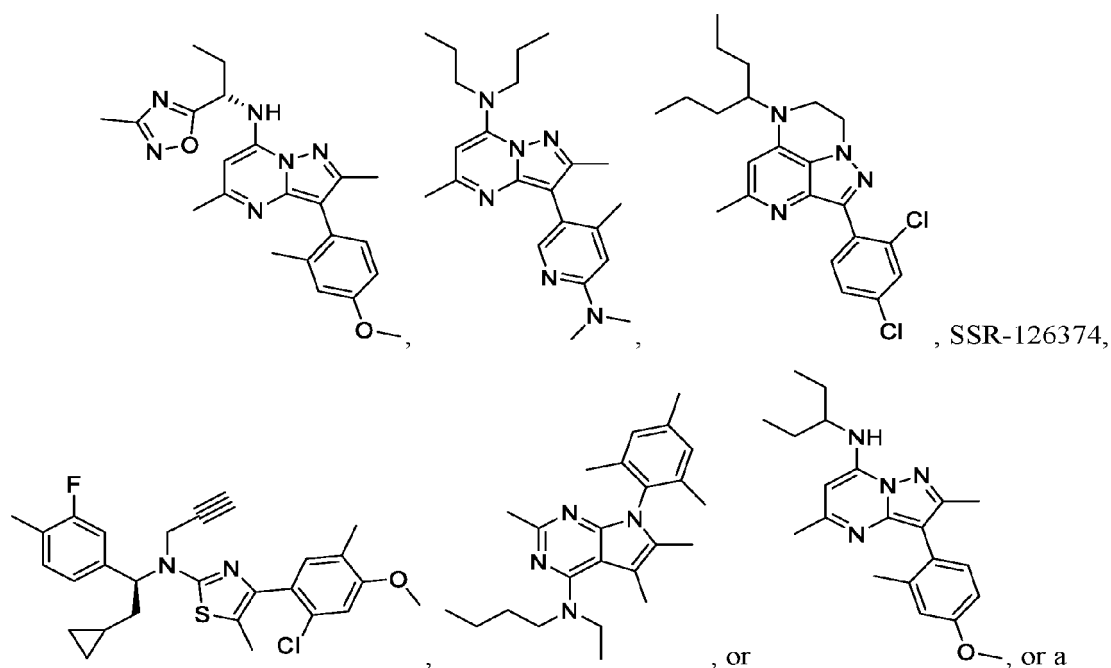
[0023] In some embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 1000 mg/day. In some embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 25 mg/day. In some embodiments, the pharmaceutical composition is administered at a dose of from about 10 mg/day to about 400 mg/day. In some embodiments, the pharmaceutical composition is administered at a dose of from about 100 mg/day to about 600 mg/day.

[0024] In some embodiments, the pharmaceutical composition is administered orally. In some embodiments, the pharmaceutical composition is formulated as capsule or a tablet. In some embodiments, the pharmaceutical composition is administered in the fed state. In some embodiments, the pharmaceutical composition is administered in the fasted state. In some embodiments, the pharmaceutical composition is administered at least once per day.

[0025] In some embodiments, the pharmaceutical composition is administered at bedtime. In some embodiments, the pharmaceutical composition is administered less than about 4 hours before sleep. In some embodiments, the pharmaceutical composition is administered less than about 2 hours before sleep. In some embodiments, the pharmaceutical composition is administered less than about 30 minutes before sleep. In some embodiments, the pharmaceutical composition is administered in the evening. In some embodiments, the pharmaceutical composition is administered at about 10 pm at night.

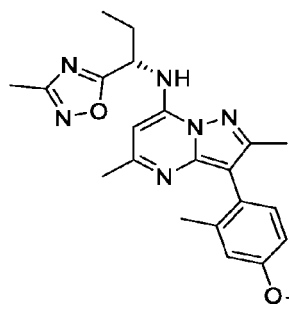
[0026] In some embodiments, the method further comprises administering to the subject an additional chemotherapeutic agent. In some embodiments, the additional chemotherapeutic agent is a glucocorticoid, a mineralocorticoid, an ACAT1 inhibitor, or an anti-androgen. In some embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In some embodiments, the mineralocorticoid is fludrocortisone.

[0027] In some embodiments, the additional chemotherapeutic agent is another CRF₁ antagonist. In some embodiments, the CRF₁ antagonist is



pharmaceutically acceptable salt thereof.

[0028] In some embodiments, the CRF₁ antagonist is



, or a pharmaceutically acceptable salt thereof.

[0029] In some embodiments, the method further comprises an additional treatment selected from surgical resection of the tumors and radiation therapy, or a combination thereof.

[0030] In some embodiments, the additional therapy is surgical resection and the surgical resection is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof.

[0031] In some embodiments, the surgical resection is prior to administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In some embodiments, the surgical resection is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In some embodiments, the surgical resection is concurrent with

administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0032] In some embodiments, the additional treatment is radiation therapy and the radiation therapy is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof. In some embodiments, the radiation therapy is prior to administration of the CRF₁ antagonist of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0033] In some embodiments, the radiation therapy is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In some embodiments, the radiation therapy is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0034] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure.

Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0035] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “Figure” and “FIG.” herein), of which:

[0037] **FIG. 1A-1B.** A schematic representation of intra-patient dose escalation study to evaluate safety, pharmacokinetics, and pharmacodynamics **(A)**. A schematic representation of inhibition of CRF₁ with a CRF₁ antagonist (Compound 1) **(B)**.

[0038] **FIG. 2.** Graphic representation of pharmacokinetics (dose-proportional, adequate exposure at safe doses) in patients with CAH following 14-days of once daily dosing at each dose level with a CRF₁ antagonist (Compound 1).

[0039] **FIG. 3.** Graphic representation of pharmacokinetics (dose-proportional, adequate exposure at safe doses) in patients with CAH following 14-days of once daily dosing at each dose level with a CRF₁ antagonist (Compound 1).

[0040] **FIG. 4.** Graphic representation of patient-level response (measuring ACTH levels) to a CRF₁ antagonist (Compound 1).

[0041] **FIG. 5A-5B.** Graphic representation of patient-level response of sample responders (measuring ACTH levels) to a CRF₁ antagonist [Subject 003001 **(A)**; Subject 007002 **(B)**].

[0042] **FIG. 6.** Graphic representation of patient-level response (measuring 17-OHP levels) to a CRF₁ antagonist (Compound 1).

[0043] **FIG. 7A-7B.** Graphic representation of patient-level response of sample responders (measuring 17-OHP levels) to a CRF₁ antagonist [Subject 003001 **(A)**; Subject 007002 **(B)**].

[0044] **FIG. 8.** Graphic representation of patient-level response (measuring androstenedione levels) to a CRF₁ antagonist (Compound 1).

[0045] **FIG. 9A-9B.** Graphic representation of patient-level response of sample responders (measuring androstenedione levels) to a CRF₁ antagonist (Compound 1) [Subject 003001 **(A)**; Subject 007002 **(B)**].

[0046] **FIG. 10A-10B.** Images of a patient with TART pre-treatment with a CRF₁ antagonist (Compound 1) **(A)** and 6-weeks post-treatment with a CRF₁ antagonist (Compound 1) **(B)**.

[0047] **FIG. 11A-11B.** Graphic representation of an *in vitro* binding assay of a CRF₁ antagonist (Compound 1); ¹²⁵I-sauvagine binding to rat cerebellar membranes one hour after oral administration of increasing Compound 1 doses **(A)** and *in vivo* inhibition of CRF-induced activity after treatment with a CRF₁ antagonist; Compound 1 (oral, 10 mg/kg) **(B)**.

[0048] **FIG. 12A-12B.** Graphic representation of ACTH levels **(A)** and 17-OHP levels **(B)** after treatment with a CRF₁ antagonist (Compound 1).

[0049] **FIG. 13.** Graphic representation of hormone responses after treatment with a CRF₁ antagonist (Compound 1).

[0050] **FIG. 14** depicts the proof of concept and escalating QD dosing at 10 pm.

[0051] **FIG. 15A-15D** are graphic representations of changes in androstenedione seen in participants in Cohort A and Cohort B.

[0052] FIG. 16A-16D are graphic representations of changes in 17-hydroxyprogesterone seen in participants in Cohort A and Cohort B.

[0053] FIG. 17A-17D are graphic representations of changes in adrenocorticotrophic hormone seen in participants in Cohort A and Cohort B.

DETAILED DESCRIPTION

[0054] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

I. Definitions

[0055] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0056] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $-\text{CH}_2\text{O}-$ is equivalent to $-\text{OCH}_2-$.

[0057] The term “alkyl,” by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched carbon chain (or carbon), or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include mono-, di- and multivalent radicals, having the number of carbon atoms designated (i.e., $\text{C}_1\text{-C}_{10}$ means one to ten carbons). Alkyl is an uncyclized chain. Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, (cyclohexyl)methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers. An alkoxy is an alkyl attached to the remainder of the molecule via an oxygen linker ($-\text{O}-$).

[0058] The term “alkylene,” by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkyl, as exemplified, but not limited by, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred herein. A “lower alkyl” or “lower alkylene” is a shorter chain alkyl or alkylene group, generally having eight or fewer

carbon atoms. The term “alkenylene,” by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkene.

[0059] The term “heteroalkyl,” by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or combinations thereof, including at least one carbon atom and at least one heteroatom (e.g., O, N, P, Si, and S), and wherein the nitrogen and sulfur atoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) (e.g., N, S, Si, or P) may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Heteroalkyl is an uncyclized chain. Examples include, but are not limited to: $-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{NH}-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{N}(\text{CH}_3)-\text{CH}_3$, $-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{S}(\text{O})-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{S}(\text{O})_2-\text{CH}_3$, $-\text{CH}=\text{CH}-\text{O}-\text{CH}_3$, $-\text{Si}(\text{CH}_3)_3$, $-\text{CH}_2-\text{CH}=\text{N}-\text{OCH}_3$, $-\text{CH}=\text{CH}-\text{N}(\text{CH}_3)-\text{CH}_3$, $-\text{O}-\text{CH}_3$, $-\text{O}-\text{CH}_2-\text{CH}_3$, and $-\text{CN}$. Up to two or three heteroatoms may be consecutive, such as, for example, $-\text{CH}_2-\text{NH}-\text{OCH}_3$ and $-\text{CH}_2-\text{O}-\text{Si}(\text{CH}_3)_3$. A heteroalkyl moiety may include one heteroatom (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include two optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include three optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include four optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include five optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include up to 8 optionally different heteroatoms (e.g., O, N, S, Si, or P).

[0060] Similarly, the term “heteroalkylene,” by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_2-$ and $-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_2-\text{NH}-\text{CH}_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-\text{C}(\text{O})_2\text{R}'$ represents both $-\text{C}(\text{O})_2\text{R}'$ and $-\text{R}'\text{C}(\text{O})_2-$. As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as $-\text{C}(\text{O})\text{R}'$, $-\text{C}(\text{O})\text{NR}'$, $-\text{NR}'\text{R}''$, $-\text{OR}'$, $-\text{SR}'$, and/or $-\text{SO}_2\text{R}'$. Where “heteroalkyl” is recited, followed by recitations of specific heteroalkyl groups, such as $-\text{NR}'\text{R}''$ or the like, it will be understood that the terms heteroalkyl and $-\text{NR}'\text{R}''$ are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term “heteroalkyl” should not be interpreted herein as excluding specific heteroalkyl groups, such as $-\text{NR}'\text{R}''$ or the like.

[0061] The terms “cycloalkyl” and “heterocycloalkyl,” by themselves or in combination with other terms, mean, unless otherwise stated, cyclic versions of “alkyl” and “heteroalkyl,”

respectively. Cycloalkyl and heterocycloalkyl are not aromatic. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidiny, 2-piperidiny, 3-piperidiny, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. A “cycloalkylene” and a “heterocycloalkylene,” alone or as part of another substituent, means a divalent radical derived from a cycloalkyl and heterocycloalkyl, respectively. “Cycloalkyl” is also meant to refer to bicyclic and polycyclic hydrocarbon rings such as, for example, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, etc.

[0062] The terms “halo” or “halogen,” by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as “haloalkyl” are meant to include monohaloalkyl and polyhaloalkyl. For example, the term “halo(C₁-C₄)alkyl” includes, but is not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0063] The term “acyl” means, unless otherwise stated, -C(O)R where R is a substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[0064] The term “aryl” means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent, which can be a single ring or multiple rings (preferably from 1 to 3 rings) that are fused together (i.e., a fused ring aryl) or linked covalently. A fused ring aryl refers to multiple rings fused together wherein at least one of the fused rings is an aryl ring. The term “heteroaryl” refers to aryl groups (or rings) that contain at least one heteroatom such as N, O, or S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Thus, the term “heteroaryl” includes fused ring heteroaryl groups (i.e., multiple rings fused together wherein at least one of the fused rings is a heteroaromatic ring). A 5,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 5 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. Likewise, a 6,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. And a 6,5-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 5 members, and wherein at least one ring is a heteroaryl ring. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-

limiting examples of aryl and heteroaryl groups include phenyl, naphthyl, pyrrolyl, pyrazolyl, pyridazinyl, triazinyl, pyrimidinyl, imidazolyl, pyrazinyl, purinyl, oxazolyl, isoxazolyl, thiazolyl, furyl, thienyl, pyridyl, pyrimidyl, benzothiazolyl, benzoxazolyl benzimidazolyl, benzofuran, isobenzofuranyl, indolyl, isoindolyl, benzothiophenyl, isoquinolyl, quinoxalyl, quinolyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalyl, 5-quinoxalyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. An “arylene” and a “heteroarylene,” alone or as part of another substituent, mean a divalent radical derived from an aryl and heteroaryl, respectively. A heteroaryl group substituent may be -O- bonded to a ring heteroatom nitrogen.

[0065] Spirocyclic rings are two or more rings wherein adjacent rings are attached through a single atom. The individual rings within spirocyclic rings may be identical or different.

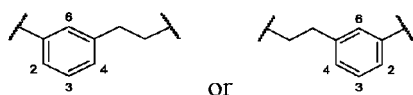
Individual rings in spirocyclic rings may be substituted or unsubstituted and may have different substituents from other individual rings within a set of spirocyclic rings. Possible substituents for individual rings within spirocyclic rings are the possible substituents for the same ring when not part of spirocyclic rings (e.g. substituents for cycloalkyl or heterocycloalkyl rings).

Spirocyclic rings may be substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkyl or substituted or unsubstituted heterocycloalkylene and individual rings within a spirocyclic ring group may be any of the immediately previous list, including having all rings of one type (e.g. all rings being substituted heterocycloalkylene wherein each ring may be the same or different substituted heterocycloalkylene). When referring to a spirocyclic ring system, heterocyclic spirocyclic rings means a spirocyclic rings wherein at least one ring is a heterocyclic ring and wherein each ring may be a different ring. When referring to a spirocyclic ring system, substituted spirocyclic rings means that at least one ring is substituted and each substituent may optionally be different.

[0066] The symbol “” denotes the point of attachment of a chemical moiety to the remainder of a molecule or chemical formula.

[0067] The term “oxo,” as used herein, means an oxygen that is double bonded to a carbon atom.

[0068] The term “alkylarylene” as an arylene moiety covalently bonded to an alkylene moiety (also referred to herein as an alkylene linker). In embodiments, the alkylarylene group has the formula:



[0069] An alkylarylene moiety may be substituted (e.g. with a substituent group) on the alkylene moiety or the arylene linker (e.g. at carbons 2, 3, 4, or 6) with halogen, oxo, $-N_3$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-Cl_3$, $-CN$, $-CHO$, $-OH$, $-NH_2$, $-COOH$, $-CONH_2$, $-NO_2$, $-SH$, $-SO_2CH_3$, $-SO_3H$, $-OSO_3H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(O)NHNH_2$, substituted or unsubstituted C_1 - C_5 alkyl or substituted or unsubstituted 2 to 5 membered heteroalkyl). In embodiments, the alkylarylene is unsubstituted.

[0070] Each of the above terms (e.g., “alkyl,” “heteroalkyl,” “cycloalkyl,” “heterocycloalkyl,” “aryl,” and “heteroaryl”) includes both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[0071] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to, $-OR'$, $=O$, $=NR'$, $=N-OR'$, $-NR'R''$, $-SR'$, $-halogen$, $-SiR'R''R'''$, $-OC(O)R'$, $-C(O)R'$, $-CO_2R'$, $-CONR'R''$, $-OC(O)NR'R''$, $-NR''C(O)R'$, $-NR'-C(O)NR''R'''$, $-NR''C(O)_2R'$, $-NR-C(NR'R''R''')=NR'''$, $-NR-C(NR'R'')=NR'''$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2NR'R''$, $-NRSO_2R'$, $-NR'NR''R'''$, $-ONR'R''$, $-NR'C(O)NR''NR'''R''''$, $-CN$, $-NO_2$, $-NR'SO_2R''$, $-NR'C(O)R''$, $-NR'C(O)-OR''$, $-NR'OR''$, in a number ranging from zero to $(2m'+1)$, where m' is the total number of carbon atoms in such radical. R , R' , R'' , R''' , and R'''' each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted heteroaryl, substituted or unsubstituted alkyl, alkoxy, or thioalkoxy groups, or arylalkyl groups. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R' , R'' , R''' , and R'''' group when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7-membered ring. For example, $-NR'R''$ includes, but is not limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term “alkyl” is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., $-CF_3$ and $-CH_2CF_3$) and acyl (e.g., $-C(O)CH_3$, $-C(O)CF_3$, $-C(O)CH_2OCH_3$, and the like).

[0072] Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are varied and are selected from, for example: $-OR'$, $-NR'R''$, $-SR'$, $-halogen$, $-$

SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR'', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -NR'NR''R''', -ONR'R'', -NR'C(O)NR''NR'''R''', -CN, -NO₂, -R', -N₃, -CH(Ph)₂, fluoro(C₁-C₄)alkoxy, and fluoro(C₁-C₄)alkyl, -NR'SO₂R'', -NR'C(O)R'', -NR'C(O)-OR'', -NR'OR'', in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R'', R''', and R'''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''', and R'''' groups when more than one of these groups is present.

[0073] Substituents for rings (e.g. cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene) may be depicted as substituents on the ring rather than on a specific atom of a ring (commonly referred to as a floating substituent). In such a case, the substituent may be attached to any of the ring atoms (obeying the rules of chemical valency) and in the case of fused rings or spirocyclic rings, a substituent depicted as associated with one member of the fused rings or spirocyclic rings (a floating substituent on a single ring), may be a substituent on any of the fused rings or spirocyclic rings (a floating substituent on multiple rings). When a substituent is attached to a ring, but not a specific atom (a floating substituent), and a subscript for the substituent is an integer greater than one, the multiple substituents may be on the same atom, same ring, different atoms, different fused rings, different spirocyclic rings, and each substituent may optionally be different. Where a point of attachment of a ring to the remainder of a molecule is not limited to a single atom (a floating substituent), the attachment point may be any atom of the ring and in the case of a fused ring or spirocyclic ring, any atom of any of the fused rings or spirocyclic rings while obeying the rules of chemical valency. Where a ring, fused rings, or spirocyclic rings contain one or more ring heteroatoms and the ring, fused rings, or spirocyclic rings are shown with one more floating substituents (including, but not limited to, points of attachment to the remainder of the molecule), the floating substituents may be bonded to the heteroatoms. Where the ring heteroatoms are shown bound to one or more hydrogens (e.g. a ring nitrogen with two bonds to ring atoms and a third bond to a hydrogen) in the structure or formula with the floating substituent, when the heteroatom is bonded to the floating substituent, the substituent will be understood to replace the hydrogen, while obeying the rules of chemical valency.

[0074] Two or more substituents may optionally be joined to form aryl, heteroaryl, cycloalkyl, or heterocycloalkyl groups. Such so-called ring-forming substituents are typically, though not

necessarily, found attached to a cyclic base structure. In one embodiment, the ring-forming substituents are attached to adjacent members of the base structure. For example, two ring-forming substituents attached to adjacent members of a cyclic base structure create a fused ring structure. In another embodiment, the ring-forming substituents are attached to a single member of the base structure. For example, two ring-forming substituents attached to a single member of a cyclic base structure create a spirocyclic structure. In yet another embodiment, the ring-forming substituents are attached to non-adjacent members of the base structure.

[0075] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally form a ring of the formula $-T-C(O)-(CRR')_q-U-$, wherein T and U are independently $-NR-$, $-O-$, $-CRR'-$, or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-A-(CH_2)_r-B-$, wherein A and B are independently $-CRR'-$, $-O-$, $-NR-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2NR'-$, or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-(CRR')_s-X'-(C''R''R''')_d-$, where s and d are independently integers of from 0 to 3, and X' is $-O-$, $-NR'-$, $-S-$, $-S(O)-$, $-S(O)_2-$, or $-S(O)_2NR'-$. The substituents R, R', R'', and R''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[0076] As used herein, the terms “heteroatom” or “ring heteroatom” are meant to include oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), and silicon (Si).

[0077] A “substituent group,” as used herein, means a group selected from the following moieties:

(A) oxo, halogen, $-CF_3$, $-CN$, $-OH$, $-NH_2$, $-COOH$, $-CONH_2$, $-NO_2$, $-SH$, $-SO_3H$, $-SO_4H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC=(O)NHNH_2$, $-NHC=(O)NH_2$, $-NHOSO_2H$, $-NHC=(O)H$, $-NHC(O)-OH$, $-NHOH$, $-OCF_3$, $-OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(B) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(i) oxo, halogen, $-CF_3$, $-CN$, $-OH$, $-NH_2$, $-COOH$, $-CONH_2$, $-NO_2$, $-SH$, $-SO_3H$, $-SO_4H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC=(O)NHNH_2$, $-NHC=(O)NH_2$, $-NHOSO_2H$, $-NHC=(O)H$, $-NHC(O)-OH$, $-NHOH$, $-OCF_3$, $-OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl,

unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(ii) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(a) oxo, halogen, -CF₃, -CN, -OH, -NH₂, -COOH, -CONH₂, -NO₂, -SH, -SO₃H, -SO₄H, -SO₂NH₂, -NHNH₂, -ONH₂, -NHC=(O)NHNH₂, -NHC=(O)NH₂, -NHSO₂H, -NHC=(O)H, -NHC(O)-OH, -NHOH, -OCF₃, -OCHF₂, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(b) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from: oxo,

halogen, -CF₃, -CN, -OH, -NH₂, -COOH, -CONH₂, -NO₂, -SH, -SO₃H, -SO₄H, -SO₂NH₂, -NHNH₂, -ONH₂, -NHC=(O)NHNH₂, -NHC=(O)NH₂, -NHSO₂H, -NHC=(O)H, -NHC(O)-OH, -NHOH, -OCF₃, -OCHF₂, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl.

[0078] A “size-limited substituent” or “size-limited substituent group,” as used herein, means a group selected from all of the substituents described above for a “substituent group,” wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C₁-C₂₀ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₃-C₈ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C₆-C₁₀ aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl.

[0079] A “lower substituent” or “lower substituent group,” as used herein, means a group selected from all of the substituents described above for a “substituent group,” wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C₁-C₈ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₃-C₇ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C₆-C₁₀ aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl.

[0080] In some embodiments, each substituted group described in the compounds herein is substituted with at least one substituent group. More specifically, in some embodiments, each substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene described in the compounds herein are substituted with at least one substituent group. In other embodiments, at least one or all of these groups are substituted with at least one size-limited substituent group. In other embodiments, at least one or all of these groups are substituted with at least one lower substituent group.

[0081] In other embodiments of the compounds herein, each substituted or unsubstituted alkyl may be a substituted or unsubstituted C₁-C₂₀ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₃-C₈ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C₆-C₁₀ aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl. In some embodiments of the compounds herein, each substituted or unsubstituted alkylene is a substituted or unsubstituted C₁-C₂₀ alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 20 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C₃-C₈ cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 8 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C₆-C₁₀ arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 10 membered heteroarylene.

[0082] In some embodiments, each substituted or unsubstituted alkyl is a substituted or unsubstituted C₁-C₈ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₃-C₇ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C₆-C₁₀ aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl. In some embodiments, each substituted or unsubstituted alkylene is a substituted or unsubstituted C₁-C₈ alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 8 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C₃-C₇ cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a

substituted or unsubstituted 3 to 7 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C₆-C₁₀ arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 9 membered heteroarylene. In some embodiments, the compound is a chemical species set forth in the Examples section, figures, or tables below.

[0083] Certain compounds of the present invention possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present invention. The compounds of the present invention do not include those that are known in art to be too unstable to synthesize and/or isolate. The present invention is meant to include compounds in racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

[0084] As used herein, the term “isomers” refers to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the structural arrangement or configuration of the atoms.

[0085] The term “tautomer,” as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

[0086] It will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the invention.

[0087] Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the invention.

[0088] Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ¹³C- or ¹⁴C-enriched carbon are within the scope of this invention.

[0089] The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the

compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I), or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

[0090] It should be noted that throughout the application that alternatives are written in Markush groups, for example, each amino acid position that contains more than one possible amino acid. It is specifically contemplated that each member of the Markush group should be considered separately, thereby comprising another embodiment, and the Markush group is not to be read as a single unit.

[0091] “Analog” or “analogue” is used in accordance with its plain ordinary meaning within Chemistry and Biology and refers to a chemical compound that is structurally similar to another compound (i.e., a so-called “reference” compound) but differs in composition, e.g., in the replacement of one atom by an atom of a different element, or in the presence of a particular functional group, or the replacement of one functional group by another functional group, or the absolute stereochemistry of one or more chiral centers of the reference compound. Accordingly, an analog is a compound that is similar or comparable in function and appearance but not in structure or origin to a reference compound.

[0092] The terms “a” or “an,” as used in herein means one or more. In addition, the phrase “substituted with a[n],” as used herein, means the specified group may be substituted with one or more of any or all of the named substituents. For example, where a group, such as an alkyl or heteroaryl group, is “substituted with an unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl, or unsubstituted 2 to 20 membered heteroalkyl,” the group may contain one or more unsubstituted $\text{C}_1\text{-C}_{20}$ alkyls, and/or one or more unsubstituted 2 to 20 membered heteroalkyls.

[0093] Moreover, where a moiety is substituted with an R substituent, the group may be referred to as “R-substituted.” Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different. Where a particular R group is present in the description of a chemical genus (such as Formula (I)), a Roman alphabetic symbol may be used to distinguish each appearance of that particular R group. For example, where multiple R^{13} substituents are present, each R^{13} substituent may be distinguished as $\text{R}^{13\text{A}}$, $\text{R}^{13\text{B}}$, $\text{R}^{13\text{C}}$, $\text{R}^{13\text{D}}$, etc., wherein each of $\text{R}^{13\text{A}}$, $\text{R}^{13\text{B}}$, $\text{R}^{13\text{C}}$, $\text{R}^{13\text{D}}$, etc. is defined within the scope of the definition of R^{13} and optionally differently.

[0094] A “detectable moiety” as used herein refers to a moiety that can be covalently or noncovalently attached to a compound or biomolecule that can be detected for instance, using techniques known in the art. In embodiments, the detectable moiety is covalently attached. The detectable moiety may provide for imaging of the attached compound or biomolecule. The detectable moiety may indicate the contacting between two compounds. Exemplary detectable

moieties are fluorophores, antibodies, reactive dies, radio-labeled moieties, magnetic contrast agents, and quantum dots. Exemplary fluorophores include fluorescein, rhodamine, GFP, coumarin, FITC, Alexa fluor, Cy3, Cy5, BODIPY, and cyanine dyes. Exemplary radionuclides include Fluorine-18, Gallium-68, and Copper-64. Exemplary magnetic contrast agents include gadolinium, iron oxide and iron platinum, and manganese.

[0095] Descriptions of compounds of the present invention are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

[0096] The term “pharmaceutically acceptable salts” is meant to include salts of the active compounds that are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, oxalic, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (*see, for example, Berge et al., “Pharmaceutical Salts”, Journal of Pharmaceutical Science, 1977, 66, 1-19*). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0097] Thus, the compounds of the present invention may exist as salts, such as with pharmaceutically acceptable acids. The present invention includes such salts. Non-limiting examples of such salts include hydrochlorides, hydrobromides, phosphates, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, propionates, tartrates (e.g., (+)-tartrates, (-)-tartrates, or mixtures thereof including racemic mixtures), succinates, benzoates, and salts with amino acids such as glutamic acid, and quaternary ammonium salts (e.g. methyl iodide, ethyl iodide, and the like). These salts may be prepared by methods known to those skilled in the art.

[0098] The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound may differ from the various salt forms in certain physical properties, such as solubility in polar solvents. In embodiments, compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in a conventional manner. The parent form of the compounds differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but, unless specifically indicated, the salts disclosed herein are equivalent to the parent form of the compound for the purposes of the present invention.

[0099] In addition to salt forms, the present invention provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Prodrugs of the compounds described herein may be converted *in vivo* after administration. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment, such as, for example, when contacted with a suitable enzyme or chemical reagent.

[0100] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

[0101] “Pharmaceutically acceptable excipient” and “pharmaceutically acceptable carrier” refer to a substance that aids the administration of a compound to and absorption by a subject and can be included in the compositions of the present invention without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable

excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates such as lactose, amylose or starch, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrrolidone, and colors, and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds of the invention. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

[0102] The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

[0103] A "CRF1 inhibitor" refers to a compound (e.g., compounds described herein) that reduces the activity of CRF1 when compared to a control, such as absence of the compound or a compound with known inactivity.

[0104] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues, wherein the polymer may optionally be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. In embodiments, the terms "polypeptide," "peptide," and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include genetically coded and non-genetically coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified polypeptide backbones. The terms include fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence; fusion proteins with heterologous and homologous leader sequences, with or without N-terminus methionine residues; immunologically tagged proteins; and the like.

[0105] A polypeptide, or a cell is "recombinant" when it is artificial or engineered, or derived from or contains an artificial or engineered protein or nucleic acid (e.g. non-natural or not wild type). For example, a polynucleotide that is inserted into a vector or any other heterologous location, e.g., in a genome of a recombinant organism, such that it is not associated with nucleotide sequences that normally flank the polynucleotide as it is found in nature is a

recombinant polynucleotide. A protein expressed *in vitro* or *in vivo* from a recombinant polynucleotide is an example of a recombinant polypeptide. Likewise, a polynucleotide sequence that does not appear in nature, for example a variant of a naturally occurring gene, is recombinant.

[0106] “Contacting” is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated; however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents that can be produced in the reaction mixture.

[0107] The term “contacting” may include allowing two species to react, interact, or physically touch, wherein the two species may be a compound as described herein and a protein or enzyme. In some embodiments contacting includes allowing a compound described herein to interact with a protein or enzyme that is involved in a signaling pathway (e.g., MAP kinase pathway).

[0108] As defined herein, the term “activation”, “activate”, “activating” and the like in reference to a protein refers to conversion of a protein into a biologically active derivative from an initial inactive or deactivated state. The terms reference activation, or activating, sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein decreased in a disease.

[0109] The terms “agonist,” “activator,” “upregulator,” etc. refer to a substance capable of detectably increasing the expression or activity of a given gene or protein. The agonist can increase expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the agonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or higher than the expression or activity in the absence of the agonist. In embodiments, an agonist is a molecule that interacts with a target to cause or promote an increase in the activation of the target. In embodiments, activators are molecules that increase, activate, facilitate, enhance activation, sensitize, or up-regulate, e.g., a gene, protein, ligand, receptor, or cell.

[0110] As defined herein, the term “inhibition”, “inhibit”, “inhibiting” and the like in reference to a protein-inhibitor interaction means negatively affecting (e.g. decreasing) the activity or function of the protein relative to the activity or function of the protein in the absence of the inhibitor. In embodiments inhibition means negatively affecting (e.g. decreasing) the concentration or levels of the protein relative to the concentration or level of the protein in the absence of the inhibitor. In embodiments inhibition refers to reduction of a disease or symptoms of disease. In embodiments, inhibition refers to a reduction in the activity of a particular protein

target. Thus, inhibition includes, at least in part, partially or totally blocking stimulation, decreasing, preventing, or delaying activation, or inactivating, desensitizing, or down-regulating signal transduction or enzymatic activity or the amount of a protein. In embodiments, inhibition refers to a reduction of activity of a target protein resulting from a direct interaction (e.g. an inhibitor binds to the target protein). In embodiments, inhibition refers to a reduction of activity of a target protein from an indirect interaction (e.g. an inhibitor binds to a protein that activates the target protein, thereby preventing target protein activation).

[0111] The terms “inhibitor,” “repressor” or “antagonist” or “downregulator” interchangeably refer to a substance capable of detectably decreasing the expression or activity of a given gene or protein. The antagonist can decrease expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the antagonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or lower than the expression or activity in the absence of the antagonist. An antagonist prevents, reduces, inhibits, or neutralizes the activity of an agonist, and an antagonist can also prevent, inhibit, or reduce constitutive activity of a target, e.g., a target receptor, even where there is no identified agonist. In embodiments, inhibitors are molecules that decrease, block, prevent, delay activation, inactivate, desensitize, or down-regulate, e.g., a gene, protein, ligand, receptor, or cell. An inhibitor may also be defined as a molecule that reduces, blocks, or inactivates a constitutive activity. An “antagonist” is a molecule that opposes the action(s) of an agonist.

[0112] The terms “corticotropin-releasing factor type-1,” “CRF₁,” “corticotropin-releasing hormone receptor 1,” and “CHHR1” refer to a protein (including homologs, isoforms, and functional fragments thereof) and is a receptor for corticotropin releasing-hormone. The terms include any recombinant or naturally-occurring form of CRF₁ variants thereof that maintain CRF₁ activity (e.g. within at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% activity compared to wildtype CRF₁). The terms include any mutant form of CRF₁ variants (e.g., frameshift mutations) thereof that maintain CRF₁ activity (e.g. within at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% activity compared to wildtype CRF₁). In embodiments, the CRF₁ protein encoded by the *CRHR1* gene has the amino acid sequence set forth in or corresponding to Entrez 1233, UniProt P34998.

[0113] The term “expression” includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion. Expression can be detected using conventional techniques for detecting protein (e.g., ELISA, Western blotting, flow cytometry, immunofluorescence, immunohistochemistry, *etc.*).

[0114] The terms “disease” or “condition” refer to a state of being or health status of a patient or subject capable of being treated with the compounds or methods provided herein. The disease may be a malignant or benign tumor (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)). The disease may be cancer. In some further instances, “cancer” refers to human cancers and carcinomas, sarcomas, adenocarcinomas, lymphomas, leukemias, etc., including solid and lymphoid cancers, kidney, breast, lung, bladder, colon, ovarian, prostate, pancreas, adrenal, stomach, brain, head and neck, skin, uterine, testicular, glioma, esophagus, and liver cancer, including hepatocarcinoma, lymphoma, including B-acute lymphoblastic lymphoma, non-Hodgkin’s lymphomas (e.g., Burkitt’s, Small Cell, and Large Cell lymphomas), Hodgkin’s lymphoma, leukemia (including MDS, AML, ALL, ATLL and CML), or multiple myeloma.

[0115] As used herein, the term “cancer” refers to all types of cancer, neoplasm or malignant tumors found in mammals (e.g. humans), including leukemia, carcinomas and sarcomas. Exemplary cancers that may be treated with a compound or method provided herein include adrenal cancer and tumors (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)) brain cancer, glioma, glioblastoma, neuroblastoma, prostate cancer, colorectal cancer, pancreatic cancer, cervical cancer, gastric cancer, ovarian cancer, lung cancer, and cancer of the head. Exemplary cancers that may be treated with a compound or method provided herein include cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus, Medulloblastoma, colorectal cancer, pancreatic cancer. Additional examples include, thyroid carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, or prostate cancer.

[0116] The term “congenital adrenal hyperplasia” or “CAH” are any of several autosomal recessive diseases resulting from mutations of genes for enzymes mediating the biochemical

steps of production of mineralocorticoids, glucocorticoids or sex steroids from cholesterol by the adrenal glands (steroidogenesis). These conditions involve excessive or deficient production of sex steroids and can alter development of primary or secondary sex characteristics in some affected infants, children, or adults.

[0117] “Adrenal rest tumor” refers to a neoplasm of the testis (or ovaries) consisting of adrenal tissue. It may be secondary to hyperplasia of an adrenal gland.

[0118] “Testicular adrenal rest tumor(s)” or “TART” and/or ovarian adrenal rest tumor(s) are a complication of congenital adrenal hyperplasia (CAH), which may develop from ectopic remnants of intra-testicular adrenal tissue stimulated by adrenocorticotrophic hormone (ACTH) hypersecretion. TART lesions are typically located within the rete testis and are bilateral, synchronous, nodular and multiple. TART can lead to testicular structural damage, spermatogenesis disorders, infertility and mass-forming lesions.

[0119] The term “leukemia” refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of (1) the duration and character of the disease-acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number abnormal cells in the blood-leukemic or aleukemic (subleukemic). Exemplary leukemias that may be treated with a compound or method provided herein include, for example, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemetic leukemia, basophilic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, multiple myeloma, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, or undifferentiated cell leukemia.

[0120] The term “sarcoma” generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas that may be treated with a compound or method

provided herein include a chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, or telangiectaltic sarcoma.

[0121] The term “melanoma” is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas that may be treated with a compound or method provided herein include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, or superficial spreading melanoma.

[0122] The term “carcinoma” refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas that may be treated with a compound or method provided herein include, for example, thyroid carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, medullary thyroid carcinoma, familial medullary thyroid carcinoma, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniformi carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma,

infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhus carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, or carcinoma villosum.

[0123] The terms “treating” or “treatment” refer to any indicia of success in the therapy or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient’s physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. The term “treating” and conjugations thereof, may include prevention of an injury, pathology, condition, or disease. In embodiments, treating is preventing. In embodiments, treating does not include preventing.

[0124] “Treating” or “treatment” as used herein (and as well-understood in the art) also broadly includes any approach for obtaining beneficial or desired results in a subject’s condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (*i.e.*, not worsening) the state of disease, prevention of a disease’s transmission or spread, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable. In other words, “treatment” as used herein includes any cure, amelioration, or prevention of a disease. Treatment may prevent the disease from occurring; inhibit the disease’s spread; relieve the disease’s symptoms (*e.g.*, ocular pain, seeing

halos around lights, red eye, very high intraocular pressure), fully or partially remove the disease's underlying cause, shorten a disease's duration, or do a combination of these things.

[0125] "Treating" and "treatment" as used herein include prophylactic treatment. Treatment methods include administering to a subject a therapeutically effective amount of a compound described herein. The administering step may consist of a single administration or may include a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the age of the patient, the concentration of the compound, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient.

[0126] The term "prevent" refers to a decrease in the occurrence of disease symptoms in a patient. As indicated above, the prevention may be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur absent treatment. In embodiments, prevent refers to slowing the progression of the disease, disorder or condition or inhibiting progression thereof to a harmful or otherwise undesired state.

[0127] "Patient" or "subject in need thereof" refers to a living organism suffering from or prone to a disease or condition that can be treated by administration of a pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a patient is human.

[0128] An "effective amount" is an amount sufficient for a compound to accomplish a stated purpose relative to the absence of the compound (e.g., achieve the effect for which it is administered, treat a disease, reduce enzyme activity, increase enzyme activity, reduce a signaling pathway, or reduce one or more symptoms of a disease or condition). An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A "prophylactically effective amount" of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of an injury, disease, pathology or condition, or reducing the likelihood of the onset (or reoccurrence) of an injury, disease, pathology, or

condition, or their symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An “activity decreasing amount,” as used herein, refers to an amount of antagonist required to decrease the activity of an enzyme relative to the absence of the antagonist. A “function disrupting amount,” as used herein, refers to the amount of antagonist required to disrupt the function of an enzyme or protein relative to the absence of the antagonist. The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see, e.g.,* Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and *Remington: The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins). The therapeutically effective amount can be ascertained by measuring relevant physiological effects, and it can be adjusted in connection with the dosing regimen and diagnostic analysis of the subject’s condition, and the like. By way of example, measurement of the serum level of a CRF1 inhibitor (or, e.g., a metabolite thereof) at a particular time post-administration may be indicative of whether a therapeutically effective amount has been administered.

[0129] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art.

[0130] Therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring compounds effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan. Adjusting the dose to achieve maximal therapeutic window efficacy or toxicity in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[0131] The term “therapeutically effective amount,” as used herein, refers to that amount of the therapeutic agent sufficient to ameliorate the disorder, as described above. For example, for the given parameter, a therapeutically effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Therapeutic efficacy can also be expressed as “-fold” increase or decrease. For example, a therapeutically

effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control.

[0132] The terms “QD,” “qd,” and “q.d.” refer to once a day, i.e., a drug or therapeutic agent is administered once a day for the duration of the therapeutic regimen.

[0133] The terms “BD,” “bd,” and “b.i.d.” refer to twice a day, i.e., a drug or therapeutic agent is administered two times a day for the duration of the therapeutic regimen.

[0134] The terms “QID,” “qid,” and “q.i.d.” refer to three times a day, i.e., a drug or therapeutic agent is administered three times a day for the duration of the therapeutic regimen.

[0135] Dosages may be varied depending upon the requirements of the patient and the compound being employed. The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. Dosage amounts and intervals can be adjusted individually to provide levels of the administered compound effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[0136] As used herein, the term “administering” means oral administration, administration as a suppository, topical contact, intravenous, parenteral, intraperitoneal, intramuscular, intralesional, intrathecal, intracranial, intranasal or subcutaneous administration, or the implantation of a slow-release device, *e.g.*, a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (*e.g.*, buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.*, intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, *etc.* By “co-administer” it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies (*e.g.* anti-cancer agent, chemotherapeutic, or treatment for a neurodegenerative disease). The compound of the invention can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compound individually or in combination (more than one compound or agent). Thus, the preparations can also be combined, when desired, with other active substances (*e.g.* to reduce metabolic degradation). The compositions of the present invention can be

delivered by transdermally, by a topical route, formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols. Oral preparations include tablets, pills, powder, dragees, capsules, liquids, lozenges, cachets, gels, syrups, slurries, suspensions, etc., suitable for ingestion by the patient. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. The compositions of the present invention may additionally include components to provide sustained release and/or comfort. Such components include high molecular weight, anionic mucomimetic polymers, gelling polysaccharides and finely-divided drug carrier substrates. These components are discussed in greater detail in U.S. Pat. Nos. 4,911,920; 5,403,841; 5,212,162; and 4,861,760. The entire contents of these patents are incorporated herein by reference in their entirety for all purposes. The compositions of the present invention can also be delivered as microspheres for slow release in the body. For example, microspheres can be administered via intradermal injection of drug-containing microspheres, which slowly release subcutaneously (see Rao, *J. Biomater Sci. Polym. Ed.* 7:623-645, 1995; as biodegradable and injectable gel formulations (see, e.g., Gao *Pharm. Res.* 12:857-863, 1995); or, as microspheres for oral administration (see, e.g., Eyles, *J. Pharm. Pharmacol.* 49:669-674, 1997). In another embodiment, the formulations of the compositions of the present invention can be delivered by the use of liposomes which fuse with the cellular membrane or are endocytosed, *i.e.*, by employing receptor ligands attached to the liposome, that bind to surface membrane protein receptors of the cell resulting in endocytosis. By using liposomes, particularly where the liposome surface carries receptor ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the compositions of the present invention into the target cells *in vivo*. (See, *e.g.*, Al-Muhammed, *J. Microencapsul.* 13:293-306, 1996; Chonn, *Curr. Opin. Biotechnol.* 6:698-708, 1995; Ostro, *Am. J. Hosp. Pharm.* 46:1576-1587, 1989). The compositions of the present invention can also be delivered as nanoparticles.

[0137] By “co-administer” it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies. The compounds of the invention can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compounds individually or in combination (more than one compound). The compositions of the present invention can be delivered transdermally, by a topical route, or formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

[0138] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art.

[0139] Utilizing the teachings provided herein, an effective prophylactic or therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred mode of administration and the toxicity profile of the selected agent.

[0140] The compounds described herein can be used in combination with one another, with other active agents known to be useful in treating cancer (e.g. colon cancer), cardiovascular disease, metabolic disease, immune or inflammatory disease or disorder.

[0141] In some embodiments, co-administration includes administering one active agent within 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24 hours, 2 days, 4 days, 1 week or 1 month of a second active agent. Co-administration includes administering two active agents simultaneously, approximately simultaneously (e.g., within about 1, 5, 10, 15, 20, or 30 minutes of each other), or sequentially in any order. In some embodiments, co-administration can be accomplished by co-formulation, i.e., preparing a single pharmaceutical composition including both active agents. In other embodiments, the active agents can be formulated separately. In another embodiment, the active and/or adjunctive agents may be linked or conjugated to one another. In some embodiments, the compounds described herein may be combined with treatments for cancer (e.g. colon cancer), cardiovascular disease, metabolic disease, immune or inflammatory disease or disorder.

[0142] The compounds described herein can be administered to treat a fertility disease or disorder and/or cancer. In this regard, the compounds disclosed herein may be administered either alone to treat such diseases or disorders or may be co-administered with another therapeutic agent to treat such diseases or disorders.

[0143] The compounds disclosed herein may be co-administered with an agonist or antagonist of CRF₁ function. The compounds described herein can be co-administered with conventional anti-cancer/chemotherapeutic.

[0144] “Anti-cancer agent” is used in accordance with its plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In some embodiments, an anti-cancer agent is a chemotherapeutic. In some embodiments, an anti-cancer agent is an agent

identified herein having utility in methods of treating cancer. In some embodiments, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer. Examples of anti-cancer agents include, but are not limited to, MEK (e.g. MEK1, MEK2, or MEK1 and MEK2) inhibitors (e.g. XL518, CI-1040, PD035901, selumetinib/ AZD6244, GSK1120212/ trametinib, GDC-0973, ARRY-162, ARRY-300, AZD8330, PD0325901, U0126, PD98059, TAK-733, PD318088, AS703026, BAY 869766), alkylating agents (e.g., cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, nitrogen mustards (e.g., mechlorethamine, cyclophosphamide, chlorambucil, melphalan), ethylenimine and methylmelamines (e.g., hexamethylmelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomustine, semustine, streptozocin), triazines (decarbazine)), anti-metabolites (e.g., 5-azathioprine, leucovorin, capecitabine, fludarabine, gemcitabine, pemetrexed, raltitrexed, folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., fluorouracil, floxouridine, Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin), *etc.*), plant alkaloids (e.g., vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, docetaxel, *etc.*), topoisomerase inhibitors (e.g., irinotecan, topotecan, amsacrine, etoposide (VP16), etoposide phosphate, teniposide, *etc.*), antitumor antibiotics (e.g., doxorubicin, adriamycin, daunorubicin, epirubicin, actinomycin, bleomycin, mitomycin, mitoxantrone, plicamycin, *etc.*), platinum-based compounds (e.g. cisplatin, oxaloplatin, carboplatin), anthracenedione (e.g., mitoxantrone), substituted urea (e.g., hydroxyurea), methyl hydrazine derivative (e.g., procarbazine), adrenocortical suppressant (e.g., mitotane, aminoglutethimide), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, bleomycin), enzymes (e.g., L-asparaginase), inhibitors of mitogen-activated protein kinase signaling (e.g. U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002, Syk inhibitors, mTOR inhibitors, antibodies (e.g., rituxan), gossypol, genasense, polyphenol E, Chlorofusin, all trans-retinoic acid (ATRA), bryostatin, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), 5-aza-2'-deoxycytidine, all trans retinoic acid, doxorubicin, vincristine, etoposide, gemcitabine, imatinib (Gleevec.RTM.), geldanamycin, 17-N-Allylamino-17-Demethoxygeldanamycin (17-AAG), flavopiridol, LY294002, bortezomib, trastuzumab, BAY 11-7082, PKC412, PD184352, 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense

oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; broprimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydridemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziqune; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9-dioxamycin; diphenyl spiromustine; docosanil; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone;

miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol;
 mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone;
 mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin;
 monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene
 inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B;
 mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides;
 nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin;
 nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide
 modulators; nitroxide antioxidant; nitrullin; O6-benzylguanine; octreotide; okicenone;
 oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer;
 ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic
 acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan
 polysulfate sodium; pentostatin; pentozole; perflubron; perfosfamide; perillyl alcohol;
 phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride;
 pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum
 complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin;
 prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based
 immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein
 tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins;
 pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists;
 raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP
 inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII
 retinamide; roglitimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol;
 saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence
 derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction
 modulators; single chain antigen-binding protein; sizofuran; sobuzoxane; sodium borocaptate;
 sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid;
 spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-
 cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive
 intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans;
 tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur;
 tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide;
 tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin
 mimetic; thymalfasin; thymopoietin receptor agonist; thymotrnan; thyroid stimulating hormone;
 tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem

cell factor; translation inhibitors; tretinoin; triacetyluridine; tricyriline; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin; stimalamer, Adriamycin, Dactinomycin, Bleomycin, Vinblastine, Cisplatin, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; broprimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziqune; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; iimofosine; interleukin II (including recombinant interleukin II, or rIL.sub.2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon gamma-1b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedpa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone

hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; tricirbine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulazole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride, agents that arrest cells in the G2-M phases and/or modulate the formation or stability of microtubules, (e.g. Taxol.TM (i.e. paclitaxel), Taxotere.TM, compounds comprising the taxane skeleton, Erbulozole (i.e. R-55104), Dolastatin 10 (i.e. DLS-10 and NSC-376128), Mivobulin isethionate (i.e. as CI-980), Vincristine, NSC-639829, Discodermolide (i.e. as NVP-XX-A-296), ABT-751 (Abbott, i.e. E-7010), Altorhyrtins (e.g. Altorhyrtin A and Altorhyrtin C), Spongistatins (e.g. Spongistatin 1, Spongistatin 2, Spongistatin 3, Spongistatin 4, Spongistatin 5, Spongistatin 6, Spongistatin 7, Spongistatin 8, and Spongistatin 9), Cemadotin hydrochloride (i.e. LU-103793 and NSC-D-669356), Epothilones (e.g. Epothilone A, Epothilone B, Epothilone C (i.e. desoxyepothilone A or dEpoA), Epothilone D (i.e. KOS-862, dEpoB, and desoxyepothilone B), Epothilone E, Epothilone F, Epothilone B N-oxide, Epothilone A N-oxide, 16-aza-epothilone B, 21-aminoepothilone B (i.e. BMS-310705), 21-hydroxyepothilone D (i.e. Desoxyepothilone F and dEpoF), 26-fluoroepothilone, Auristatin PE (i.e. NSC-654663), Soblidotin (i.e. TZT-1027), LS-4559-P (Pharmacia, i.e. LS-4577), LS-4578 (Pharmacia, i.e. LS-477-P), LS-4477 (Pharmacia), LS-4559 (Pharmacia), RPR-112378 (Aventis), Vincristine sulfate, DZ-3358 (Daiichi), FR-182877 (Fujisawa, i.e. WS-9885B), GS-164 (Takeda), GS-198 (Takeda), KAR-2 (Hungarian Academy of Sciences), BSF-223651 (BASF, i.e. ILX-651 and LU-223651), SAH-49960 (Lilly/Novartis), SDZ-268970 (Lilly/Novartis), AM-97 (Armad/Kyowa Hakko), AM-132 (Armad), AM-138 (Armad/Kyowa Hakko), IDN-5005 (Indena), Cryptophycin 52 (i.e. LY-355703), AC-7739 (Ajinomoto, i.e. AVE-8063A and CS-39.HCl), AC-7700 (Ajinomoto, i.e. AVE-8062, AVE-8062A, CS-39-L-Ser.HCl, and RPR-258062A), Vitilevuamide, Tubulysin A, Canadensol, Centaureidin (i.e. NSC-106969), T-138067 (Tularik, i.e. T-67, TL-138067 and TI-138067), COBRA-1 (Parker Hughes Institute, i.e. DDE-261 and WHI-261), H10 (Kansas State University), H16 (Kansas State University), Oncocidin A1 (i.e. BTO-956 and DIME), DDE-313 (Parker Hughes Institute), Fijianolide B, Laulimalide, SPA-2 (Parker Hughes Institute), SPA-1 (Parker Hughes Institute, i.e. SPIKET-P), 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-569), Narcosine (also known as NSC-5366), Nascapine, D-24851 (Asta Medica), A-105972 (Abbott), Hemiasterlin, 3-BAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-191), TMPN (Arizona State University), Vanadocene acetylacetonate, T-138026 (Tularik), Monsatrol, Inanocine (i.e. NSC-698666), 3-IAABE (Cytoskeleton/Mt. Sinai

School of Medicine), A-204197 (Abbott), T-607 (Tuiarik, i.e. T-900607), RPR-115781 (Aventis), Eleutherobins (such as Desmethyleleutherobin, Desaeyleleutherobin, Isoeleutherobin A, and Z-Eleutherobin), Caribaeoside, Caribaeolin, Halichondrin B, D-64131 (Asta Medica), D-68144 (Asta Medica), Diazonamide A, A-293620 (Abbott), NPI-2350 (Nereus), Taccalonolide A, TUB-245 (Aventis), A-259754 (Abbott), Diozostatin, (-)-Phenylahistin (i.e. NSCL-96F037), D-68838 (Asta Medica), D-68836 (Asta Medica), Myoseverin B, D-43411 (Zentaris, i.e. D-81862), A-289099 (Abbott), A-318315 (Abbott), HTI-286 (i.e. SPA-110, trifluoroacetate salt) (Wyeth), D-82317 (Zentaris), D-82318 (Zentaris), SC-12983 (NCI), Resverastatin phosphate sodium, BPR-OY-007 (National Health Research Institutes), and SSR-250411 (Sanofi)), steroids (e.g., dexamethasone), finasteride, aromatase inhibitors, gonadotropin-releasing hormone agonists (GnRH) such as goserelin or leuprolide, adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate, megestrol acetate, medroxyprogesterone acetate), estrogens (e.g., diethylstilbestrol, ethinyl estradiol), antiestrogen (e.g., tamoxifen), androgens (e.g., testosterone propionate, fluoxymesterone), antiandrogen (e.g., flutamide), immunostimulants (e.g., Bacillus Calmette-Guérin (BCG), levamisole, interleukin-2, alpha-interferon, *etc.*), monoclonal antibodies (e.g., anti-CD20, anti-HER2, anti-CD52, anti-HLA-DR, and anti-VEGF monoclonal antibodies), immunotoxins (e.g., anti-CD33 monoclonal antibody-calicheamicin conjugate, anti-CD22 monoclonal antibody-pseudomonas exotoxin conjugate, *etc.*), radioimmunotherapy (e.g., anti-CD20 monoclonal antibody conjugated to ^{111}In , ^{90}Y , or ^{131}I , *etc.*), triptolide, homoharringtonine, dactinomycin, doxorubicin, epirubicin, topotecan, itraconazole, vindesine, cerivastatin, vincristine, deoxyadenosine, sertraline, pitavastatin, irinotecan, clofazimine, 5-nonyloxytryptamine, vemurafenib, dabrafenib, erlotinib, gefitinib, EGFR inhibitors, epidermal growth factor receptor (EGFR)-targeted therapy or therapeutic (e.g. gefitinib (Iressa™), erlotinib (Tarceva™), cetuximab (Erbix™), lapatinib (Tykerb™), panitumumab (Vectibix™), vandetanib (Caprelsa™), afatinib/BIBW2992, CI-1033/canertinib, neratinib/HKI-272, CP-724714, TAK-285, AST-1306, ARRY334543, ARRY-380, AG-1478, dacomitinib/PF299804, OSI-420/desmethyl erlotinib, AZD8931, AEE788, pelitinib/EKB-569, CUDC-101, WZ8040, WZ4002, WZ3146, AG-490, XL647, PD153035, BMS-599626), sorafenib, imatinib, sunitinib, dasatinib, or the like.

[0145] “Chemotherapeutic” or “chemotherapeutic agent” is used in accordance with its plain ordinary meaning and refers to a chemical composition or compound having antineoplastic properties or the ability to inhibit the growth or proliferation of cells.

[0146] The compounds disclosed herein may be co-administered with an antiproliferative/antineoplastic drug or a combination thereof, as used in medical oncology, such as an alkylating agent (for example cis-platin, carboplatin, cyclophosphamide, nitrogen mustard,

melphalan, chlorambucil, busulphan or a nitrosourea); an antimetabolite (for example an antifolate such as a fluoropyrimidine like 5-fluorouracil or tegafur, raltitrexed, methotrexate, cytosine arabinoside, hydroxyurea, gemcitabine or paclitaxel); an antitumour antibiotic (for example an anthracycline such as adriamycin, bleomycin, doxorubicin, daunomycin, epirubicin, idarubicin, mitomycin-C, dactinomycin or mithramycin); an antimitotic agent (for example a vinca alkaloid such as vincristine, vinblastine, vindesine or vinorelbine, or a taxoid such as taxol or taxotere); or a topoisomerase inhibitor (for example an epipodophyllotoxin such as etoposide, teniposide, amsacrine, topotecan or a camptothecin); (ii) a cytostatic agent such as an antioestrogen (for example tamoxifen, toremifene, raloxifene, droloxifene or idoxifene), an oestrogen receptor down regulator (for example fulvestrant), an antiandrogen (for example bicalutamide, flutamide, nilutamide or cyproterone acetate), a LHRH antagonist or LHRH agonist (for example goserelin, leuporelin or buserelin), a progestogen (for example megestrol acetate), an aromatase inhibitor (for example as anastrozole, letrozole, vorazole or exemestane) or an inhibitor of 5.alpha.-reductase such as finasteride; (iii) an agent which inhibits cancer cell invasion (for example a metalloproteinase inhibitor like marimastat or an inhibitor of urokinase plasminogen activator receptor function); (iv) an inhibitor of growth factor function, for example: a growth factor antibody (for example the anti-erbB2 antibody trastuzumab, or the anti-erbB1 antibody cetuximab [C225]), a farnesyl transferase inhibitor, a tyrosine kinase inhibitor or a serine/threonine kinase inhibitor, an inhibitor of the epidermal growth factor family (for example an EGFR family tyrosine kinase inhibitor such as N-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholinopropoxy)quinazolin-4-amine (gefitinib, AZD 1839), N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (erlotinib, OSI-774) or 6-acrylamido-N-(3-chloro-4-fluorophenyl)-7-(3-morpholinopropoxy)quinazolin-4-amine (CI 1033)), an inhibitor of the platelet-derived growth factor family, or an inhibitor of the hepatocyte growth factor family; (v) an antiangiogenic agent such as one which inhibits the effects of vascular endothelial growth factor (for example the anti-vascular endothelial cell growth factor antibody bevacizumab, a compound disclosed in WO 97/22596, WO 97/30035, WO 97/32856 or WO 98/13354), or a compound that works by another mechanism (for example linomide, an inhibitor of integrin .alpha.v.beta.3 function or an angiostatin); (vi) a vascular damaging agent such as combretastatin A4, or a compound disclosed in WO 99/02166, WO 00/40529, WO 00/41669, WO 01/92224, WO 02/04434 or WO 02/08213; (vii) an agent used in antisense therapy, for example one directed to one of the targets listed above, such as ISIS 2503, an anti-ras antisense; (viii) an agent used in a gene therapy approach, for example approaches to replace aberrant genes such as aberrant p53 or aberrant BRCA1 or BRCA2, GDEPT (gene-directed enzyme pro-drug therapy) approaches such as those using cytosine deaminase, thymidine kinase or a

bacterial nitroreductase enzyme and approaches to increase patient tolerance to chemotherapy or radiotherapy such as multi-drug resistance gene therapy; or (ix) an agent used in an immunotherapeutic approach, for example ex-vivo and in-vivo approaches to increase the immunogenicity of patient tumour cells, such as transfection with cytokines such as interleukin 2, interleukin 4 or granulocyte-macrophage colony stimulating factor, approaches to decrease T-cell anergy, approaches using transfected immune cells such as cytokine-transfected dendritic cells, approaches using cytokine-transfected tumour cell lines and approaches using anti-idiotypic antibodies.

[0147] In embodiments, the compounds disclosed herein can be co-administered with an antibody, such as a monoclonal antibody targeting B-Lymphocytes (such as CD20 (rituximab), MRA-aIL16R and T-Lymphocytes, CTLA4-Ig, HuMax II-15) or antibody modulating Ig function such as anti-IgE (for example omalizumab).

[0148] In embodiments, treatment of cancer includes administration of an effective amount of at least two of the following: a CRF1 inhibitor, an inhibitor of the PD-L1/PD-1 pathway, an inhibitor of CTLA-4, an agonistic antibody of CD137 (4-1BB). In some embodiments, the method may comprise the use of two or more combinations.

[0149] In a further embodiment, the compounds described herein can be co-administered with conventional radiotherapeutic agents including, but not limited to, radionuclides such as ⁴⁷Sc, ⁶⁴Cu, ⁶⁷Cu, ⁸⁹Sr, ⁸⁶Y, ⁸⁷Y, ⁹⁰Y, ¹⁰⁵Rh, ¹¹¹Ag, ¹¹¹In, ^{117m}Sn, ¹⁴⁹Pm, ¹⁵³Sm, ¹⁶⁶Ho, ¹⁷⁷Lu, ¹⁸⁶Re, ¹⁸⁸Re, ²¹¹At, and ²¹²Bi, optionally conjugated to antibodies directed against tumor antigens.

[0150] In addition, a CRF₁ inhibitor may be combined with the therapeutic administration of immune cells, sometimes referred to as adoptive cell transfer. These cells may be cells from the patient, a genetically related or unrelated donor, they may be genetically modified (e.g. CAR-T cells, NK cells, etc), cell lines, genetically modified cell lines and live or dead versions of the above. CRF1 inhibitors may also be combined with vaccines of any kind (e.g. protein/peptide, viral, bacterial, cellular) to stimulate immune responses to cancer.

[0151] The CRF₁ inhibitors/CRF₁ antagonists disclosed herein can be administered by any acceptable route, such oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramusculay, intranasal, intraocularal, intrapericardial, intraperitoneal, intrapleural, intraprostatic, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravaginal, intravenousl, intravesicullar, intravitreal, liposomal, local, mucosal, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, in cremes, in lipid compositions, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, via localized perfusion, bathing target cells directly, or any combination thereof.

[0152] The CRF₁ inhibitors/CRF₁ antagonists disclosed herein can be administered by any acceptable route, such oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramuscular, intranasal, intraocular, intrapericardial, intraperitoneal, intrapleural, intraprostatic, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravaginal, intravenous, intravesicular, intravitreal, liposomal, local, mucosal, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, in cremes, in lipid compositions, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, via localized perfusion, bathing target cells directly, or any combination thereof.

[0153] The CRF₁ inhibitors/CRF₁ antagonists disclosed herein may be administered once daily until study reached endpoint. The CRF₁ inhibitors/CRF₁ antagonists disclosed herein may be administered at least three times but in some studies four or more times depending on the length of the study and/or the design of the study.

[0154] The methods disclosed herein may be used in combination with additional cancer therapy. In some embodiments, the distinct cancer therapy comprises surgery, radiotherapy, chemotherapy, toxin therapy, immunotherapy, cryotherapy or gene therapy. In some embodiments, the cancer is a chemotherapy-resistant or radio-resistant cancer.

[0155] A “cell” as used herein, refers to a cell carrying out metabolic or other function sufficient to preserve or replicate its genomic DNA. A cell can be identified by well-known methods in the art including, for example, presence of an intact membrane, staining by a particular dye, ability to produce progeny or, in the case of a gamete, ability to combine with a second gamete to produce a viable offspring. Cells may include prokaryotic and eukaryotic cells. Prokaryotic cells include but are not limited to bacteria. Eukaryotic cells include but are not limited to yeast cells and cells derived from plants and animals, for example mammalian, insect (*e.g.*, spodoptera) and human cells. Cells may be useful when they are naturally nonadherent or have been treated not to adhere to surfaces, for example by trypsinization.

[0156] “Control” or “control experiment” is used in accordance with its plain ordinary meaning and refers to an experiment in which the subjects or reagents of the experiment are treated as in a parallel experiment except for omission of a procedure, reagent, or variable of the experiment. In some instances, the control is used as a standard of comparison in evaluating experimental effects. In some embodiments, a control is the measurement of the activity of a protein in the absence of a compound as described herein (including embodiments and examples).

[0157] The term “modulator” refers to a composition that increases or decreases the level of a target molecule or the function of a target molecule or the physical state of the target of the molecule. In some embodiments, a CRF₁ associated disease modulator is a compound that

reduces the severity of one or more symptoms of a disease associated with CRF₁ (e.g. an endocrine disorder, a cancer including but not limited to TART or OART). A CRF₁ modulator is a compound that increases or decreases the activity or function or level of activity or level of function of CRF₁. A modulator may act alone, or it may use a cofactor, e.g., a protein, metal ion, or small molecule. Examples of modulators include small molecule compounds and other bioorganic molecules. Numerous libraries of small molecule compounds (e.g., combinatorial libraries) are commercially available and can serve as a starting point for identifying a modulator. The skilled artisan is able to develop one or more assays (e.g., biochemical or cell-based assays) in which such compound libraries can be screened in order to identify one or more compounds having the desired properties; thereafter, the skilled medicinal chemist is able to optimize such one or more compounds by, for example, synthesizing and evaluating analogs and derivatives thereof. Synthetic and/or molecular modeling studies can also be utilized in the identification of an activator.

[0158] The term “modulate” is used in accordance with its plain ordinary meaning and refers to the act of changing or varying one or more properties. “Modulation” refers to the process of changing or varying one or more properties. For example, as applied to the effects of a modulator on a target protein, to modulate means to change by increasing or decreasing a property or function of the target molecule or the amount of the target molecule. In embodiments, the terms “modulate”, “modulation” and the like refer to the ability of a molecule (e.g., an activator or an inhibitor) to increase or decrease the function or activity of CRF₁, either directly or indirectly, relative to the absence of the molecule.

[0159] The term “associated” or “associated with” in the context of a substance or substance activity or function associated with a disease (e.g. a protein associated disease, an endocrine disorder, a disease associated with CRF₁ activity, a CRF₁ associated disease (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)) means that the disease (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)) is caused by (in whole or in part), or a symptom of the disease is caused by (in whole or in part) the substance or substance activity or function. For example, an endocrine disorder associated with CRF₁ activity or function may be an endocrine disorder that results (entirely or partially) from aberrant CRF₁ function (e.g. enzyme activity, protein-protein interaction, signaling pathway) or an endocrine disorder wherein a particular symptom of the disease is caused (entirely or partially) by aberrant CRF₁ activity or function. As used herein, what is described as being associated with a disease, if a causative agent, could be a target for treatment of the disease. For example, an endocrine disorder associated with CRF₁ activity or function or a CRF₁ associated disease (e.g., an endocrine disorder including but not limited to TART or OART), may be treated with a

compound described herein (e.g., CRF₁ modulator, CRF₁ inhibitor, or CRF₁ antagonist), in the instance where increased CRF₁ activity or function (e.g. signaling pathway activity) causes the disease (e.g., an endocrine disorder such as TART or OART). For example, an endocrine disorder associated with CRF₁ activity or function or a CRF₁ associated endocrine disorder, may be treated with an CRF₁ modulator or CRF₁ inhibitor, in the instance where increased CRF₁ activity or function (e.g. signaling pathway activity) causes the disease.

[0160] The term “aberrant” as used herein refers to different from normal. When used to describe enzymatic activity or protein function, aberrant refers to activity or function that is greater or less than a normal control or the average of normal non-diseased control samples. Aberrant activity may refer to an amount of activity that results in a disease, wherein returning the aberrant activity to a normal or non-disease-associated amount (e.g. by administering a compound or using a method as described herein), results in reduction of the disease or one or more disease symptoms.

[0161] The term “signaling pathway” as used herein refers to a series of interactions between cellular and optionally extra-cellular components (e.g. proteins, nucleic acids, small molecules, ions, lipids) that conveys a change in one component to one or more other components, which in turn may convey a change to additional components, which is optionally propagated to other signaling pathway components. For example, binding of a CRF₁ with a compound as described herein may reduce the level of a product of the CRF₁ catalyzed reaction or the level of a downstream derivative of the product or binding may reduce the interactions between the CRF₁ or a reaction product and downstream effectors or signaling pathway components (e.g., MAP kinase pathway), resulting in changes in cell growth, proliferation, or survival.

[0162] As used herein, the terms “corticotropin-releasing factor type-1,” “CRF₁,” “corticotropin-releasing hormone receptor 1,” and “CHHR1” and all other related art-accepted terms, many of which are set forth below, refer to a compound capable of modulating, either directly or indirectly, the CRF₁ receptor in an *in vitro* assay, an *in vivo* model, and/or other means indicative of therapeutic efficacy. The terms also refer to a compound that exhibits at least some therapeutic benefit in a human subject.

[0163] The phrase “in a sufficient amount to effect a change” means that there is a detectable difference between a level of an indicator measured before (e.g., a baseline level) and after administration of a particular therapy. Indicators include any objective parameter (e.g., serum concentration) or subjective parameter (e.g., a subject’s feeling of well-being).

[0164] The “activity” of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor; to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity; to the modulation of activities of

other molecules; and the like. The term “proliferative activity” encompasses an activity that promotes, that is necessary for, or that is specifically associated with, for example, normal cell division, as well as cancer, tumors, dysplasia, cell transformation, metastasis, and angiogenesis.

[0165] “Substantially pure” indicates that a component makes up greater than about 50% of the total content of the composition, and typically greater than about 60% of the total polypeptide content. More typically, “substantially pure” refers to compositions in which at least 75%, at least 85%, at least 90% or more of the total composition is the component of interest. In some cases, the polypeptide will make up greater than about 90%, or greater than about 95% of the total content of the composition (percentage in a weight per weight basis).

[0166] The terms “specifically binds” and “selectively binds,” when referring to a ligand/receptor, antibody/antigen, or other binding pair, indicates a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a specified ligand binds to a particular receptor and does not bind in a significant amount to other proteins present in the sample. The antibody, or binding composition derived from the antigen-binding site of an antibody, of the contemplated method binds to its antigen, or a variant or mutein thereof, with an affinity that is at least two-fold greater, at least 10-times greater, at least 20-times greater, or at least 100-times greater than the affinity with any other antibody, or binding composition derived therefrom. In embodiments, the antibody will have an affinity that is greater than about 10^9 liters/mol, as determined by, e.g., Scatchard analysis (Munsen, et al. (1980) *Analyt. Biochem.* 107:220-239).

[0167] The terms “DNA,” “nucleic acid,” “nucleic acid molecule,” “polynucleotide” and the like are used interchangeably herein to refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include linear and circular nucleic acids, messenger RNA (mRNA), complementary DNA (cDNA), recombinant polynucleotides, vectors, probes, primers and the like.

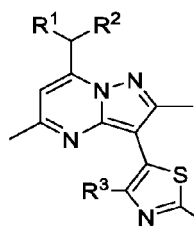
[0168] As used herein, the terms “variants” and “homologs” are used interchangeably to refer to amino acid or nucleic acid sequences that are similar to reference amino acid or nucleic acid sequences, respectively. The term encompasses naturally-occurring variants and non-naturally-occurring variants. Naturally-occurring variants include homologs (polypeptides and nucleic acids that differ in amino acid or nucleotide sequence, respectively, from one species to another), and allelic variants (polypeptides and nucleic acids that differ in amino acid or nucleotide sequence, respectively, from one individual to another within a species). Thus, variants and homologs encompass naturally occurring amino acid and nucleic acid sequences encoded thereby and their isoforms, as well as splice variants of a protein or gene. The terms also

encompass nucleic acid sequences that vary in one or more bases from a naturally-occurring nucleic acid sequence but still translate into an amino acid sequence that corresponds to the naturally-occurring protein due to degeneracy of the genetic code. Non-naturally-occurring variants and homologs include polypeptides and nucleic acids that comprise a change in amino acid or nucleotide sequence, respectively, where the change in sequence is artificially introduced (e.g., muteins); for example, the change is generated in the laboratory by human intervention (“hand of man”). Therefore, non-naturally occurring variants and homologs may also refer to those that differ from the naturally-occurring sequences by one or more conservative substitutions and/or tags and/or conjugates.

[0169] The term “muteins” as used herein refers broadly to mutated recombinant proteins. These proteins usually carry single or multiple amino acid substitutions and are frequently derived from cloned genes that have been subjected to site-directed or random mutagenesis, or from completely synthetic genes.

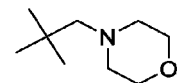
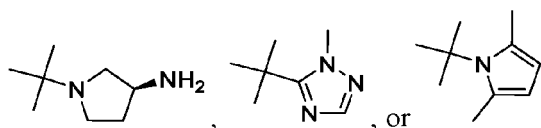
II. Compounds

[0170] In an aspect provided herein, is a compound of Formula (I):



, or a pharmaceutically acceptable salt thereof. R¹ and R² are independently ethyl or n-propyl. R³ is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy. R⁴ is hydrogen,

Br, R^aR^bN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,

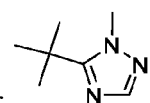


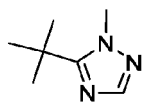
. R^a and R^b are independently hydrogen, C₁-C₃alkyl, H₂NCH₂CH₂-, (CH₃)₃COC(O)NHCH₂CH₂-, or CH₃CH₂CH₂NHCH₂CH₂-. In embodiments, the inhibitor forms an irreversible bond with the amino acid residue. In embodiments, the bond is a covalent bond.

[0171] In certain embodiments, R³ is Cl, Br, methyl, or trifluoromethyl.

[0172] In certain embodiments, R³ is Cl, Br, or methyl.

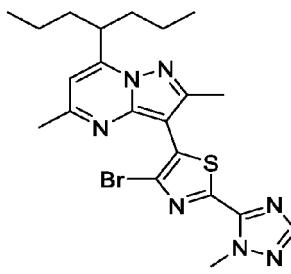
[0173] In certain embodiments, R⁴ is Br, R^aR^bN-, pyridin-4-yl, morpholin-4-yl, or





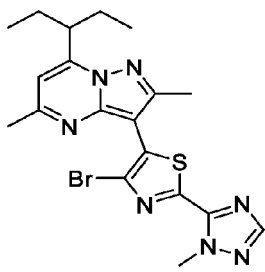
[0174] In certain embodiments, R^4 is morpholin-4-yl or

[0175] In certain embodiments, R^4 is hydrogen, Br, R^aR^bN- and R^a and R^b are independently C_1 - C_3 alkyl.



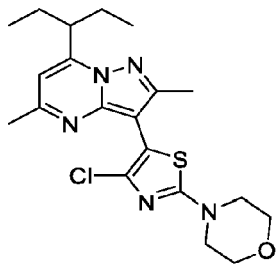
[0176] In certain embodiments, the compound is pharmaceutically acceptable salt thereof.

[0177] In certain embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0178] In certain embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0179] In one aspect described herein, there is a method of treating a subject in need thereof comprising administering an astressin or a pharmaceutically acceptable salt or solvate thereof. An astressin generally refers to a nonselective corticotropin releasing hormone antagonist that reduces the synthesis of ACTH and cortisol.

[0180] In one aspect described herein, there is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, the method comprising: (i) measuring a hormone

level in the subject in need thereof; (ii) administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof; and repeating steps (i) and (ii) until the hormone level reaches a pre-determined range followed by a maintenance therapy of a daily dosing of said astressin.

[0181] In one aspect described herein, there is a method of improving hyperandrogenic symptoms in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a method of treating menstrual irregularity, ovulatory dysfunction or infertility, in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a method of improving metabolic symptoms in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto; or a pharmaceutically acceptable salt or solvate thereof.

[0182] In one aspect described herein, there is a method of improving quality of life in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof.

III. Pharmaceutical Compositions

[0183] In an aspect, there is provided a pharmaceutical composition, including a compound as described herein, including embodiments, or the structural Formula (I), and a pharmaceutically acceptable excipient.

[0184] The compounds (e.g., CRF₁ inhibitors/antagonists) of the present disclosure may be in the form of compositions suitable for administration to a subject. In general, such compositions are “pharmaceutical compositions” comprising a compound (e.g., CRF₁ inhibitor(s)/antagonist(s)) and one or more pharmaceutically acceptable or physiologically acceptable diluents, carriers or excipients. In certain embodiments, the compound (e.g., CRF₁ inhibitor/antagonist) are present in a therapeutically acceptable amount. The pharmaceutical compositions may be used in the methods of the present disclosure; thus, for example, the pharmaceutical compositions can be administered *ex vivo* or *in vivo* to a subject in order to practice the therapeutic and prophylactic methods and uses described herein.

[0185] In one aspect described herein, there is a pharmaceutical composition in the form of a capsule comprising an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a pharmaceutical composition in the form of a tablet comprising an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof.

[0186] The pharmaceutical compositions of the present disclosure can be formulated to be compatible with the intended method or route of administration; exemplary routes of administration are set forth herein.

[0187] The pharmaceutical compositions containing the active ingredient (e.g., an inhibitor of CRF₁ function) may be in a form suitable for oral use, for example, as tablets, capsules, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups, solutions, microbeads or elixirs. Pharmaceutical compositions intended for oral use may be prepared for the manufacture of pharmaceutical compositions, and such compositions may contain one or more agents such as, for example, sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets, capsules and the like contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture thereof. These excipients may be, for example, diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc.

[0188] The tablets, capsules and the like suitable for oral administration may be uncoated or coated to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action. For example, a time-delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated to form osmotic therapeutic tablets for controlled release. Additional agents include biodegradable or biocompatible particles or a polymeric substance such as polyesters, polyamine acids, hydrogel, polyvinyl pyrrolidone, polyanhydrides, polyglycolic acid, ethylene-vinylacetate, methylcellulose, carboxymethylcellulose, protamine sulfate, or lactide/glycolide copolymers, polylactide/glycolide copolymers, or ethylenevinylacetate copolymers in order to control delivery of an administered composition. For example, the oral agent can be entrapped in microcapsules prepared by coacervation techniques or by interfacial polymerization, by the use of hydroxymethylcellulose or gelatin-microcapsules or poly(methylmethacrolate) microcapsules, respectively, or in a colloid drug delivery system. Colloidal dispersion systems include macromolecule complexes, nano-capsules, microspheres, microbeads, and lipid-based systems, including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Methods for the preparation of the above-mentioned formulations will be apparent to those skilled in the art.

[0189] Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium

phosphate, kaolin or microcrystalline cellulose, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

[0190] Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture thereof. Such excipients can be suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents, for example a naturally-occurring phosphatide (e.g., lecithin), or condensation products of an alkylene oxide with fatty acids (e.g., polyoxy-ethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols (e.g., for heptadecaethyleneoxycetanol), or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol (e.g., polyoxyethylene sorbitol monooleate), or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides (e.g., polyethylene sorbitan monooleate). The aqueous suspensions may also contain one or more preservatives.

[0191] Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation.

[0192] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, and optionally one or more suspending agents and/or preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified herein.

[0193] The pharmaceutical compositions of the present disclosure may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example, liquid paraffin, or mixtures of these. Suitable emulsifying agents may be naturally occurring gums, for example, gum acacia or gum tragacanth; naturally occurring phosphatides, for example, soy bean, lecithin, and esters or partial esters derived from fatty acids; hexitol anhydrides, for example, sorbitan monooleate; and condensation products of partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate.

[0194] The pharmaceutical compositions typically comprise a therapeutically effective amount of a CRF₁ inhibitor/antagonist contemplated by the present disclosure and one or more pharmaceutically and physiologically acceptable formulation agents. Suitable pharmaceutically acceptable or physiologically acceptable diluents, carriers or excipients include, but are not limited to, antioxidants (e.g., ascorbic acid and sodium bisulfate), preservatives (e.g., benzyl

alcohol, methyl parabens, ethyl or n-propyl, p-hydroxybenzoate), emulsifying agents, suspending agents, dispersing agents, solvents, fillers, bulking agents, detergents, buffers, vehicles, diluents, and/or adjuvants. For example, a suitable vehicle may be physiological saline solution or citrate-buffered saline, possibly supplemented with other materials common in pharmaceutical compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Those skilled in the art will readily recognize a variety of buffers that can be used in the pharmaceutical compositions and dosage forms contemplated herein. Typical buffers include, but are not limited to, pharmaceutically acceptable weak acids, weak bases, or mixtures thereof. As an example, the buffer components can be water soluble materials such as phosphoric acid, tartaric acids, lactic acid, succinic acid, citric acid, acetic acid, ascorbic acid, aspartic acid, glutamic acid, and salts thereof. Acceptable buffering agents include, for example, a Tris buffer; N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES); 2-(N-Morpholino)ethanesulfonic acid (MES); 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES); 3-(N-Morpholino)propanesulfonic acid (MOPS); and N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS).

[0195] After a pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form, a lyophilized form requiring reconstitution prior to use, a liquid form requiring dilution prior to use, or other acceptable form. In some embodiments, the pharmaceutical composition is provided in a single-use container (e.g., a single-use vial, ampule, syringe, or autoinjector (similar to, e.g., an EpiPen®)), whereas a multi-use container (e.g., a multi-use vial) is provided in other embodiments.

[0196] Formulations can also include carriers to protect the composition against rapid degradation or elimination from the body, such as a controlled release formulation, including liposomes, hydrogels, prodrugs and microencapsulated delivery systems. For example, a time-delay material such as glyceryl monostearate or glyceryl stearate alone, or in combination with a wax, may be employed. Any drug delivery apparatus may be used to deliver a CRF₁ inhibitor/antagonist, including implants (e.g., implantable pumps) and catheter systems, slow injection pumps and devices, all of which are well known to the skilled artisan.

[0197] Depot injections, which are generally administered subcutaneously or intramuscularly, may also be utilized to release the compound (e.g., CRF₁ inhibitor/antagonist) disclosed herein over a defined period of time. Depot injections are usually either solid- or oil-based and generally comprise at least one of the formulation components set forth herein. One of ordinary skill in the art is familiar with possible formulations and uses of depot injections.

[0198] The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents mentioned herein. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Acceptable diluents, solvents and dispersion media that may be employed include water, Ringer's solution, isotonic sodium chloride solution, Cremophor[®] EL (BASF, Parsippany, NJ) or phosphate buffered saline (PBS), ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. In addition, sterile fixed oils are conventionally employed as a solvent or suspending medium; for this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. Moreover, fatty acids, such as oleic acid, find use in the preparation of injectables. Prolonged absorption of particular injectable formulations can be achieved by including an agent that delays absorption (e.g., aluminum monostearate or gelatin).

[0199] The present disclosure contemplates the administration of the compound (e.g., CRF₁ inhibitor/antagonist) in the form of suppositories for rectal administration. The suppositories can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include, but are not limited to, cocoa butter and polyethylene glycols.

[0200] The compound (e.g., CRF₁ inhibitor/antagonist) contemplated by the present disclosure may be in the form of any other suitable pharmaceutical composition (e.g., sprays for nasal or inhalation use) currently known or developed in the future.

IV. Methods

[0201] In certain embodiments, provided herein are methods for treating or preventing a disease comprising administering an effective amount of a compound of Formula (I), or an isotopic variant thereof or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.

[0202] In one embodiment, provided herein are methods for treating or preventing a tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing a benign tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing a malignant tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing an adrenal

tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing an adrenal rest tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.

[0203] In one embodiment, provided herein are methods for treating or preventing infertility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing male infertility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for improving or increasing sperm count, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for improving or increasing sperm motility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for improving or increasing sperm morphology, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing female infertility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.

[0204] In one embodiment, provided herein are methods for treating or preventing a proliferative or hyperproliferative disease or disorder, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In certain embodiments, the proliferative disease is cancer. In certain embodiments, the proliferative disease is a cancer of the breast, skin, prostate, cervix, uterus, ovary, testes, bladder, lung, liver, larynx, oral cavity, colon and gastrointestinal tract (e.g., esophagus, stomach, pancreas), brain, thyroid, blood, and lymphatic system.

[0205] In certain embodiments, the cancer treatable with the methods provided herein includes, but is not limited to, (1) leukemias, including, but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myelodysplastic syndrome or a symptom thereof (such as anemia, thrombocytopenia, neutropenia, bicytopenia or pancytopenia), refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess blasts (RAEB),

RAEB in transformation (RAEB-T), preleukemia, and chronic myelomonocytic leukemia (CMML); (2) chronic leukemias, including, but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, and hairy cell leukemia; (3) polycythemia vera; (4) lymphomas, including, but not limited to, Hodgkin's disease and non-Hodgkin's disease; (5) multiple myelomas, including, but not limited to, smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma, and extramedullary plasmacytoma; (6) Waldenstrom's macroglobulinemia; (7) monoclonal gammopathy of undetermined significance; (8) benign monoclonal gammopathy; (9) heavy chain disease; (10) bone and connective tissue sarcomas, including, but not limited to, bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, metastatic cancers, neurilemmoma, rhabdomyosarcoma, and synovial sarcoma; (11) brain tumors, including, but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, and primary brain lymphoma; (12) breast cancer, including, but not limited to, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, primary cancers, Paget's disease, and inflammatory breast cancer; (13) adrenal cancer, including, but not limited to, pheochromocytoma and adrenocortical carcinoma; (14) thyroid cancer, including, but not limited to, papillary or follicular thyroid cancer, medullary thyroid cancer, and anaplastic thyroid cancer; (15) pancreatic cancer, including, but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; (16) pituitary cancer, including, but limited to, Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipidus; (17) eye cancer, including, but not limited, to ocular melanoma such as iris melanoma, choroidal melanoma, and ciliary body melanoma, and retinoblastoma; (18) vaginal cancer, including, but not limited to, squamous cell carcinoma, adenocarcinoma, and melanoma; (19) vulvar cancer, including, but not limited to, squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; (20) cervical cancers, including, but not limited to, squamous cell carcinoma, and adenocarcinoma; (21) uterine cancer, including, but not limited to, endometrial carcinoma and uterine sarcoma; (22) ovarian cancer, including, but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; (23) esophageal cancer, including, but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous

carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; (24) stomach cancer, including, but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; (25) colon cancer; (26) rectal cancer; (27) liver cancer, including, but not limited to, hepatocellular carcinoma and hepatoblastoma; (28) gallbladder cancer, including, but not limited to, adenocarcinoma; (29) cholangiocarcinomas, including, but not limited to, papillary, nodular, and diffuse; (30) lung cancer, including, but not limited to, non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma, and small-cell lung cancer; (31) testicular cancer, including, but not limited to, germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, and choriocarcinoma (yolk-sac tumor); (32) prostate cancer, including, but not limited to, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; (33) penile cancer; (34) oral cancer, including, but not limited to, squamous cell carcinoma; (35) basal cancer; (36) salivary gland cancer, including, but not limited to, adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; (37) pharynx cancer, including, but not limited to, squamous cell cancer and verrucous; (38) skin cancer, including, but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, and acral lentiginous melanoma; (39) kidney cancer, including, but not limited to, renal cell cancer, adenocarcinoma, hypernephroma, fibrosarcoma, and transitional cell cancer (renal pelvis and/or ureter); (40) Wilms' tumor; (41) bladder cancer, including, but not limited to, transitional cell carcinoma, squamous cell cancer, adenocarcinoma, and carcinosarcoma; and other cancer, including, not limited to, myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangi endotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, and papillary adenocarcinomas (*See Fishman et al.*, 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy *et al.*, 1997, *Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery*, Viking Penguin, Penguin Books U.S.A., Inc., United States of America).

[0206] Depending on the disorder, disease, or condition to be treated, and the subject's condition, the compounds or pharmaceutical compositions provided herein can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), inhalation, nasal, vaginal, rectal, sublingual, or topical (e.g., transdermal or local) routes of administration and can be formulated, alone or

together, in suitable dosage unit with pharmaceutically acceptable excipients, carriers, adjuvants, and vehicles appropriate for each route of administration as described elsewhere herein.

[0207] The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. Recommended routes of administration for the second active agents are known to those of ordinary skill in the art. See, e.g., Physicians' Desk Reference, 1755-1760 (56th ed., 2002).

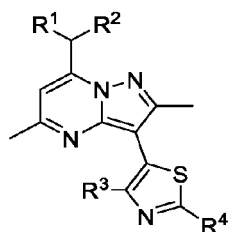
[0208] In one embodiment, provided herein are methods for treating or preventing testicular adrenal rest tumors (TART) in a subject, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof, wherein the subject has congenital adrenal hyperplasia (CAH).

[0209] In one embodiment, provided herein are methods for treating or preventing ovarian adrenal rest tumors (OART) in a subject, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof, wherein the subject has congenital adrenal hyperplasia (CAH).

[0210] In an aspect provided herein, is a method of treating or preventing testicular adrenal rest tumors (TART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF₁) antagonist or a pharmaceutically acceptable salt thereof.

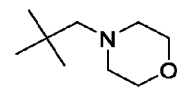
[0211] In an aspect provided herein, is a method of treating or preventing ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF₁) antagonist or a pharmaceutically acceptable salt thereof.

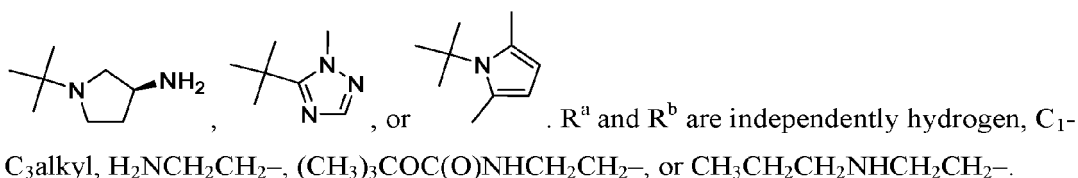
[0212] In an aspect provided herein, is a method of treating or preventing testicular adrenal rest tumors (TART), comprising administering to a subject in need thereof a compound of structural Formula (I):



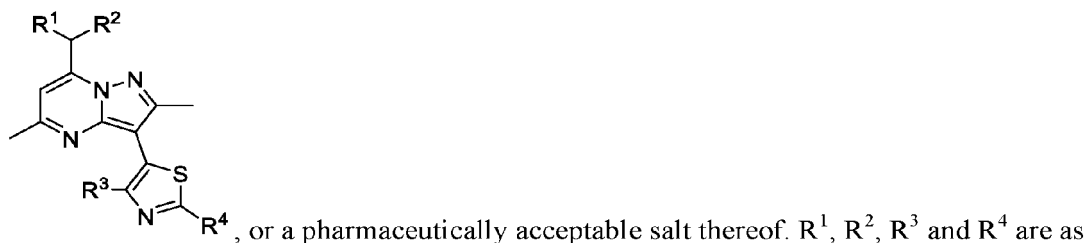
, or a pharmaceutically acceptable salt thereof. R¹ and R² are independently ethyl or n-propyl. R³ is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy. R⁴ is hydrogen,

Br, R^aR^bN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,



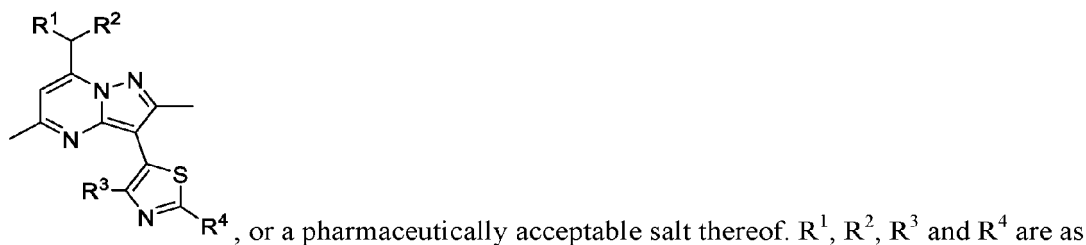


[0213] In an aspect provided herein, is a method of treating or preventing ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a compound of structural Formula (I):



described herein, including embodiments.

[0214] In an aspect provided herein, is a method of decreasing or reducing adrenocorticotrophic hormone (ACTH) levels in a subject in need thereof, comprising administering to the subject contacting the ACTH with a compound of structural Formula (I):

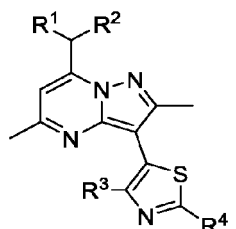


described herein, including embodiments. R^1 , R^2 , R^3 and R^4 are as described herein, including embodiments. In certain embodiments, at least about 50% of the subjects demonstrate reduction in ACTH. In certain embodiments, at least about 60% of the subjects demonstrate reduction in ACTH. In certain embodiments, at least about 70% of the subjects demonstrate reduction in ACTH. In certain embodiments, at least about 80% of the subjects demonstrate reduction in ACTH. In embodiments, at least about 10% of subjects were in the standard or normal range for ACTH after treatment.

[0215] In embodiments, at least about 20% of subjects were in the standard or normal range for ACTH after treatment. In embodiments, at least about 30% of subjects were in the standard or normal range for ACTH after treatment. In certain embodiments, ACTH levels are decreased or reduced within about 30 minutes post dosing. In certain embodiments, ACTH levels are decreased or reduced within about 1 hour post dosing. In certain embodiments, ACTH levels are

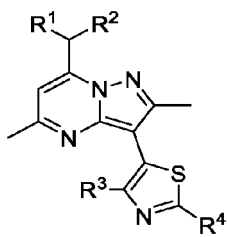
decreased or reduced within about 90 minutes post dosing. In certain embodiments, ACTH levels are decreased or reduced within about 2 hours post dosing.

[0216] In an aspect provided herein, is a method of decreasing or reducing 17 α -hydroxyprogesterone (17-OHP) levels in a subject in need thereof, comprising administering to the subject a compound of structural Formula (I):



, or a pharmaceutically acceptable salt thereof. R¹, R², R³ and R⁴ are as described herein, including embodiments. R¹, R², R³ and R⁴ are as described herein, including embodiments. In certain embodiments, at least about 50% of the subjects demonstrate reduction in 17-OHP. In certain embodiments, at least about 60% of the subjects demonstrate reduction in 17-OHP. In certain embodiments, at least about 70% of the subjects demonstrate reduction in 17-OHP. In certain embodiments, at least about 80% of the subjects demonstrate reduction in 17-OHP. In embodiments, at least about 30% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 40% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 50% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 60% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 30% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment. In embodiments, at least about 40% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment. In embodiments, at least about 50% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment. In embodiments, at least about 60% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment.

[0217] In an aspect provided herein, is a method of decreasing or reducing androstenedione levels in a subject in need thereof, comprising administering to the subject a compound of structural Formula (I):

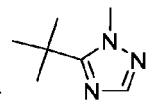


, or a pharmaceutically acceptable salt thereof. R^1 , R^2 , R^3 and R^4 are as described herein, including embodiments. R^1 , R^2 , R^3 and R^4 are as described herein, including embodiments. In certain embodiments, at least about 50% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 60% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 70% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 80% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 90% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 100% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 30% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In certain embodiments, at least about 40% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In certain embodiments, at least about 50% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In certain embodiments, at least about 60% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In certain embodiments, at least about 30% of subjects were in the standard or normal range for androstenedione after treatment. In certain embodiments, at least about 40% of subjects were in the standard or normal range for androstenedione after treatment. In certain embodiments, at least about 50% of subjects were in the standard or normal range for androstenedione after treatment.

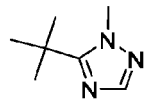
[0218] In certain embodiments, R^3 is Cl, Br, methyl, or trifluoromethyl.

[0219] In certain embodiments, R^3 is Cl, Br, or methyl.

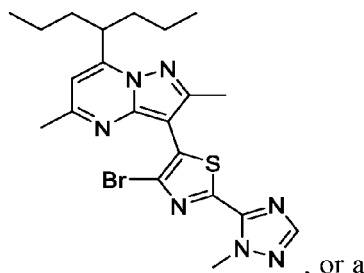
[0220] In certain embodiments, R^4 is Br, $R^a R^b N-$, pyridin-4-yl, morpholin-4-yl, or



[0221] In certain embodiments, R^4 is morpholin-4-yl or

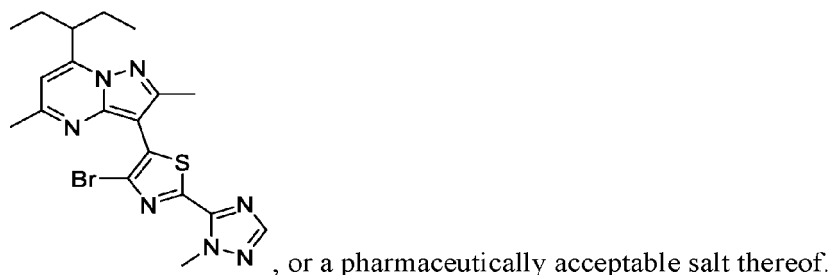


[0222] In certain embodiments, R^4 is hydrogen, Br, $R^a R^b N-$ and R^a and R^b are independently C_1 - C_3 alkyl.

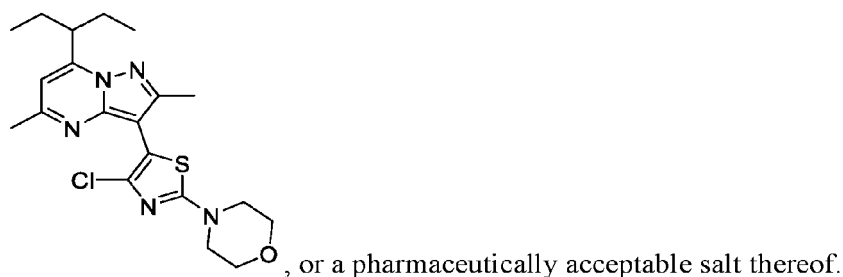


[0223] In certain embodiments, the compound is pharmaceutically acceptable salt thereof.

[0224] In certain embodiments, the compound is



[0225] In certain embodiments, the compound is



[0226] In certain embodiments, a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is cyclically administered to a patient. Cycling therapy involves the administration of an active agent or a combination of active agents for a period of time, followed by a rest for a period of time, and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

[0227] In certain embodiments, in the treatment, prevention, or amelioration of one or more symptoms of the disorders, diseases, or conditions described herein (e.g., treatment or prevention of testicular adrenal rest tumors (TART) or treatment or prevention of ovarian adrenal rest tumors (OART)), an appropriate dosage level of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof generally is ranging from about 1 mg to 3000 mg, from about 1 mg to 2000 mg, from about 1 mg to 1000 mg, from about 1 mg to about 500 mg, from about 5 mg to about 500 mg,

from about 5 mg to about 400 mg, from about 5 mg to about 300 mg, from about 5 mg to about 250 mg, from about 5 mg to about 200 mg, from about 50 mg to about 200 mg, from about 10 mg to about 400 mg, from about 5 mg to about 25 mg, from about 10 mg to about 25 mg, or from about 25 to about 200 mg, which can be administered in single or multiple doses. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 450 mg, about 500 mg, about 600 mg, about 800 mg, or about 1000 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 450 mg, about 500 mg, about 600 mg, about 800 mg, or about 1000 mg/day.

[illegible]

[illegible]

administered in an amount of about 600 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg.

[0229] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, or about 1000 mg.

[0230] For oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing from about 1.0 mg to about 1,000 mg of a compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In certain embodiments, for oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about

350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg, about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 980 mg, about 990 mg, about 990 mg, or about 1000 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof for the symptomatic adjustment of the dosage to the patient to be treated. In certain embodiments, for oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing about 50 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In certain embodiments, for oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing about 200 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. The pharmaceutical compositions can be administered on a regimen of one (1) to four (4) times per day, including once, twice, three times, and four times per day. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered once per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about

310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg, about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 990 mg, about 990 mg, or about 1000 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered once per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg,

about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, or about 1000 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered twice per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg, about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, or about 1000 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered three times per day.

[0231] In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered for a period of one day. In some embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of two days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of three days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of four days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of five days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of six days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of seven days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of eight days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of nine days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of ten days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 11 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 12 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 13 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 14 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered for a period of 15 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 16 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 17 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically

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hydrate, or prodrug thereof is administered over a period of 34 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 35 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 36 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 37 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 38 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 39 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 40 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 41 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 42 days or more.

[0232] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 14 days.

[0233] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically

acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 140 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg once daily for a period of up to at least

about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically

acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg once daily for a period of up to at least about 14 days.

[0234] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg twice daily

for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 140 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a

pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg twice daily

for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg twice daily for a period of up to at least about 14 days.

[0235] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 280 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330

mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg once daily for a period of up to at least about 28 days.

[0236] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 220 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 280 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula

(I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a

pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg twice daily for a period of up to at least about 28 days.

[0237] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 255 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230

mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 420 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg once daily for a period of up to at least about 42 days.

[0238] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 5 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula

(I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 420 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 370 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg twice daily for a period of up to at least about 42 days.

[0239] In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, or about 400 mg of the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered twice per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200

mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, or about 800 mg of the compound of Formula (I), or an isotopic variant thereof, or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered at least once per day.

[0240] In certain embodiments, from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

[0241] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered orally.

[0242] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is formulated as a capsule or tablet.

[0243] In certain embodiments, from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

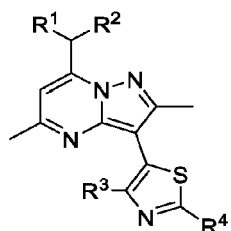
[0244] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 5 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 25 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 5 mg/day to about 25 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 10 mg/day to about 400 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 100 mg/day to about 600 mg/day.

[0245] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fed state. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fasted state. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at least once per day.

[0246] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at bedtime. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 6 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 5 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 4 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 3 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 2 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 1 hour before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 30 minutes before sleep.

[0247] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the evening. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at about 10 pm at night.

[0248] In another aspect is provided a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a pharmaceutical composition, comprising a compound of structural Formula (I): and a pharmaceutically acceptable excipient comprising with a compound as described herein, including embodiments, or the structural Formulae (I) and a pharmaceutically acceptable excipient:



, or a pharmaceutically acceptable salt thereof. R^1 , R^2 , R^3 , and R^4 are as described herein, including embodiments.

[0249] In certain embodiments, the pharmaceutical composition comprises from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments,

the pharmaceutical composition comprises from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0250] In certain embodiments, the pharmaceutical composition comprises about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg about 500 mg, or about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0251] In certain embodiments, the pharmaceutical composition is administered orally.

[0252] In certain embodiments, the pharmaceutical composition is formulated as a capsule or tablet. In certain embodiments, the capsule or tablet comprises about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, or about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the capsule or tablet comprises about 50 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the capsule or tablet comprises about 200 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0253] In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically

acceptable salt thereof, is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

[0254] In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 25 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 25 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 10 mg/day to about 400 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 100 mg/day to about 600 mg/day.

[0255] In certain embodiments, the pharmaceutical composition is administered in the fed state. In certain embodiments, the pharmaceutical composition is administered in the fasted state. In certain embodiments, the pharmaceutical composition is administered at least once per day.

[0256] In certain embodiments, the pharmaceutical composition is administered at bedtime. In certain embodiments, the pharmaceutical composition is administered less than about 6 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 5 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 4 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 3 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 2 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 1 hour before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 30 minutes before sleep.

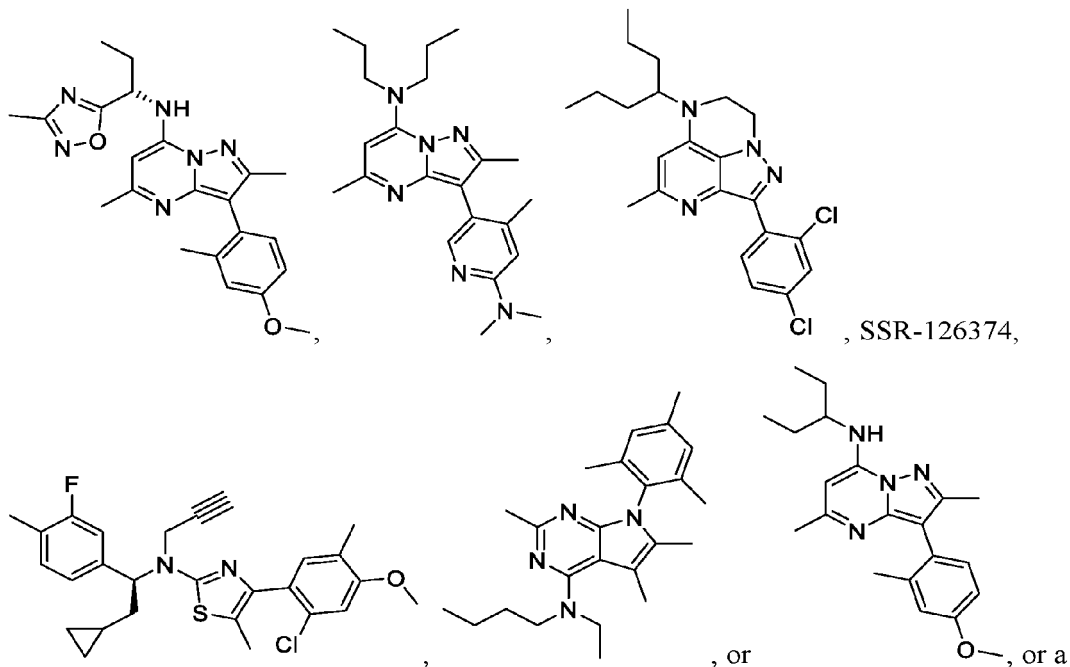
[0257] In certain embodiments, the pharmaceutical composition is administered in the evening. In certain embodiments, the pharmaceutical composition is administered at about 10 pm at night.

[0258] In certain embodiments, the subject has congenital adrenal hyperplasia (CAH).

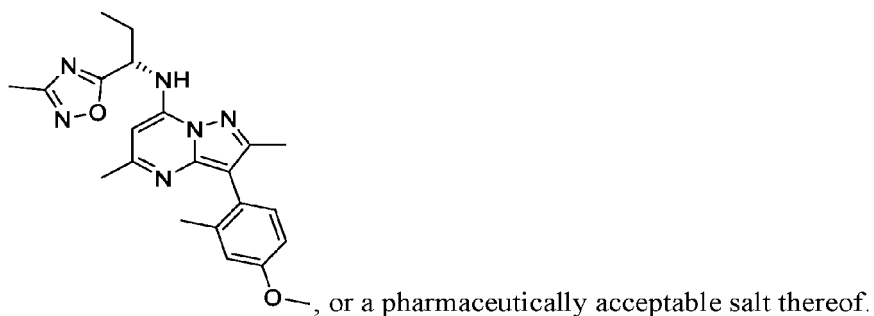
[0259] In certain embodiments, the size and/or number of the tumors is decreased or reduced. In certain embodiments, the size of the tumors is decreased or reduced. In certain embodiments, the size of the tumors is decreased or reduced.

[0260] In certain embodiments of the methods disclosed herein, the method further comprises administering to the subject an additional chemotherapeutic agent.

[0261] In certain embodiments, the the additional chemotherapeutic agent is a glucocorticoid, a mineralocorticoid, an ACAT1 inhibitor, or an anti-androgen. In certain embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In certain embodiments, the mineralocorticoid is fludrocortisone. In certain embodiments, the additional chemotherapeutic agent is another CRF₁ antagonist. In certain embodiments, the CRF₁ antagonist is:



pharmaceutically acceptable salt thereof. In certain embodiments, the CRF₁ antagonist is



[0262] In certain embodiments of the methods disclosed herein, the method further comprises an additional treatment selected from surgical resection of the tumors and radiation therapy, or a combination thereof. In certain embodiments, the additional therapy is surgical resection and the surgical resection is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof. In certain embodiments, the surgical resection is prior

to administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the surgical resection is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the surgical resection is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0263] In certain embodiments, the additional treatment is radiation therapy and the radiation therapy is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof. In certain embodiments, the radiation therapy is prior to administration of the CRF₁ antagonist of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the radiation therapy is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the radiation therapy is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0264] In one embodiment, the pharmaceutical compositions are provided in a dosage form for oral administration, which comprise a compound provided herein, and one or more pharmaceutically acceptable excipients or carriers. The pharmaceutical compositions provided herein that are formulated for oral administration may be in tablet, capsule, powder, or liquid form. In some embodiments, a tablet comprises a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil, or synthetic oil. Physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol, or polyethylene glycol may be included. In some embodiments, a capsule comprises a solid carrier such as gelatin.

[0265] In another embodiment, the pharmaceutical compositions are provided in a dosage form for parenteral administration, which comprise a compound provided herein, and one or more pharmaceutically acceptable excipients or carriers. Where pharmaceutical compositions may be formulated for intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution, which is pyrogen-free and has a suitable pH, isotonicity, and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride injection, Ringer's injection, or Lactated Ringer's injection. In some embodiments, preservatives, stabilisers, buffers, antioxidants, and/or other additives are included as required.

[0266] In yet another embodiment, the pharmaceutical compositions are provided in a dosage form for topical administration, which comprise a compound provided herein, and one or more pharmaceutically acceptable excipients or carriers.

[0267] The pharmaceutical compositions can also be formulated as modified release dosage forms, including delayed-, extended-, prolonged-, sustained-, pulsatile-, controlled-, accelerated-, fast-, targeted-, and programmed-release, and gastric retention dosage forms. These dosage forms can be prepared according to conventional methods and techniques known to those skilled in the art (*see, Remington: The Science and Practice of Pharmacy, supra; Modified-Release Drug Delivery Technology*, 2nd Edition, Rathbone *et al.*, Eds., Marcel Dekker, Inc.: New York, NY, 2008).

[0268] The pharmaceutical compositions provided herein can be provided in a unit-dosage form or multiple-dosage form. A unit-dosage form, as used herein, refers to physically discrete a unit suitable for administration to a human and animal subject, and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of an active ingredient(s) sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carriers or excipients. Examples of a unit-dosage form include an ampoule, syringe, and individually packaged tablet and capsule. A unit-dosage form may be administered in fractions or multiples thereof. A multiple-dosage form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dosage form. Examples of a multiple-dosage form include a vial, bottle of tablets or capsules, or bottle of pints or gallons.

[0269] The pharmaceutical compositions provided herein can be administered at once, or multiple times at intervals of time. It is understood that the precise dosage and duration of treatment may vary with the age, weight, and condition of the patient being treated, and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test or diagnostic data. It is further understood that for any particular individual, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations.

[0270] In certain embodiments, the pharmaceutical compositions provided herein further comprise one or more chemotherapeutic agents as defined herein.

A. Oral Administration

[0271] The pharmaceutical compositions provided herein for oral administration can be provided in solid, semisolid, or liquid dosage forms for oral administration. As used herein, oral administration also includes buccal, lingual, and sublingual administration. Suitable oral dosage

forms include, but are not limited to, tablets, fastmelts, chewable tablets, capsules, pills, strips, troches, lozenges, pastilles, cachets, pellets, medicated chewing gum, bulk powders, effervescent or non-effervescent powders or granules, oral mists, solutions, emulsions, suspensions, wafers, sprinkles, elixirs, and syrups. In addition to the active ingredient(s), the pharmaceutical compositions can contain one or more pharmaceutically acceptable carriers or excipients, including, but not limited to, binders, fillers, diluents, disintegrants, wetting agents, lubricants, glidants, coloring agents, dye-migration inhibitors, sweetening agents, flavoring agents, emulsifying agents, suspending and dispersing agents, preservatives, solvents, non-aqueous liquids, organic acids, and sources of carbon dioxide.

[0272] Binders or granulators impart cohesiveness to a tablet to ensure the tablet remaining intact after compression. Suitable binders or granulators include, but are not limited to, starches, such as corn starch, potato starch, and pre-gelatinized starch (e.g., STARCH 1500); gelatin; sugars, such as sucrose, glucose, dextrose, molasses, and lactose; natural and synthetic gums, such as acacia, alginic acid, alginates, extract of Irish moss, panwar gum, ghatti gum, mucilage of isabgol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone (PVP), Veegum, larch arabogalactan, powdered tragacanth, and guar gum; celluloses, such as ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose, methyl cellulose, hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), hydroxypropyl methyl cellulose (HPMC); microcrystalline celluloses, such as AVICEL-PH-101, AVICEL-PH-103, AVICEL RC-581, AVICEL-PH-105 (FMC Corp., Marcus Hook, PA); and mixtures thereof. Suitable fillers include, but are not limited to, talc, calcium carbonate, microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The amount of a binder or filler in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The binder or filler may be present from about 50 to about 99% by weight in the pharmaceutical compositions provided herein.

[0273] Suitable diluents include, but are not limited to, dicalcium phosphate, calcium sulfate, lactose, sorbitol, sucrose, inositol, cellulose, kaolin, mannitol, sodium chloride, dry starch, and powdered sugar. Certain diluents, such as mannitol, lactose, sorbitol, sucrose, and inositol, when present in sufficient quantity, can impart properties to some compressed tablets that permit disintegration in the mouth by chewing. Such compressed tablets can be used as chewable tablets. The amount of a diluent in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art.

[0274] Suitable disintegrants include, but are not limited to, agar; bentonite; celluloses, such as methylcellulose and carboxymethylcellulose; wood products; natural sponge; cation-exchange

resins; alginic acid; gums, such as guar gum and Veegum HV; citrus pulp; cross-linked celluloses, such as croscarmellose; cross-linked polymers, such as crospovidone; cross-linked starches; calcium carbonate; microcrystalline cellulose, such as sodium starch glycolate; polacrilin potassium; starches, such as corn starch, potato starch, tapioca starch, and pre-gelatinized starch; clays; aligns; and mixtures thereof. The amount of a disintegrant in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The amount of a disintegrant in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The pharmaceutical compositions provided herein may contain from about 0.5 to about 15% or from about 1 to about 5% by weight of a disintegrant.

[0275] Suitable lubricants include, but are not limited to, calcium stearate; magnesium stearate; mineral oil; light mineral oil; glycerin; sorbitol; mannitol; glycols, such as glycerol behenate and polyethylene glycol (PEG); stearic acid; sodium lauryl sulfate; talc; hydrogenated vegetable oil, including peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil; zinc stearate; ethyl oleate; ethyl laureate; agar; starch; lycopodium; silica or silica gels, such as AEROSIL® 200 (W.R. Grace Co., Baltimore, MD) and CAB-O-SIL® (Cabot Co. of Boston, MA); and mixtures thereof. The pharmaceutical compositions provided herein may contain about 0.1 to about 5% by weight of a lubricant.

[0276] Suitable glidants include, but are not limited to, colloidal silicon dioxide, CAB-O-SIL® (Cabot Co. of Boston, MA), and asbestos-free talc. Suitable coloring agents include, but are not limited to, any of the approved, certified, water soluble FD&C dyes, and water insoluble FD&C dyes suspended on alumina hydrate, and color lakes and mixtures thereof. A color lake is the combination by adsorption of a water-soluble dye to a hydrous oxide of a heavy metal, resulting in an insoluble form of the dye. Suitable flavoring agents include, but are not limited to, natural flavors extracted from plants, such as fruits, and synthetic blends of compounds which produce a pleasant taste sensation, such as peppermint and methyl salicylate. Suitable sweetening agents include, but are not limited to, sucrose, lactose, mannitol, syrups, glycerin, and artificial sweeteners, such as saccharin and aspartame. Suitable emulsifying agents include, but are not limited to, gelatin, acacia, tragacanth, bentonite, and surfactants, such as polyoxyethylene sorbitan monooleate (TWEEN® 20), polyoxyethylene sorbitan monooleate 80 (TWEEN® 80), and triethanolamine oleate. Suitable suspending and dispersing agents include, but are not limited to, sodium carboxymethylcellulose, pectin, tragacanth, Veegum, acacia, sodium carbomethylcellulose, hydroxypropyl methylcellulose, and polyvinylpyrrolidone. Suitable preservatives include, but are not limited to, glycerin, methyl and propylparaben, benzoic add,

sodium benzoate and alcohol. Suitable wetting agents include, but are not limited to, propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate, and polyoxyethylene lauryl ether. Suitable solvents include, but are not limited to, glycerin, sorbitol, ethyl alcohol, and syrup. Suitable non-aqueous liquids utilized in emulsions include, but are not limited to, mineral oil and cottonseed oil. Suitable organic acids include, but are not limited to, citric and tartaric acid. Suitable sources of carbon dioxide include, but are not limited to, sodium bicarbonate and sodium carbonate.

[0277] It should be understood that many carriers and excipients may serve several functions, even within the same formulation.

[0278] The pharmaceutical compositions provided herein for oral administration can be provided as compressed tablets, tablet triturates, chewable lozenges, rapidly dissolving tablets, multiple compressed tablets, or enteric-coating tablets, sugar-coated, or film-coated tablets. Enteric-coated tablets are compressed tablets coated with substances that resist the action of stomach acid but dissolve or disintegrate in the intestine, thus protecting the active ingredients from the acidic environment of the stomach. Enteric-coatings include, but are not limited to, fatty acids, fats, phenyl salicylate, waxes, shellac, ammoniated shellac, and cellulose acetate phthalates. Sugar-coated tablets are compressed tablets surrounded by a sugar coating, which may be beneficial in covering up objectionable tastes or odors and in protecting the tablets from oxidation. Film-coated tablets are compressed tablets that are covered with a thin layer or film of a water-soluble material. Film coatings include, but are not limited to, hydroxyethylcellulose, sodium carboxymethylcellulose, polyethylene glycol 4000, and cellulose acetate phthalate. Film coating imparts the same general characteristics as sugar coating. Multiple compressed tablets are compressed tablets made by more than one compression cycle, including layered tablets, and press-coated or dry-coated tablets.

[0279] The tablet dosage forms can be prepared from the active ingredient in powdered, crystalline, or granular forms, alone or in combination with one or more carriers or excipients described herein, including binders, disintegrants, controlled-release polymers, lubricants, diluents, and/or colorants. Flavoring and sweetening agents are especially useful in the formation of chewable tablets and lozenges.

[0280] The pharmaceutical compositions provided herein for oral administration can be provided as soft or hard capsules, which can be made from gelatin, methylcellulose, starch, or calcium alginate. The hard gelatin capsule, also known as the dry-filled capsule (DFC), consists of two sections, one slipping over the other, thus completely enclosing the active ingredient. The soft elastic capsule (SEC) is a soft, globular shell, such as a gelatin shell, which is plasticized by the addition of glycerin, sorbitol, or a similar polyol. The soft gelatin shells may contain a

preservative to prevent the growth of microorganisms. Suitable preservatives are those as described herein, including methyl- and propyl-parabens, and sorbic acid. The liquid, semisolid, and solid dosage forms provided herein may be encapsulated in a capsule. Suitable liquid and semisolid dosage forms include solutions and suspensions in propylene carbonate, vegetable oils, or triglycerides. Capsules containing such solutions can be prepared as described in U.S. Pat. Nos. 4,328,245; 4,409,239; and 4,410,545. The capsules may also be coated as known by those of skill in the art in order to modify or sustain dissolution of the active ingredient.

[0281] Coloring and flavoring agents can be used in all of the above dosage forms.

[0282] The pharmaceutical compositions provided herein for oral administration can be formulated as immediate or modified release dosage forms, including delayed-, sustained, pulsed-, controlled, targeted-, and programmed-release forms.

B. Parenteral Administration

[0283] The pharmaceutical compositions provided herein can be administered parenterally by injection, infusion, or implantation, for local or systemic administration. Parenteral administration, as used herein, include intravenous, intraarterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular, intrasynovial, intravesical, and subcutaneous administration.

[0284] The pharmaceutical compositions provided herein for parenteral administration can be formulated in any dosage forms that are suitable for parenteral administration, including solutions, suspensions, emulsions, micelles, liposomes, microspheres, nanosystems, and solid forms suitable for solutions or suspensions in liquid prior to injection. Such dosage forms can be prepared according to conventional methods known to those skilled in the art of pharmaceutical science (*see, Remington: The Science and Practice of Pharmacy, supra*).

[0285] The pharmaceutical compositions intended for parenteral administration can include one or more pharmaceutically acceptable carriers and excipients, including, but not limited to, aqueous vehicles, water-miscible vehicles, non-aqueous vehicles, antimicrobial agents or preservatives against the growth of microorganisms, stabilizers, solubility enhancers, isotonic agents, buffering agents, antioxidants, local anesthetics, suspending and dispersing agents, wetting or emulsifying agents, complexing agents, sequestering or chelating agents, cryoprotectants, lyoprotectants, thickening agents, pH adjusting agents, and inert gases.

[0286] Suitable aqueous vehicles include, but are not limited to, water, saline, physiological saline or phosphate buffered saline (PBS), sodium chloride injection, Ringers injection, isotonic dextrose injection, sterile water injection, dextrose and lactated Ringers injection. Suitable non-aqueous vehicles include, but are not limited to, fixed oils of vegetable origin, castor oil, corn oil, cottonseed oil, olive oil, peanut oil, peppermint oil, safflower oil, sesame oil, soybean oil,

hydrogenated vegetable oils, hydrogenated soybean oil, and medium-chain triglycerides of coconut oil, and palm seed oil. Suitable water-miscible vehicles include, but are not limited to, ethanol, 1,3-butanediol, liquid polyethylene glycol (e.g., polyethylene glycol 300 and polyethylene glycol 400), propylene glycol, glycerin, *N*-methyl-2-pyrrolidone, *N,N*-dimethylacetamide, and dimethyl sulfoxide.

[0287] Suitable antimicrobial agents or preservatives include, but are not limited to, phenols, cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl *p*-hydroxybenzoates, thimerosal, benzalkonium chloride (e.g., benzethonium chloride), methyl- and propyl-parabens, and sorbic acid. Suitable isotonic agents include, but are not limited to, sodium chloride, glycerin, and dextrose. Suitable buffering agents include, but are not limited to, phosphate and citrate. Suitable antioxidants are those as described herein, including bisulfite and sodium metabisulfite. Suitable local anesthetics include, but are not limited to, procaine hydrochloride. Suitable suspending and dispersing agents are those as described herein, including sodium carboxymethylcellulose, hydroxypropyl methylcellulose, and polyvinylpyrrolidone. Suitable emulsifying agents are those described herein, including polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate 80, and triethanolamine oleate. Suitable sequestering or chelating agents include, but are not limited to EDTA. Suitable pH adjusting agents include, but are not limited to, sodium hydroxide, hydrochloric acid, citric acid, and lactic acid. Suitable complexing agents include, but are not limited to, cyclodextrins, including α -cyclodextrin, β -cyclodextrin, hydroxypropyl- β -cyclodextrin, sulfobutylether- β -cyclodextrin, and sulfobutylether 7- β -cyclodextrin (CAPTISOL®, CyDex, Lenexa, KS).

[0288] When the pharmaceutical compositions provided herein are formulated for multiple dosage administration, the multiple dosage parenteral formulations must contain an antimicrobial agent at bacteriostatic or fungi static concentrations. All parenteral formulations must be sterile, as known and practiced in the art.

[0289] In one embodiment, the pharmaceutical compositions for parenteral administration are provided as ready-to-use sterile solutions. In another embodiment, the pharmaceutical compositions are provided as sterile dry soluble products, including lyophilized powders and hypodermic tablets, to be reconstituted with a vehicle prior to use. In yet another embodiment, the pharmaceutical compositions are provided as ready-to-use sterile suspensions. In yet another embodiment, the pharmaceutical compositions are provided as sterile dry insoluble products to be reconstituted with a vehicle prior to use. In still another embodiment, the pharmaceutical compositions are provided as ready-to-use sterile emulsions.

[0290] The pharmaceutical compositions provided herein for parenteral administration can be formulated as immediate or modified release dosage forms, including delayed-, sustained, pulsed-, controlled, targeted-, and programmed-release forms.

[0291] The pharmaceutical compositions provided herein for parenteral administration can be formulated as a suspension, solid, semi-solid, or thixotropic liquid, for administration as an implanted depot. In one embodiment, the pharmaceutical compositions provided herein are dispersed in a solid inner matrix, which is surrounded by an outer polymeric membrane that is insoluble in body fluids but allows the active ingredient in the pharmaceutical compositions diffuse through.

[0292] Suitable inner matrixes include, but are not limited to, polymethylmethacrylate, polybutyl-methacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethylene terephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinyl acetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers, such as hydrogels of esters of acrylic and metliacrylic acid, collagen, cross-linked polyvinyl alcohol, and cross-linked partially hydrolyzed polyvinyl acetate.

[0293] Suitable outer polymeric membranes include but are not limited to, polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinyl acetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinyl chloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinyloxyethanol copolymer.

V. Examples

[0294] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

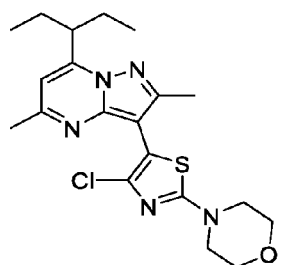
Example 1. A Phase 2, Open-Label Study to Evaluate the Efficacy and Safety of Compound 1 in Subjects with Testicular Adrenal Rest Tumors

[0295] Testicular adrenal rest tumors (TART) is a common complication in congenital adrenal hyperplasia (CAH) that is caused by adrenocorticotropin hormone (ACTH)–driven

overstimulation of aberrant adrenal cells within the testes and results in impaired spermatogenesis and infertility. TARTs can be found anywhere on the testes, though a typical (very common) location is within the mediastinum. This is a particularly problematic location. Because of the central location of TARTs being in the mediastinum, compression of the nearby seminiferous tubules is common and can lead to obstructive azoospermia, irreversible damage to surrounding tissue, and permanent infertility in males.

[0296] Current treatment for TART consists of chronic high-dose glucocorticoids, a problematic therapy with significant side effects and a narrow therapeutic window. Given the serious nature of TART and the limitations and risks of chronic steroid therapy, new treatment modalities are needed for patients with TART. Compound 1, a high-affinity and selective small-molecule antagonist of the corticotropin-releasing factor type-1 (CRF₁) receptor, may be effective in blocking ACTH synthesis, thereby reducing the size of testicular lesions in TART and reducing the need for chronic supraphysiologic doses of glucocorticoids. This mechanism of action has been validated in a previous proof-of-concept study using a similar small-molecule CRF₁ receptor antagonist, which produced dose-dependent reductions in ACTH and 17-hydroxyprogesterone (17-OHP) in subjects with CAH. Compound 1 has been shown to have an acceptable safety and tolerability profile in nonclinical toxicology studies and in 2 completed Phase 1 clinical studies in healthy volunteers. These promising results provide a rationale for investigating the use of Compound 1 in the treatment of TART, in combination with replacement glucocorticoids and mineralocorticoids.

[0297] The structure of Compound 1 is:



[0298] Building upon the 2 completed Phase 1 studies of Compound 1 in healthy volunteers and the ongoing Phase 2 proof-of-concept study of Compound 1 in subjects with CAH, this Phase 2 will study Compound 1 specifically in male CAH subjects with TART for the first time. A sample size of approximately 12 subjects was selected to provide sufficient data for an initial evaluation of efficacy and safety in subjects with TART while maintaining feasible subject recruitment goals for this rare condition. Given the rarity of the disease and thus the small sample size that can be recruited, this study will be open label, and all enrolled subjects will

receive active study drug. Subjects will serve as their own controls in that their outcome measures after 12 weeks of study drug dosing will be compared to their baseline measures.

[0299] The schedule of study visits (every 2 weeks through Week 4, then every 4 weeks for the remainder of the study) provides an appropriate balance between minimizing subject burden and closely monitoring subject safety and response to therapy. The 4-week post-treatment washout period will allow for adequate safety follow-up and analysis of any HPA axis rebound after the last dose of study drug.

[0300] The specification of study drug dosing at 10pm and after consumption of a snack is based on information from the Phase 1 studies in healthy volunteers. The plasma $C_{\max}$ (maximum concentration) for Compound 1 in the Phase 1 studies occurred approximately 5 hours after dosing. Therefore, Compound 1 will be dosed daily at 10pm in this study in order to achieve peak plasma study drug concentration at the time of day when most adrenal androgens are produced. Subjects will consume a standardized snack before taking study drug because the Phase 1 studies showed minimal study drug absorption when subjects were dosed in a fasted state. Specific dose reduction criteria, strict individual treatment stopping criteria, and appropriate laboratory and medical assessments have been established to ensure subject safety. (FIGS. 1A-1B).

Objectives	Endpoints
Primary	
<ul style="list-style-type: none"> To assess the efficacy of Compound 1 in subjects with TART 	<ul style="list-style-type: none"> Reduction in the size/volume of TART on testicular ultrasound from screening to Week 12
Secondary	
<ul style="list-style-type: none"> To evaluate the safety of Compound 1 in subjects with TART To assess the pharmacodynamic (PD) biomarker effects of Compound 1 	<ul style="list-style-type: none"> Adverse events (AEs), serious adverse events (SAEs), AEs leading to withdrawal, AEs of special interest (AESIs) Physical examination findings Vital signs Changes in electrocardiogram (ECG) Changes in clinical laboratory values Changes in plasma concentrations of key PD biomarkers, including ACTH, 17-OHP, testosterone, androstenedione, luteinizing hormone (LH),

follicle-stimulating hormone (FSH), inhibin B, sex hormone-binding globulin (SHBG), renin, and aldosterone from baseline to Week 12

- To assess changes on semen analysis
- To assess changes in metabolism
- To evaluate changes in quality of life (QoL) and mood
- Changes in sperm count, morphology, and motility from baseline to Week 12
- Changes in metabolic parameters, including fasting glucose, HbA1c, insulin sensitivity, and lipid profile (including LDL cholesterol, HDL cholesterol, and triglycerides), from baseline to Week 12
- Changes in body weight, body mass index (BMI), waist circumference, and blood pressure
- Changes in QoL measured using the Short Form 36 (SF-36)
- Changes in mood measured using the Hospital Anxiety and Depression Scale (HADS)

Study Population

[0301] Approximately 12 male subjects with TART will be enrolled at approximately 6 investigative sites in the United States, United Kingdom, and possibly other countries. Up to 12 additional subjects may be added to further evaluate specific safety, PD, or efficacy findings.

Eligibility Criteria

Inclusion Criteria

- Adult male subjects aged 18 to 30, inclusive
- Documented historical diagnosis of CAH based on documented genetic mutation or historical documentation of elevated 17-OHP
- Diagnosis of TART via testicular ultrasound and/or magnetic resonance imaging (MRI) at screening with ≥ 1 lesion(s) measuring ≥ 2 cm in diameter
- On a stable regimen of GC replacement for ≥ 30 days before baseline
- Agree to follow contraception guidelines and refrain from donating sperm throughout the treatment period and for 90 days after the last dose of study drug
- Subject is able to understand all study procedures and risks involved and provides written informed consent indicating willingness to comply with all aspects of the protocol

Exclusion Criteria

- Any clinically significant unstable medical condition, medically significant illness, or chronic disease within 30 days of screening, including but not limited to:

- A malignancy or <3 years of remission history from any malignancy, other than successfully treated localized skin cancer
 - Presence of clinically significant renal disease, as evidenced by an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73m²
 - Current or chronic history of liver disease or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones)
 - Confirmed positive test at screening for active hepatitis B, hepatitis C, or human immunodeficiency virus (HIV)
- Any clinically significant psychiatric disorders, including any current major depressive episode, bipolar disorder, schizophrenia, schizoaffective disorder, major depressive disorder with psychotic features, or any other psychotic disorder within the preceding 6 months
- HADS score >12 for either depression or anxiety
- At increased risk of suicide on the basis of the Investigator's judgment or the results of the Columbia-Suicide Severity Rating Scale (C-SSRS) conducted at screening and baseline (eg, C-SSRS Type 3, 4, or 5 ideation during the preceding 6 months or any suicidal behavior within the past 12 months)
- Clinically significant and abnormal clinical or laboratory assessments must be discussed with the Medical Monitor to determine eligibility for this study. Abnormal assessments that must be reviewed to determine eligibility include but are not limited to:
 - Clinically meaningful abnormal ECG results, in the opinion of the Investigator
 - Fridericia-corrected QT interval (QTcF) >450 msec
 - Alanine aminotransferase (ALT) >2x upper limit of normal (ULN)
 - Bilirubin >1.5x ULN (isolated bilirubin >1.5x ULN is acceptable if bilirubin is fractionated and direct bilirubin is <35%)
- A history that includes bilateral adrenalectomy or hypopituitarism
- Use of any other investigational drug within 30 days or 5 half-lives, whichever is longer, before initial screening
- Require use of prohibited concomitant medications, including strong inhibitors and/or inducers of CYP3A4 (with the exception of glucocorticoids, hydrocortisone, and dexamethasone) within 30 days or 5 half-lives (whichever is longer) of first dose of study drug. Medications that are sensitive substrates or substrates with narrow therapeutic ranges (metabolized by CYP3A4, 2C8, 2C9, or 2C19) should be discussed on a case-by-case basis with the Medical Monitor to determine whether the medication should be discontinued or may be continued with caution. If washout is feasible, then the medication should be

withdrawn at least 30 days or 5 half-lives (whichever is longer) before the first dose of study drug.

- Donation of blood within 60 days before first dose of study drug, or donation of platelets, white blood cells, or plasma within 15 days before first dose of study drug.

Study Duration

[0302] The expected duration of study participation for each subject is up to approximately 20 weeks. This includes a screening period of ≤ 4 weeks, a treatment period of 12 weeks, and a safety follow-up period of 4 weeks.

Study Design

[0303] This is a Phase 2, open-label efficacy and safety study of Compound 1 for the treatment of TART. All subjects will receive a daily oral dose of Compound 1 for 12 weeks. In-clinic study visits will occur for screening and at Day 1 (baseline) and Weeks 2, 4, 8, 12 (end of treatment), and 16 (follow-up).

Dose, Route, Regimen

[0304] All subjects will receive a daily oral dose of Compound 1 for 12 weeks. Subjects will receive 600 mg/day during the first 2 weeks and 1000 mg/day during the remaining 10 weeks of the treatment period. Study drug will be taken daily at 10pm (or bedtime, if earlier), 5 to 15 minutes after consumption of a standardized snack.

[0305] On mornings of study visits with 8am blood draws for laboratory assessments, subjects should take their morning glucocorticoid replacement after the blood draws. For all other days during the study, subjects may take their morning glucocorticoid replacement at their usual time (generally between 6am and 8am).

[0306] If a subject experiences a Grade 3 or higher TEAE considered at least possibly related to study drug, the Investigator may reduce the subject's daily dose of Compound 1 down in increments of 200 mg. Study drug will be discontinued in subjects who experience clinically significant liver chemistry, QTcF, or other individual treatment stopping criteria.

Investigational Drug

[0307] Compound 1 is a small-molecule CRF₁ receptor antagonist and will be supplied as 200-mg, size 1, white, hard gelatin capsules.

Example 2.

[0308] Since elevated ACTH is the primary driver for TART growth, blocking ACTH synthesis using Compound 1, a small-molecule antagonist of the CRF₁ receptor, may be effective in reducing ACTH levels, thereby reducing the size of testicular lesions in TART and reducing the need for chronic supraphysiologic doses of GCs. In CAH, this mechanism of action has been

validated in a previous proof-of-concept study using a similar small-molecule CRF₁ receptor antagonist, which produced dose-dependent reductions in ACTH and 17-OHP in subjects with CAH. The safety profile of Compound 1 has been established in nonclinical toxicology studies and in 2 completed Phase 1 clinical studies in healthy volunteers. A Phase 2 study in subjects with CAH is currently ongoing.

Nonclinical Toxicology of Compound 1

[0309] The nonclinical safety profile of Compound 1 has been evaluated in repeat-dose range-finding and definitive toxicology studies of up to 91 days in duration in rats and dogs using the clinically relevant route (oral) and schedule of administration (once daily). In rats and beagle dogs administered oral Compound 1 once daily for 91 consecutive days at doses up to 2000 mg/kg/day, toxicologic findings were all mild and limited to the male reproductive tract, and all Compound 1-related findings were reversible following a 6-week treatment-free recovery period.

[0310] Additionally, a fertility toxicology study was conducted in rats. In this study, male rats were administered oral Compound 1 once daily at 0, 5, 20, or 1000 mg/kg for 85 to 87 days (70 days before mating, during mating, and ≤ 4 days between mating and necropsy). Male reproductive performance (mean day of mating, mating and fertility indices, and conception rates) was unaffected by Compound 1 treatment. Untreated females mated with the Compound 1-treated males had no ovarian and uterine effects.

[0311] In addition, a complete battery of standard genotoxicity tests consisting of in vitro bacterial reverse mutation and chromosome aberration assays and an in vivo (rat) bone marrow micronucleus assay has been conducted. Exploratory and definitive embryo-fetal development studies in rabbits and a bovine corneal opacity and permeability assay have also been conducted.

Clinical Experience with Compound 1

[0312] To date, a total of 53 subjects, all healthy adults aged 21 to 65 years, have been treated with at least 1 dose of Compound 1 in 2 Phase 1 studies. The first-in-human study of Compound 1 (Study I3C-FW-BLAA) evaluated single ascending doses of 2 mg to 800 mg. In this study, a small number of subjects experienced diarrhea and headache of mild or moderate severity. A subsequent study (Study I3C-FW-BLAB) evaluated repeat doses of 50 mg, 100 mg, or 200 mg Compound 1 once daily for 14 consecutive days. A small number of subjects in the repeat-dose study experienced dyspnea, rhinorrhea, palpitations, and headache. The Phase 1 studies showed no clinically significant alterations in safety laboratory values, vital signs, or electrocardiograms (ECGs). Compound 1 has been well tolerated in healthy human subjects, with no SAEs, AEs leading to discontinuation, or dose-limiting toxicities (DLTs). A maximum tolerated dose has not been reached in humans.

[0313] An ongoing Phase 2 open-label study (Study Compound 2) is currently evaluating the safety and efficacy of Compound 1 dosed once daily for 6 weeks in adults with classic CAH. In the initial Cohort A of this study, all subjects are dosed starting at 200 mg/day for 14 days, escalating for each subject to 600 mg/day for 14 days, then 1000 mg/day for 14 days if the subject experiences no DLTs at the previous dose level. (**FIGS. 2-3**).

Known Potential Risks

[0314] Because the reversible testicular effects (degeneration/atrophy) observed in rats and dogs treated with high-dose Compound 1 (2,000 mg/kg/day) are likely associated with on-target (mechanism-driven) effects, these effects are likely to be relevant to human risk. However, male reproductive studies in rodents showed no impairment in fertility at the highest dose tested (1,000 mg/kg/day). Additionally, there were no effects on clinical laboratory markers of testicular function (LH and FSH) when Compound 1 was administered to healthy male volunteers at doses up to 200 mg per day for 14 consecutive days. Clinical laboratory measures of testicular function (LH, FSH, inhibin B, and SHBG) will be assessed throughout this study to monitor the risk of testicular injury. Testicular ultrasounds and semen analysis will also be performed at screening/baseline and the end of the treatment period.

[0315] Laboratory abnormalities in liver function tests (LFTs) have been observed at significantly higher doses in nonclinical studies. However, these events were not observed in the Phase 1 clinical studies. Clinical laboratory measures of liver chemistry will be assessed throughout this study, and strict individual treatment stopping criteria for liver chemistry findings are in place.

[0316] Compound 1-induced effects on the thyroid gland were noted in dogs after 91 days of high-dose (2,000 mg/kg/day) treatment but not in any other nonclinical studies. Clinical pathology parameters of thyroid function (eg, T4, T3, thyroid-stimulating hormone [TSH]) were not evaluated in the Phase 1 studies but will be monitored throughout this study.

Known Potential Benefits

[0317] Early clinical studies suggests that Compound 1 is effective as a CRF₁ receptor antagonist, a mechanism of action that supports the clinical investigation of Compound 1 for treatment of TART. In CAH, this mechanism of action has been validated in a previous proof-of-concept study using a similar small-molecule CRF₁ receptor antagonist, which produced dose-dependent reductions in ACTH and 17-OHP in subjects with CAH.

Assessment of Potential Risks and Benefits

[0318] The mild and reversible toxicologic effects of Compound 1 on the male reproductive tract in nonclinical studies occurred in the context of animals with normal, rather than elevated, baseline hormone function. As a CRF₁ receptor antagonist, Compound 1 is intended to reduce

abnormally elevated ACTH levels, which in TART drives overstimulation of aberrant adrenal cells within the testes. In nonclinical models where baseline hormone levels are within normal ranges, clinical benefits are not expected, and indeed, toxicities may be expected as a result of androgen reduction outside of the normal range. In TART, where baseline ACTH and androgen levels are pathologically elevated, androgen reduction would be considered a positive, therapeutic effect.

[0319] Given the serious nature of TART and the limitations (and risks) of chronic steroid therapy, new treatment modalities are needed for patients with TART. Given the acceptable safety and tolerability profile of Compound 1 in healthy volunteers from the Phase 1 program, the low probability of demonstrating meaningful PD marker changes in healthy subjects, and the lack of any clear benefit to exposing more healthy subjects to study drug, the Sponsor believes the benefit-to-risk profile favors the entry of Compound 1 into Phase 2 in subjects with TART.

Example 3. Congenital Adrenal Hyperplasia (CAH)

[0320] CAH results from a genetic defect in the 21-hydroxylase enzyme. CAH patients are thus unable to produce cortisol. Without cortisol to provide negative feedback, patients over-produce ACTH. High ACTH promotes the overproduction of potent androgens (male sex hormones). Without wishing to be bound by theory, Compound 1 blocks CRF₁ receptor to prevent ACTH release by the pituitary and associated drive on the hypothalamic-pituitary-adrenal axis. Replacement of cortisol to physiological levels will correct cortisol deficiency, however, will not lower ACTH, resulting in hyperplasia of adrenal gland and associated overproduction of adrenal androgens.

[0321] As shown in **FIGS. 4** and **5A-5B**, the attenuation of ACTH demonstrates target engagement and functional CRF₁ receptor antagonism. 80% of subjects demonstrated reduction in ACTH. 70% of subjects demonstrated > 25% reduction in ACTH. 30% of subjects were in the normal range (*) after treatment with a CRF₁ receptor antagonist.

[0322] **FIGS. 6** and **7A-7B** demonstrate “control” of disease based on standard guidelines, which would then allow steroid taper. 80% of subjects demonstrated reduction in 17-OHP. 50% of subjects demonstrated > 25% reduction in ACTH. 50% of subjects were in the guideline range (*; 1200 ng/dL) after treatment with a CRF₁ receptor antagonist.

[0323] **FIGS. 8** and **9A-9B** demonstrate attenuation of morning rise in A4 upon treatment with a CRF₁ antagonist, indicative of its ability to control excess androgen production and associated symptoms. 100% of subjects demonstrated reduction in androstenedione (at various doses). 60% of subjects demonstrated > 25% reduction in androstenedione. 50% of subjects were within the normal reference range after treatment with a CRF₁ receptor antagonist.

[0324] FIGS. 10A-10B show approximately 25% shrinkage in TART in a 25 year old male with classic congenital adrenal hyperplasia following 6 weeks treatment with a CRF₁ receptor antagonist.

[0325] FIGS. 11A-11B demonstrate Compound 1 is a potent, selective, and functional CRF₁ receptor antagonist. In in vitro and ex vivo studies, Compound 1 was determined to be a potent and selective CRF₁ receptor antagonist with high affinity and selective for the hCRF₁ receptor. Compound 1 was found to have functional potency to inhibit adenylate cyclase activity (CRF₁ receptor-mediated downstream cascade), demonstrating in vitro efficacy as a CRF₁ receptor antagonist. Compound 1 was found to significantly inhibit CRF-induced locomotor behavior in response to i.c.v. administration of CRF (FIG. 11B).

[0326] FIGS. 12A-12B demonstrate single pill of CRF₁ receptor antagonist improves ACTH and 17-OHP in CAH patients.

[0327] FIG. 13 shows the subject level hormone changes (ACTH, 17-OHP, and androstenedione) by dose of a CRF₁ receptor antagonist.

Example 4. Assess reduction in excess ACTH and adrenal steroid production

[0328] The objectives of the study are: to assess the ability of Compound 1 to reduce excess ACTH and adrenal steroid production in participants with classic CAH on a stable glucocorticoid regimen; to evaluate the safety and tolerability of tildacerfont in participants with classic CAH; and to evaluate the pharmacokinetics (PK) of tildacerfont.

ELIGIBILITY CRITERIA

[0329] Inclusion Criteria:

- Adult males and females 18 years or older
- Diagnosis of classic CAH due to 21-hydroxylase deficiency
- Morning 17OHP $\geq$ 800 ng/dL, before taking any morning GC dose
- On a stable regimen of glucocorticoid replacement for $\geq$ 30 days before enrollment

[0330] Exclusion Criteria:

- Clinically significant unstable medical condition, illness, or chronic disease within 30 days of screening
- Clinically significant psychiatric disorder
- History of bilateral adrenalectomy or hypopituitarism

STUDY DESIGN

[0331] FIG. 14 depicts the proof of concept and escalating QD dosing at 10 PM for Cohort A and the single dose level and BID dosing at 10 AM and 10 pm for Cohort B.

BASELINE CHARACTERISTICS

[0332] Table 1 provides a description of the baseline characteristics displayed by Cohort A and Cohort B. In Cohort A, 10 participants were enrolled and received at least 1 dose of Compound 1. One participant withdrew early because of an occupation-related scheduling conflict. In Cohort B, 9 participants were enrolled and received at least one dose of Compound 1. One participant was withdrawn upon discovery of a major protocol violation at Baseline and did not have postbaseline efficacy/PK data. Thus, baseline characteristics are calculated for n=8 in Cohort B. One participant in Cohort B was taking both HC and prednisone.

Table 1 Baseline characteristics of each cohort

Parameter	Cohort A (n=10)	Cohort B (n=8)
Demographics		
Age (years), mean (range)	39.3 (24-66)	36.8 (19-66)
Sex	5M, 5F	1M, 7F
Bmi (kg/m ²), mean $\pm$ SD	31.5 $\pm$ 12.0	31.1 $\pm$ 6.7
Daily GC dose (mg HC equivalents), mean range	28 (13-40)	35 (20-50)
Types of GCs	4 JC, 3 pred, 3 dex	2HC, 4 pred, 3 dex
17OHP at screening ^a (ng/dL), mean $\pm$ SD	5,985 $\pm$ 6,347	4,592 $\pm$ 4,624

Abbreviations: 17OHP: 17-hydroxyprogesterone; BMI: body mass index; dex: dexamethasone; F: female; GC: glucocorticoid; HC: hydrocortisone; M: male; pred: prednisone; SD: standard deviation.
^a 17OHP reference ranges: 27-199 ng/dL males, 15-290 ng/dL females.

SAFETY

[0333] Across both cohorts, Compound 1 was generally well tolerated. The most common type of adverse event (AE) was gastrointestinal disorders. There were no serious AEs and no AEs leading to withdrawal.

PHARMACOKINETICS

[0334] Over a wide range of doses (200 to 1000 mg QD), Compound 1 exhibited a predictable, dose-dependent PK profile.

EFFICACY

[0335] FIG. 15A-15D show the changes in levels of Androstenedione (A4) across both cohorts. Levels of A4 improved at ≥ 1 dose level for 10 out of 10 participants in Cohort A and 6 out of 8 participants in Cohort B. Excess A4 is associated with clinical sequelae in CAH. Attenuation of A4 demonstrates control of adrenal androgen.

[0336] FIG. 16A-16D show the changes in levels of 17-hydroxyprogesterone (17-OHP) across both cohorts. Levels of 17OHP improved at ≥ 1 dose level for 8 out of 10 participants in Cohort A and 6 out of 7 participants in Cohort B. 17OHP is a critical hormone in CAH diagnosis and management. Attenuation of 17OHP demonstrates control of adrenal steroid production.

[0337] FIG. 17A-17D show the changes in levels of adrenocorticotrophic hormone (ACTH) across both cohorts. Levels of ACTH improved at ≥ 1 dose level for 8 out of 10 participants in

Cohort A and 5 out of 7 participants in Cohort B. Attenuation of ACTH demonstrates target engagement and functional CRF₁ receptor antagonism.

Table 2 – Percentage change from baseline in ACTH, 17OHP and A4.

	Cohort A			Cohort B
	200 mg QD (Week 2)	600 mg QD (Week 4)	1000 mg QD (Week 6)	200 mg BID (Week 2)
ACTH	-25.7 (±51.6)	-60.1 (±37.5)	-32.7 (±47.0)	-36.5 (±58.0)
17OHP	+22.2 (±172.3)	-23.7 (±64.2)	-13.8 (±74.7)	-47.8 (±63.3)
A4	-12.1 (±47.4)	-33.2 (±36.5)	-28.6 (±44.6)	-16.0 (±81.4)

CONCLUSIONS

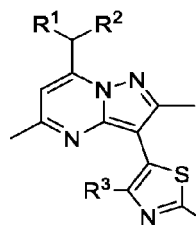
[0338] Compound 1, a potent and selective CRF₁ receptor antagonist, offers a novel nonsteroidal oral treatment approach for CAH. Compound 1 was well tolerated and efficacious in reducing excess ACTH, 17OHP, and androstenedione in patients with classic CAH. Compound 1 exhibited a predictable, dose-dependent PK profile suitable for the target population.

[0339] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF₁) antagonist or a pharmaceutically acceptable salt thereof.
2. The method of claim 1, wherein the subject has congenital adrenal hyperplasia (CAH).
3. The method of claim 1 or 2, wherein the size and/or number of the tumors is decreased or reduced.
4. A method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a compound of structural Formula (I):



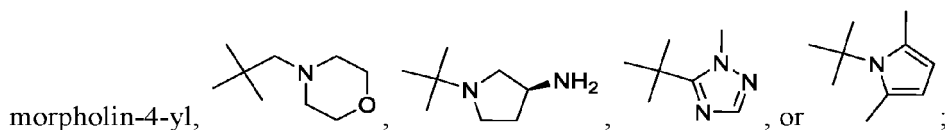
(I), or a pharmaceutically acceptable salt thereof,

wherein:

R¹ and R² are independently ethyl or n-propyl;

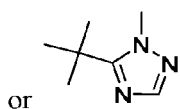
R³ is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R⁴ is hydrogen, Br, R^aR^bN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl,



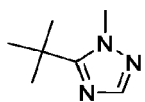
R^a and R^b are independently hydrogen, C₁-C₃alkyl, H₂NCH₂CH₂-, (CH₃)₃COC(O)NHCH₂CH₂-, or CH₃CH₂CH₂NHCH₂CH₂-.

5. The method of claim 4, wherein R³ is Cl, Br, methyl, or trifluoromethyl.
6. The method of claim 4, wherein R³ is Cl, Br, or methyl.
7. The method of claim 4, wherein R⁴ is Br, R^aR^bN-, pyridin-4-yl, morpholin-4-yl,



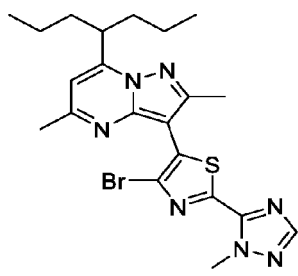
or

8. The method of claim 4, wherein R⁴ is morpholin-4-yl or



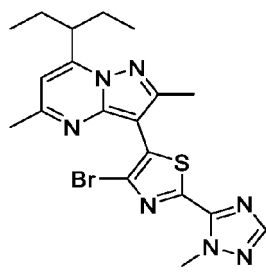
9. The method of claim 4, wherein R^4 is hydrogen, Br, R^aR^bN- and R^a and R^b are independently C_1 - C_3 alkyl.

10. The method of claim 4, wherein the compound is



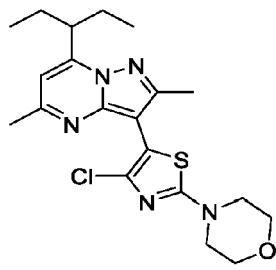
, or a pharmaceutically acceptable salt thereof.

11. The method of claim 4, wherein the compound is



, or a pharmaceutically acceptable salt thereof.

12. The method of claim 4, wherein the compound is



, or a pharmaceutically acceptable salt thereof.

13. The method of claim 4, wherein the subject has congenital adrenal hyperplasia (CAH).

14. The method of claim 4 or 13, wherein the size and/or number of the tumors is decreased or reduced.

15. The method of any one of claims 4-14, wherein from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

16. The method of any one of claims 4-15, wherein from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

17. The method of any one of claims 4-15, wherein from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

18. The method of any one of claims 4-15, wherein from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

19. The method of any one of claims 4-15, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 5 mg/day to about 1000 mg/day is administered to the subject.

20. The method of claim 19, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose from about 5 mg/day to about 25 mg/day is administered to the subject.

21. The method of claim 19, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose from about 10 mg/day to about 400 mg/day is administered to the subject.

22. The method of claim 19, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 100 mg/day to about 600 mg/day is administered to the subject.

23. The method of any one of claims 4-22, wherein the compound is administered orally.

24. The method of any one of claims 4-23, wherein the compound is formulated as a capsule or tablet.

25. The method of any one of claims 4-24, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fed state.

26. The method of any one of claims 4-24, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fasted state.

27. The method of any one of claims 4-26, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at least once per day.

28. The method of any one of claims 4-27, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at bedtime.

29. The method of any one of claims 4-27, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 4 hours before sleep.

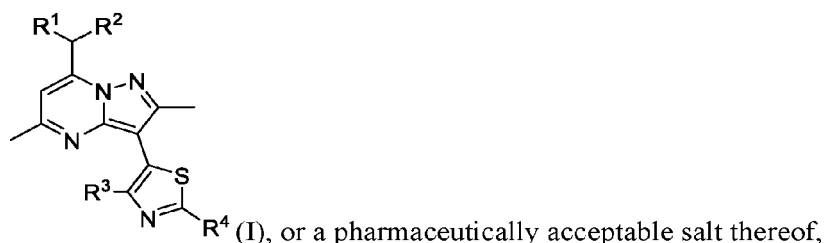
30. The method of any one of claims 4-27, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 2 hours before sleep.

31. The method of any one of claims 4-27, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 30 minutes before sleep.

32. The method of any one of claims 4-27, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the evening.

33. The method of any one of claims 4-27, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at about 10 pm at night.

34. A method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a pharmaceutical composition, comprising a compound of structural Formula (I) and a pharmaceutically acceptable excipient:

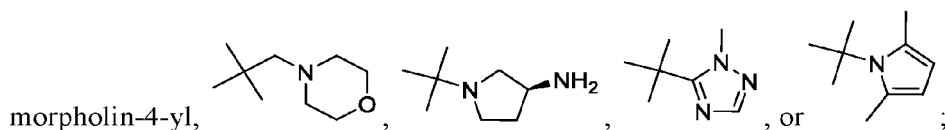


wherein:

R^1 and R^2 are independently ethyl or n-propyl;

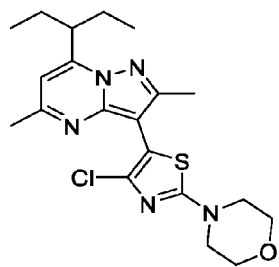
R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R^4 is hydrogen, Br, $R^a R^b N-$, methoxymethyl, n-butyl, acetamido, pyridin-4-yl,



R^a and R^b are independently hydrogen, C_1 - C_3 alkyl, $H_2NCH_2CH_2-$, $(CH_3)_3COC(O)NHCH_2CH_2-$, or $CH_3CH_2CH_2NHCH_2CH_2-$.

35. The method of claim 31, wherein the compound of Formula (I) has the structure:



, or a pharmaceutically acceptable salt thereof.

36. The method of claim 31 or 32, wherein the pharmaceutical composition comprises from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

37. The method of any one of claims 34-36, wherein the pharmaceutical composition comprises from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

38. The method of any one of claims 34-36, wherein the pharmaceutical composition comprises from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

39. The method of any one of claims 34-36, wherein the pharmaceutical composition comprises from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

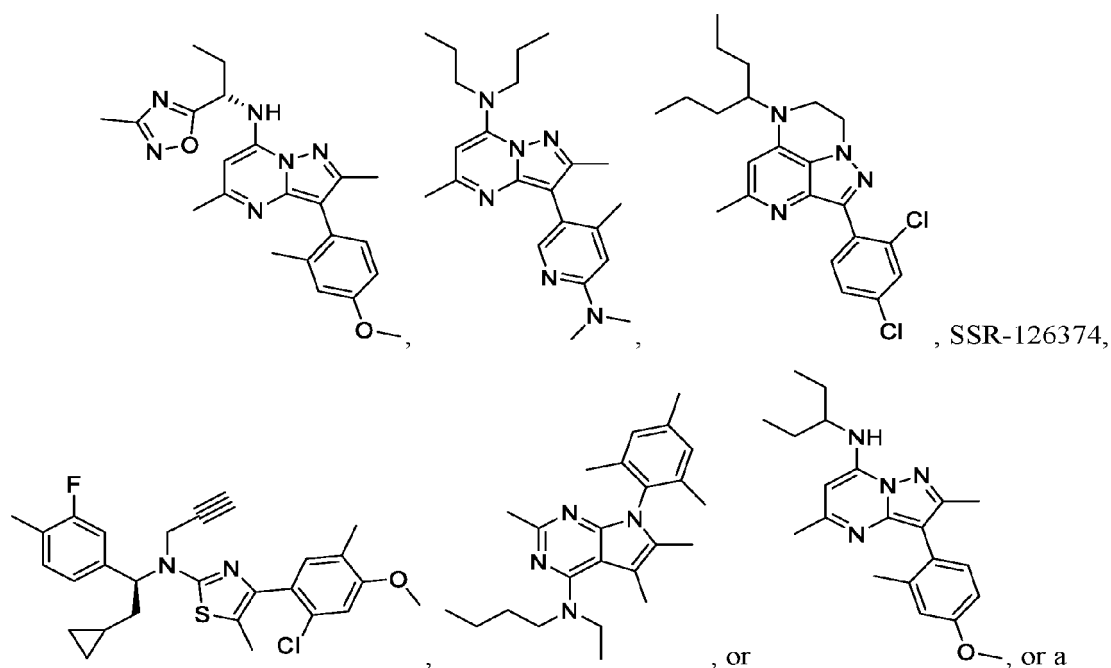
40. The method of any one of claims 34-36, wherein the pharmaceutical composition comprises about 5 mg, about 10 mg, about 15 mg, about 20 mg, 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg about 500 mg, or about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

41. The method of any one of claims 34-36, wherein the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 1000 mg/day.

42. The method of claim 41, wherein the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 25 mg/day.

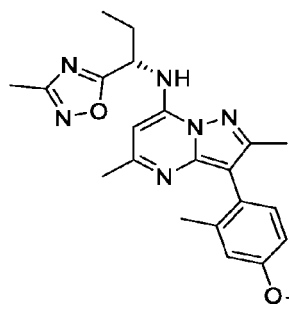
43. The method of claim 41, wherein the pharmaceutical composition is administered at a dose of from about 10 mg/day to about 400 mg/day.

44. The method of claim 41, wherein the pharmaceutical composition is administered at a dose of from about 100 mg/day to about 600 mg/day.
45. The method of any one of claims 34-44, wherein the pharmaceutical composition is administered orally.
46. The method of any one of claims 34-45, wherein the pharmaceutical composition is formulated as capsule or a tablet.
47. The method of any one of claims 34-46, wherein the pharmaceutical composition is administered in the fed state.
48. The method of any one of claims 34-46, wherein the pharmaceutical composition is administered in the fasted state.
49. The method of any one of claims 34-48, wherein the pharmaceutical composition is administered at least once per day.
50. The method of any one of claims 34-49, wherein the pharmaceutical composition is administered at bedtime.
51. The method of any one of claims 34-50, wherein the pharmaceutical composition is administered less than about 4 hours before sleep.
52. The method of any one of claims 34-51, wherein the pharmaceutical composition is administered less than about 2 hours before sleep.
53. The method of any one of claims 34-52, wherein the pharmaceutical composition is administered less than about 30 minutes before sleep.
54. The method of any one of claims 34-53, wherein the pharmaceutical composition is administered in the evening.
55. The method of any one of claims 34-54, wherein the pharmaceutical composition is administered at about 10 pm at night.
56. The method of any one of claims 1-55, further comprising administering to the subject an additional chemotherapeutic agent.
57. The method of claim 56, wherein the additional chemotherapeutic agent is a glucocorticoid, a mineralocorticoid, an ACAT1 inhibitor, or an anti-androgen.
58. The method of claim 57, wherein the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone.
59. The method of claim 57, wherein the mineralocorticoid is fludrocortisone.
60. The method of claim 56, wherein the additional chemotherapeutic agent is another CRF₁ antagonist.
61. The method of claim 60, wherein the CRF₁ antagonist is



pharmaceutically acceptable salt thereof.

62. The method of claim 61, wherein the CRF₁ antagonist is



, or a pharmaceutically acceptable salt thereof.

63. The method of any one of claims 1-62 further comprising an additional treatment selected from surgical resection of the tumors and radiation therapy, or a combination thereof.

64. The method of claim 63, wherein the additional therapy is surgical resection and the surgical resection is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof.

65. The method of claim 64, wherein the surgical resection is prior to administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

66. The method of claim 65, wherein the surgical resection is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

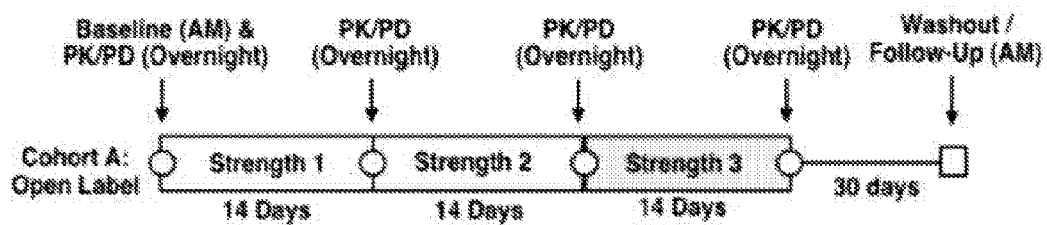
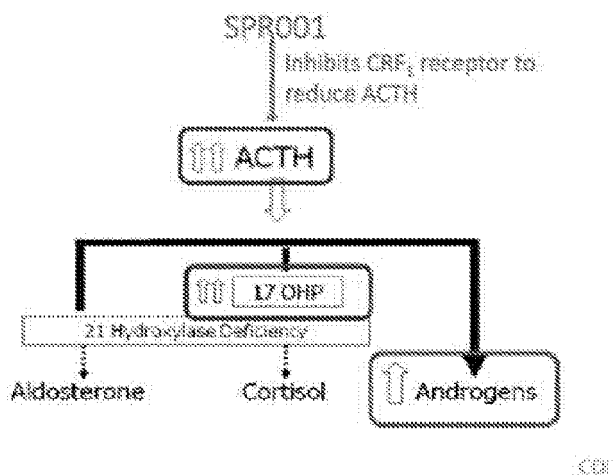
67. The method of claim 66, wherein the surgical resection is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

68. The method of claim 63, wherein the additional treatment is radiation therapy and the radiation therapy is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof.

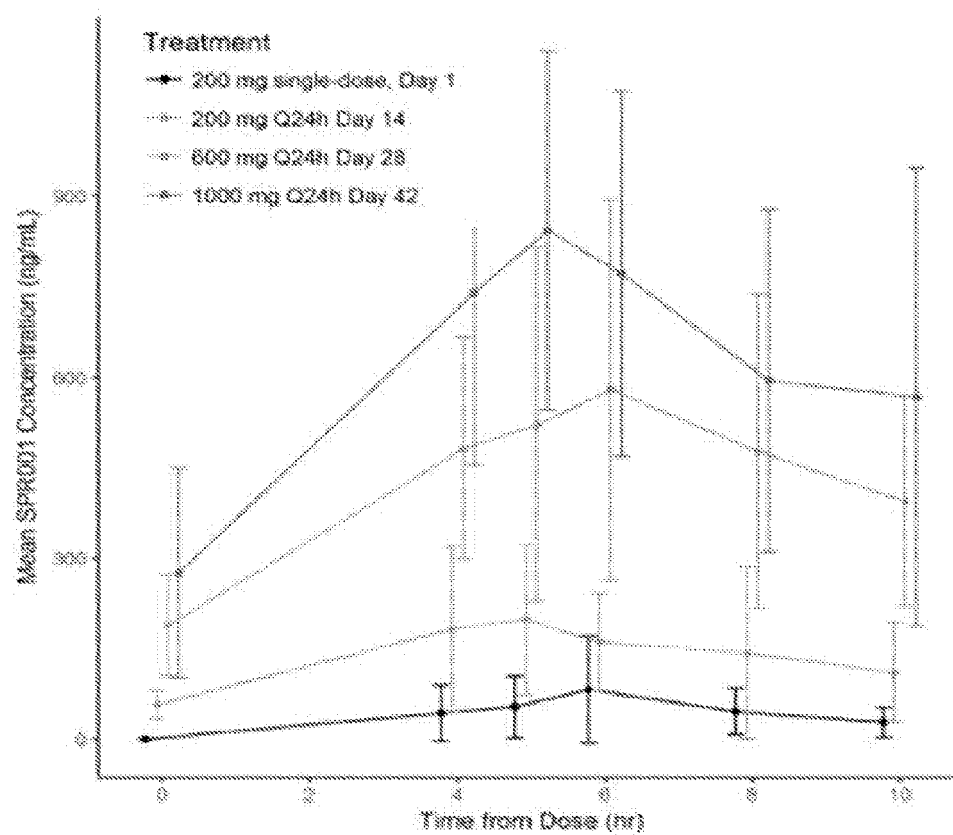
69. The method of claim 68, wherein the radiation therapy is prior to administration of the CRF₁ antagonist of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

70. The method of claim 68, wherein the radiation therapy is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

71. The method of claim 68, wherein the radiation therapy is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

**FIG. 1A****FIG. 1B**

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**FIG. 2**

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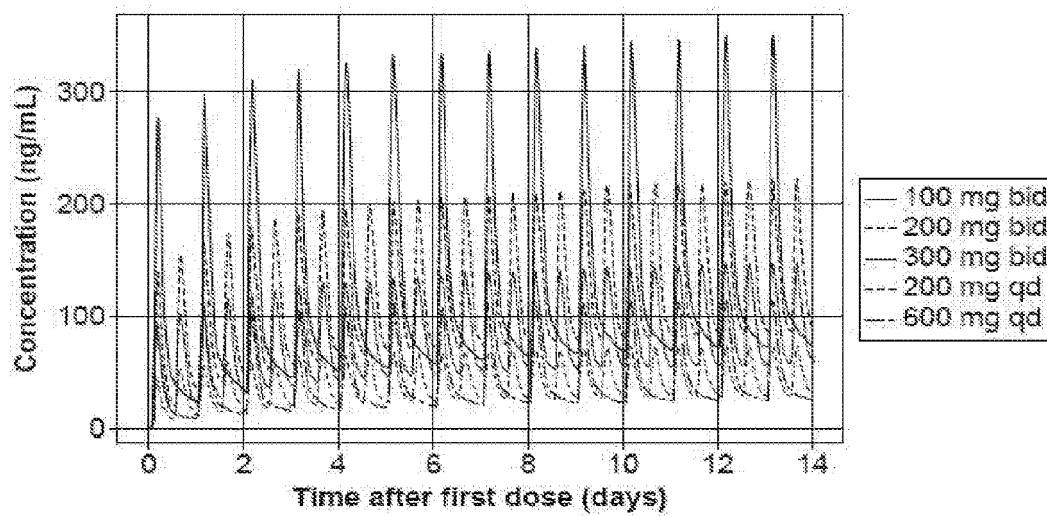


FIG. 3

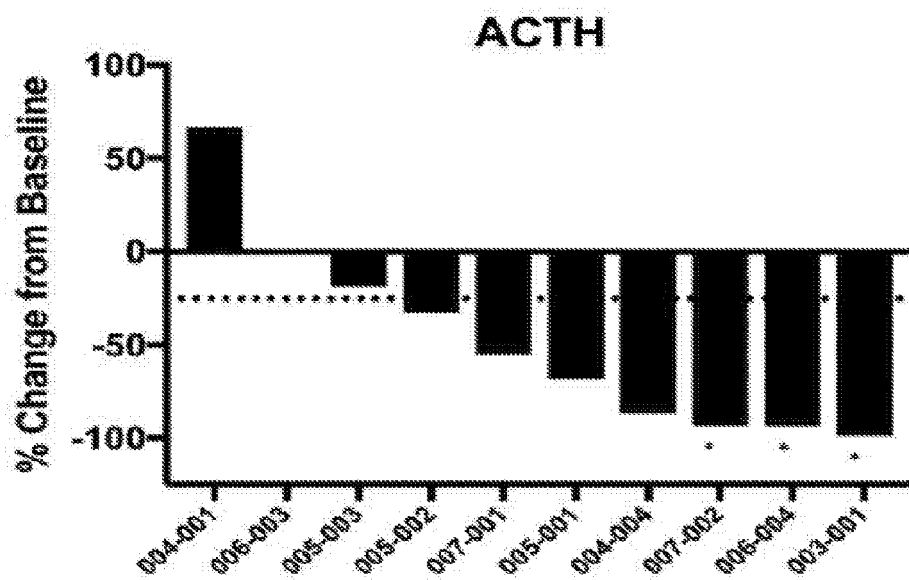


FIG. 4

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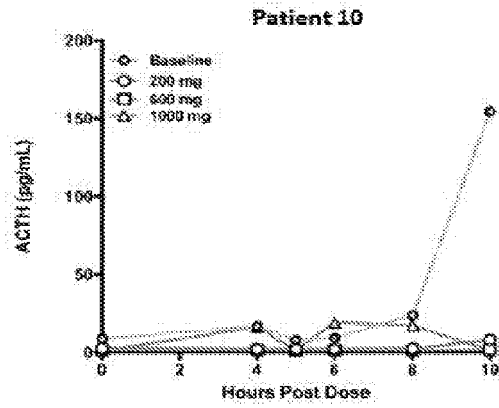


FIG. 5A

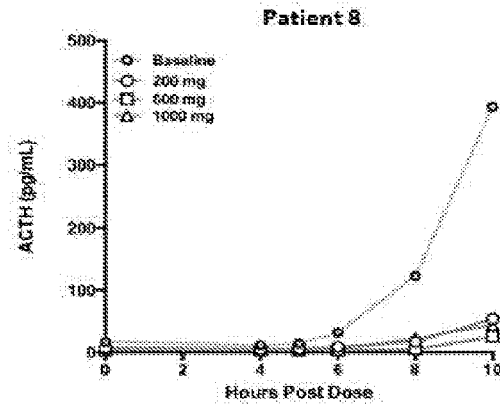


FIG. 5B

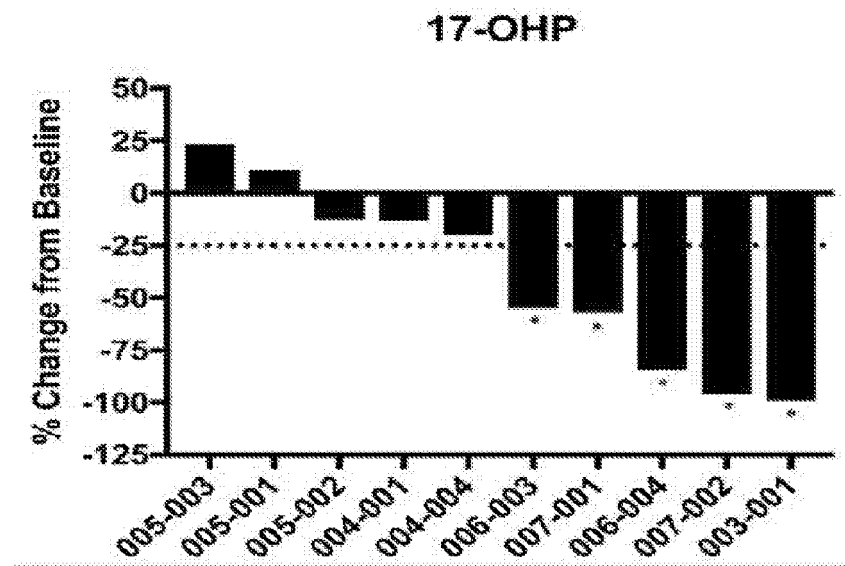
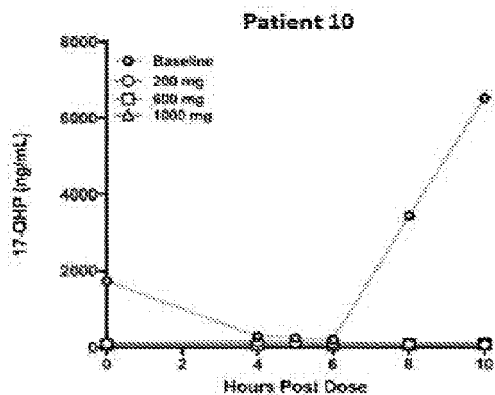
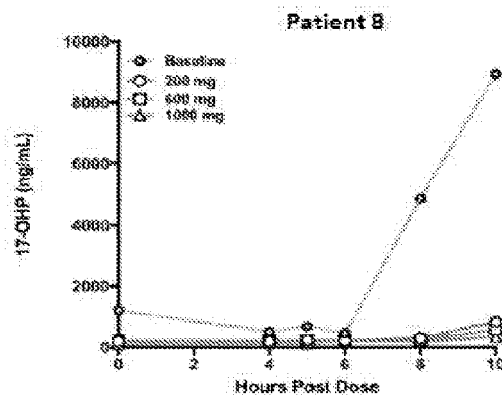
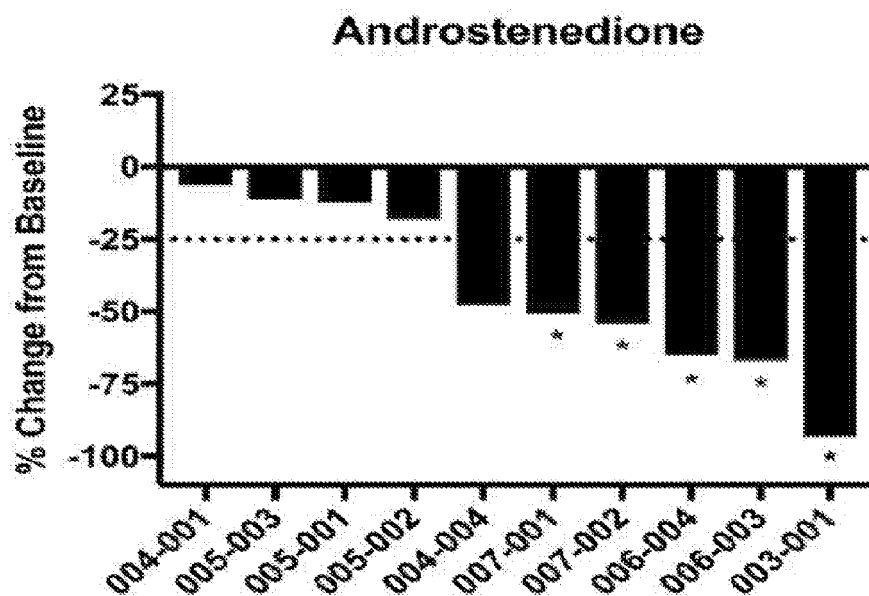


FIG. 6

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**FIG. 7A****FIG. 7B****FIG. 8**

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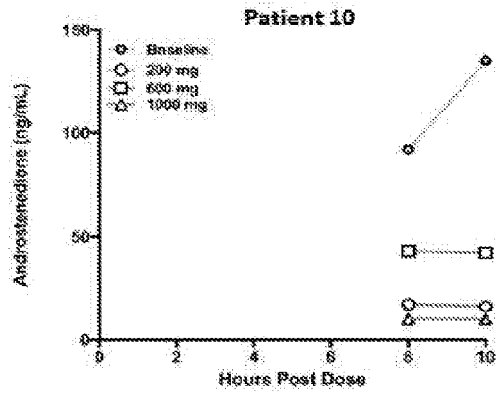


FIG. 9A

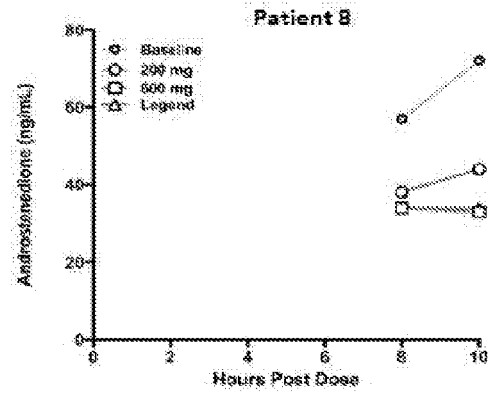


FIG. 9B

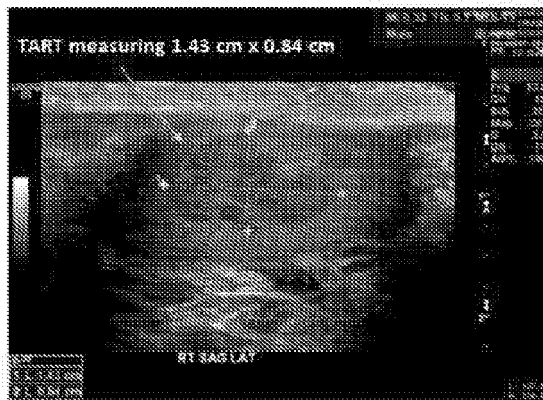


FIG. 10A

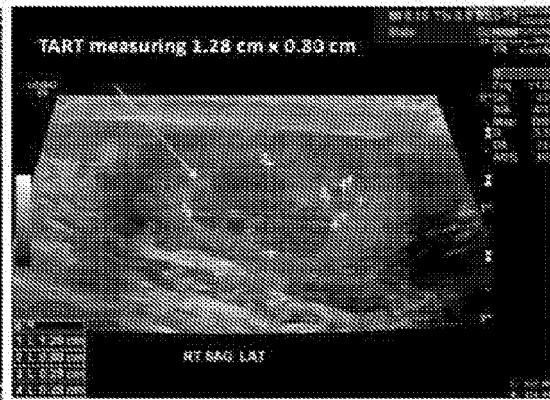


FIG. 10B

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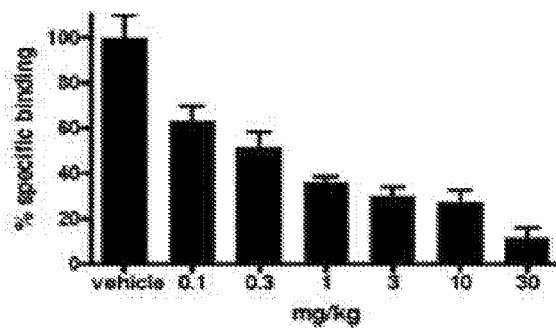
In Vitro Binding Assay

FIG. 11A

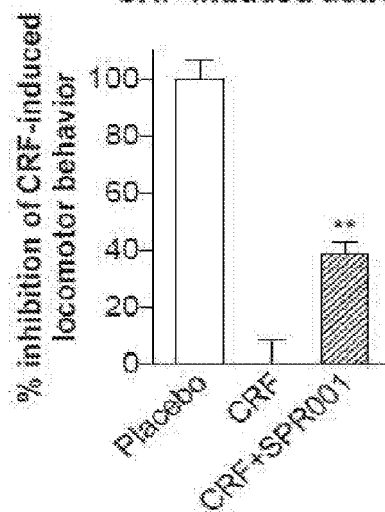
In vivo inhibition of
CRF-induced activity

FIG. 11B

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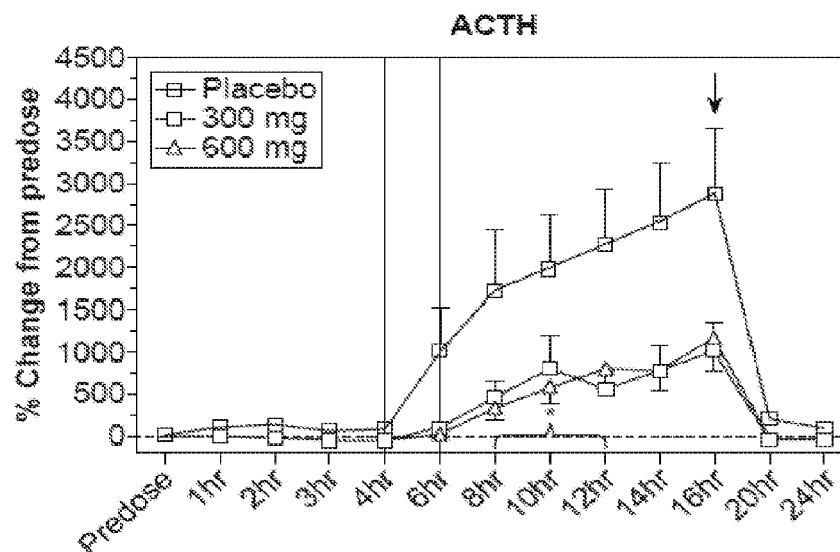


FIG. 12A

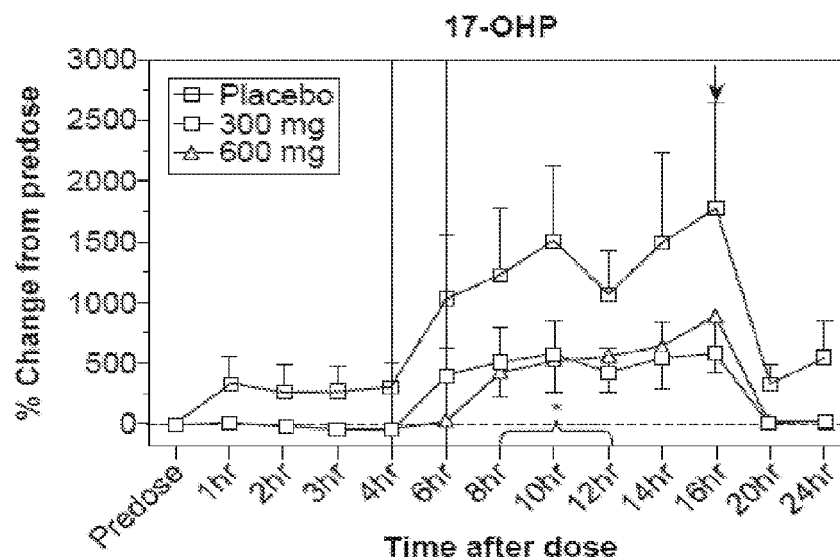


FIG. 12B

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SPR001-201 Hormone responses

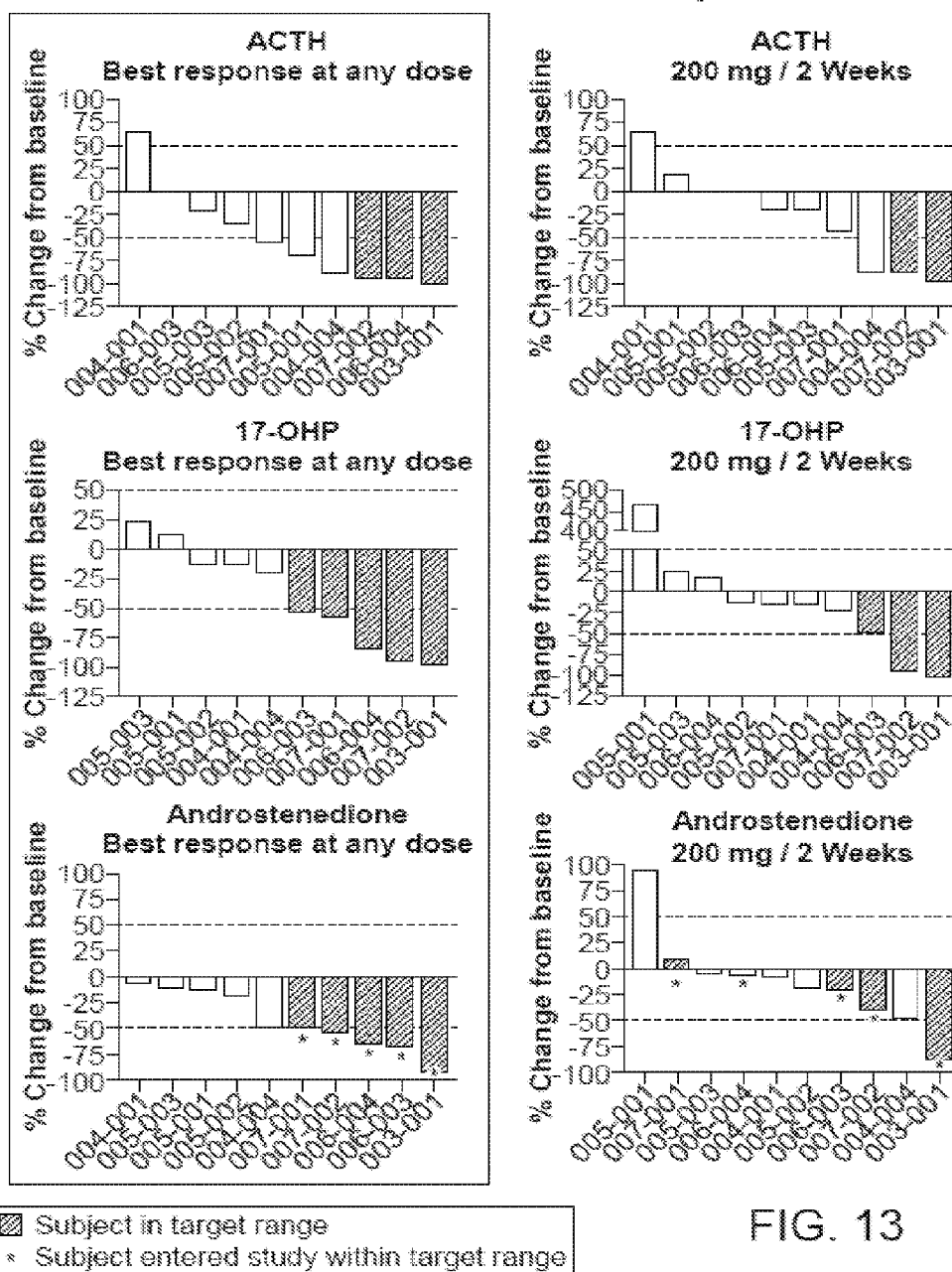


FIG. 13

ACTH: 7.2-63.3 pg/mL 17-OHP: <1200 ng/dL A4: 41-262 ng/dL (M) 27-152 ng/dL (F)

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SPR001-201 Hormone responses

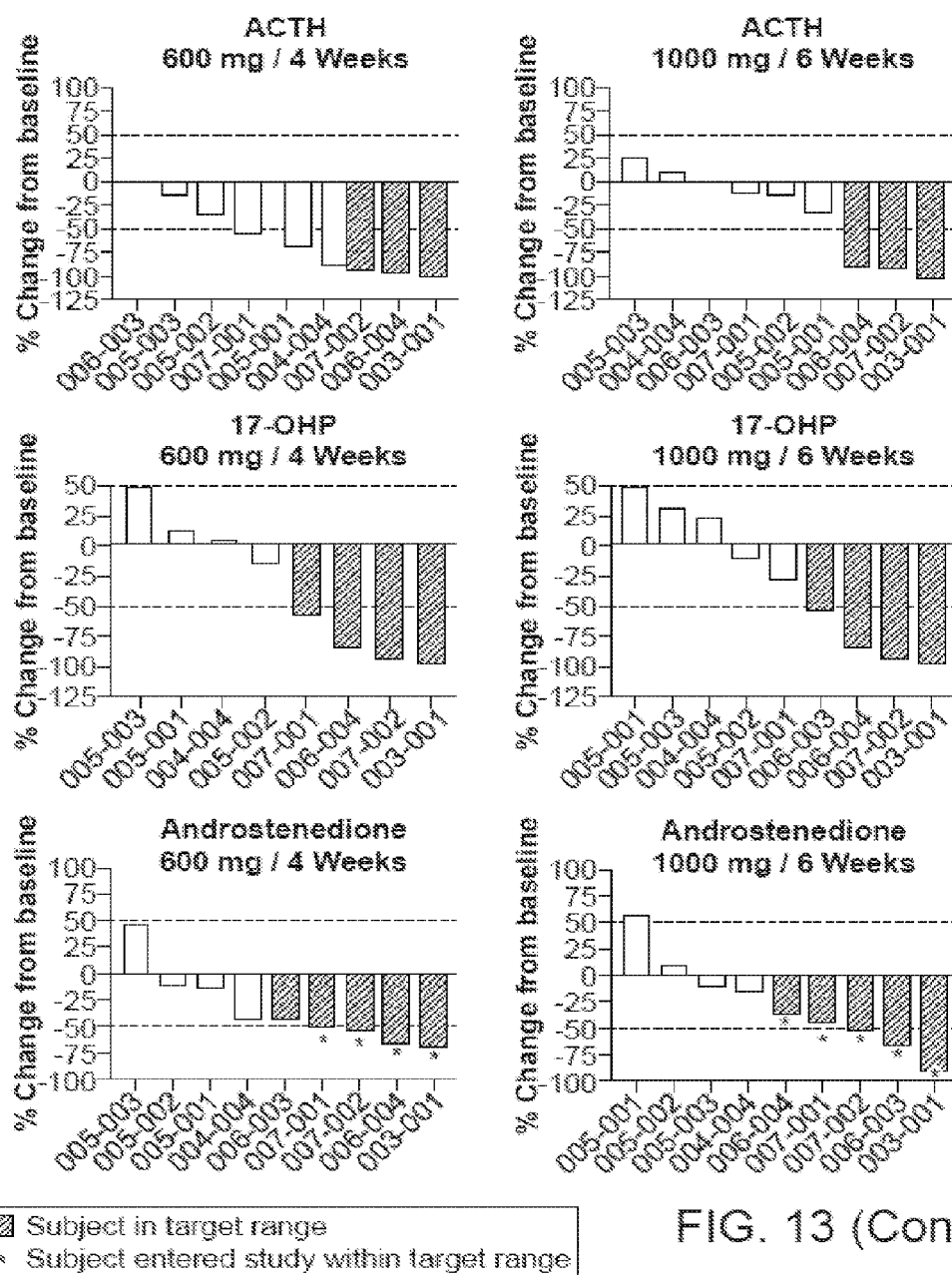


FIG. 13 (Cont.)

ACTH: 7.2-63.3 pg/mL 17-OHP: <1200 ng/dL A4: 41-262 ng/dL (M) 27-152 ng/dL (F)

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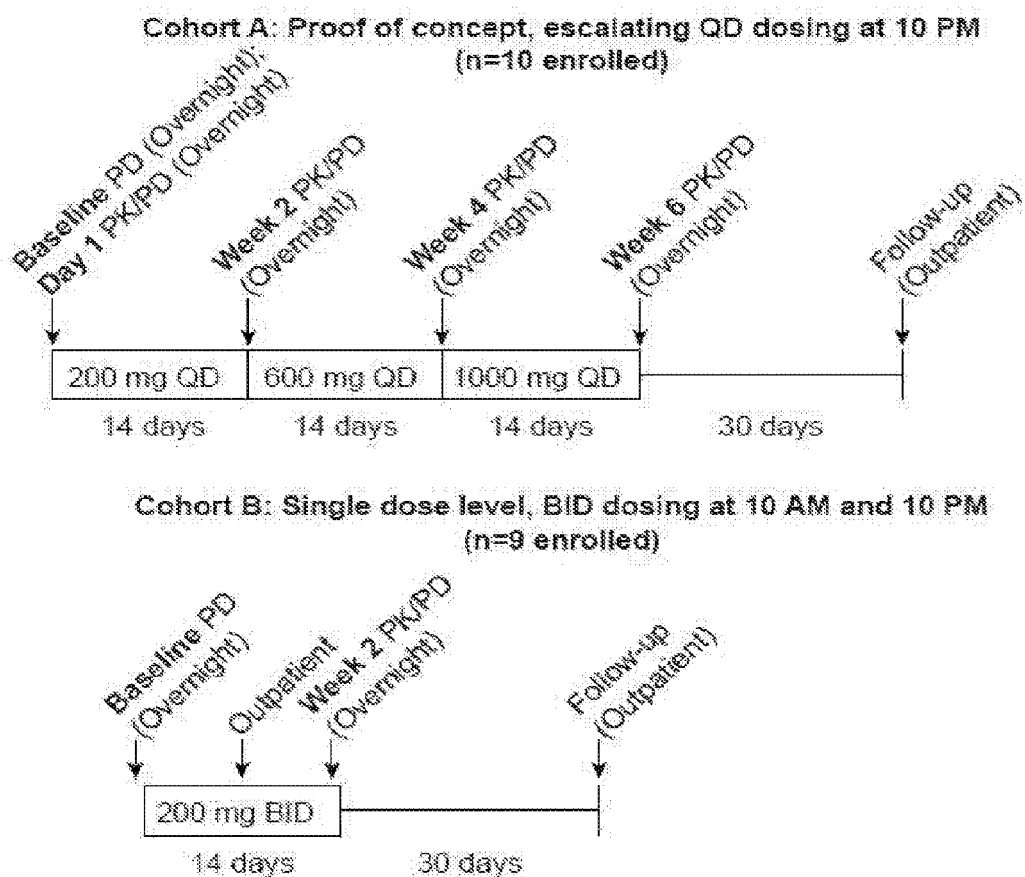
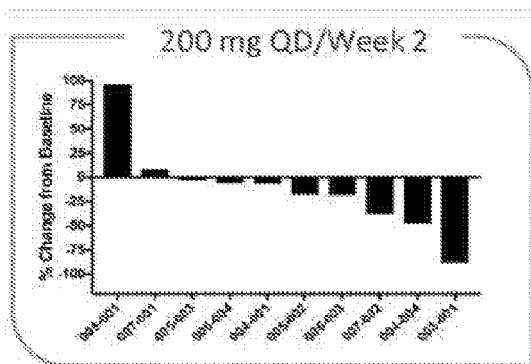
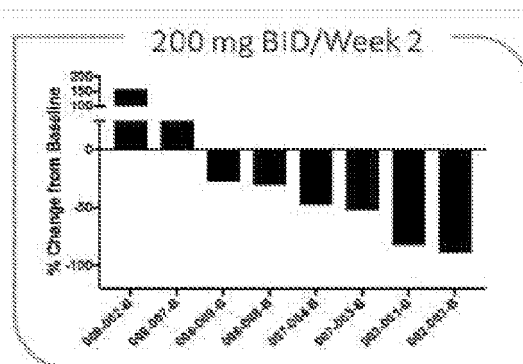
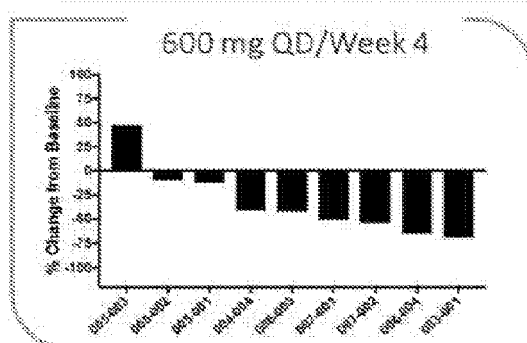
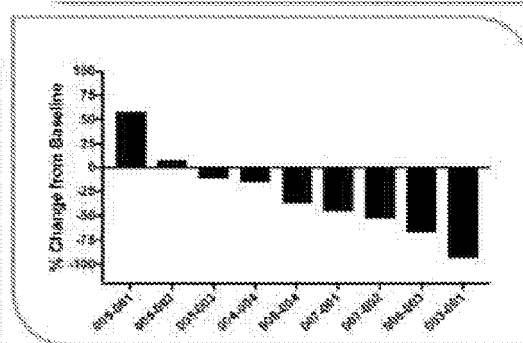


FIG. 14

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**FIG. 15A****FIG. 15B****FIG. 15C****FIG. 15D**

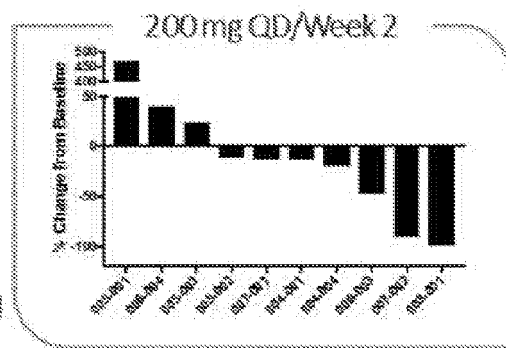


FIG. 16A

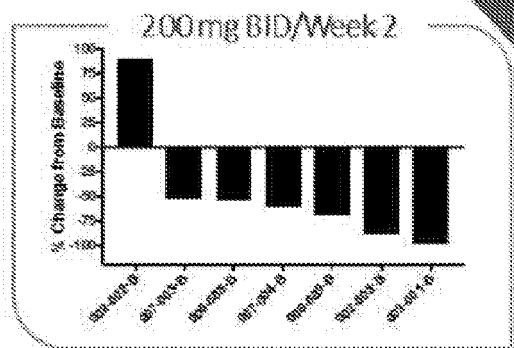


FIG. 16B

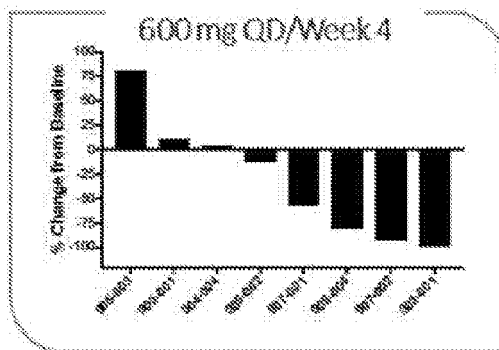


FIG. 16C

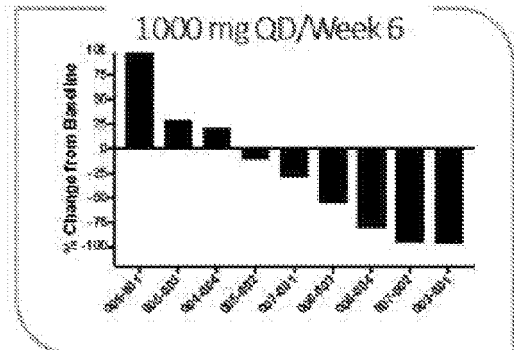
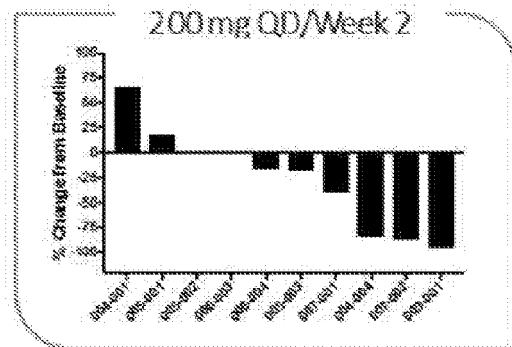
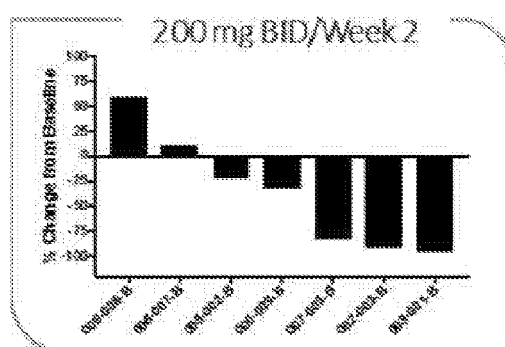
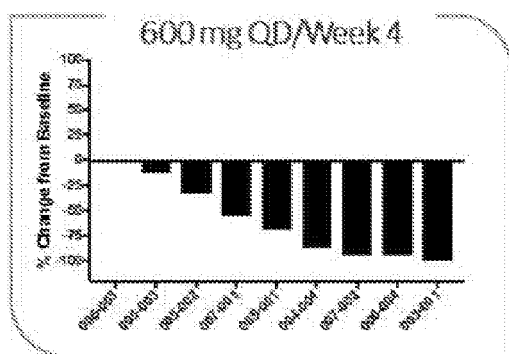
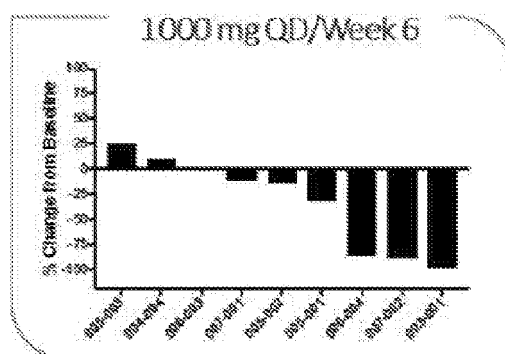


FIG. 16D

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**FIG. 17A****FIG. 17B****FIG. 17C****FIG. 17D**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/029486

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/4162; A61K 31/505; A61K 31/519; C07D 231/54; C07D 233/70; C07D 247/02 (2019.01)

CPC - A61K 31/4162; A61K 31/505; A61K 31/519; C07D 231/54; C07D 233/70; C07D 247/02 (2019.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 514/228.8; 514/230.2; 514/230.5 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2017/0020877 A1 (NEUROCRINE BIOSCIENCES, INC.) 26 January 2017 (26.01.2017) entire document	1-4, 9, 13, 14, 34
Y	US 2010/0222339 A1 (CHEN et al) 02 September 2010 (02.09.2010) entire document	1-4, 9, 13, 14, 34
Y	US 2010/0196389 A1 (EVANS-FREKE) 05 August 2010 (05.08.2010) entire document	3, 14

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 July 2019

Date of mailing of the international search report

12 AUG 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/029486

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 15-33, 35-71
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 9, 13, 14, 34

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/029486

Continued from Box No. III Observations where unity of invention is lacking

Claims 1-4, 9, 13, 14, and 34 have been analyzed subject to the restriction that the claims read on a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; and R4 is hydrogen.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-14 and 34 are drawn to methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART).

The first invention of Group I+ is restricted to a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; R4 is hydrogen; and methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART). It is believed that claims 1-4, 9, 13, 14, and 34 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is Br; R4 is hydrogen; and methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART). Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables R1, R2, R3, R4, and accordingly these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a compound of structural Formula (I); and a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a pharmaceutical composition, comprising a compound of structural Formula (I) and a pharmaceutically acceptable excipient, these shared technical features do not represent a contribution over the prior art as disclosed by US 2017/0020877 A1 to Neurocrine Biosciences, Inc. and US 2010/0222339 A1 to Chen et al.

US 2017/0020877 A1 to Neurocrine Biosciences, Inc. teaches a method of treating or preventing testicular adrenal rest tumors (TART) (Para. [0044]),...glucocorticoid treatment include cortisol replacement and suppression of ACTH to prevent virilization and menstrual disturbances in women and to inhibit testicular adrenal rest tumors in men.; Para. [0047], Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids...; Claim 1, A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF1 receptor antagonist...), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist (Claim 1; Para. [0007]); and a method of treating or preventing testicular adrenal rest tumors (TART) (Paras. [0044] and [0047]; Claim 1), comprising administering to a subject in need thereof a pharmaceutical composition (Paras. [0061] and [0065]). The pharmaceutical compositions described herein that comprise at least one of the CRF1 antagonist compounds described herein may be administered to a subject in need by any one of several routes that effectively deliver an effective amount of the compound.; Claim 1).

US 2010/0222339 A1 to Chen et al., in the field of treating congenital adrenal hyperplasia (Para. [0014]), teach a corticotropin-releasing factor type-1 (CRF1) antagonist (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)- 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine; Para. [0057], The compounds of Formula I are CRF-1 antagonists and, as such, are useful for treating a condition which is treatable by reducing CRF1 receptor tone or stimulation.); a compound of structural Formula (I) wherein R1 and R2 are independently ethyl; R3 is Br; and R4 is Br (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)- 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine); and a pharmaceutical composition, comprising a compound of structural Formula (I) (Claim 10, A pharmaceutical composition comprising: a compound according to claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.; (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)- 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: SIMREN DELANEY
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

(PCT Article 17(3)(a) and Rules 40.1 and 40.2(e))

Date of mailing
(day/month/year)

04 JUN 2019

Applicant's or agent's file reference
50535-710601

PAYMENT DUE within **ONE MONTH** from
the above date of mailing

International application No.
PCT/US2019/029486

International filing date
(day/month/year) 26 April 2019

Applicant **SPRUCE BIOSCIENCES, INC.**

1. This International Searching Authority

(i) considers that there are 1+ (number of) inventions claimed in the international application covered by the claims indicated below/on an extra sheet:
see Extra Sheet(s)

(ii) therefore considers that **the international application does not comply with the requirement of unity of invention** (Rules 13.1, 13.2 and 13.3) for the reasons indicated below/on an extra sheet:
see Extra Sheet(s)

(iii) ☐ has carried out a partial international search (see Annex) ☒ will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:
1-4, 9, 13, 14, 34

(iv) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid.

2. Consequently, the applicant is hereby **invited to pay**, within the time limit indicated above, additional fees in the amount indicated below:

\$2,080.00 x 1+ = \$2,080.00+ (See Item 2 of annex)
Fee per additional invention number of additional inventions total amount of additional fees/currency

3. The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fees may be made under protest**, that is, a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fees is excessive, where applicable, subject to the payment of a protest fee.

Where the applicant pays additional fees under protest, the applicant is hereby invited, within the time limit indicated above, to pay a protest fee (Rule 40.2(e)) in the amount of _____ (amount/currency)

Where the applicant has not, within the time limit indicated above, paid the required protest fee, the protest will be considered not to have been made and the International Searching Authority will so declare.

4. ☒ Claim(s) Nos. 15-33, 35-71 have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

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Form PCT/ISA/206 (April 2005)

INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

International application No.
PCT/US2019/029486

Item 2 (continued). For International Applications filed on or after 01 January 2014, Applicant is reminded that the search fee per additional invention indicated in item 2 is the undiscounted fee per additional invention. An Applicant may pay the search fee per additional invention fee reduced by 50% (small entity assertion) or 75% (micro entity certification), as appropriate. See 37 CFR 1.27 and 1.29.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-14 and 34 are drawn to methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART).

The first invention of Group I+ is restricted to a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; R4 is hydrogen; and methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART). It is believed that claims 1-4, 9, 13, 14, and 34 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is Br; R4 is hydrogen; and methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART). Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables R1, R2, R3, R4, and accordingly these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof; a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a compound of structural Formula (I); and a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a pharmaceutical composition, comprising a compound of structural Formula (I) and a pharmaceutically acceptable excipient, these shared technical features do not represent a contribution over the prior art as disclosed by US 2017/0020877 A1 to Neurocrine Biosciences, Inc. and US 2010/0222339 A1 to Chen et al.

US 2017/0020877 A1 to Neurocrine Biosciences, Inc. teaches a method of treating or preventing testicular adrenal rest tumors (TART) (Para. [0044]), glucocorticoid treatment include cortisol replacement and suppression of ACTH to prevent virilization and menstrual disturbances in women and to inhibit testicular adrenal rest tumors in men.; Para. [0047], Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids...; Claim 1, A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF1 receptor antagonist..., comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist (Claim 1; Para. [0007]); and a method of treating or preventing testicular adrenal rest tumors (TART) (Paras. [0044] and [0047]; Claim 1), comprising administering to a subject in need thereof a pharmaceutical composition (Paras. [0061] and [0065]). The pharmaceutical compositions described herein that comprise at least one of the CRF1 antagonist compounds described herein may be administered to a subject in need by any one of several routes that effectively deliver an effective amount of the compound.; Claim 1).

US 2010/0222339 A1 to Chen et al., in the field of treating congenital adrenal hyperplasia (Para. [0014]), teach a corticotropin-releasing factor type-1 (CRF1) antagonist (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine; Para. [0057]. The compounds of Formula I are CRF-1 antagonists and, as such, are useful for treating a condition which is treatable by reducing CRF1 receptor tone or stimulation.; a compound of structural Formula (I) wherein, R1 and R2 are independently ethyl; R3 is Br; and R4 is Br (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine); and a pharmaceutical composition, comprising a compound of structural Formula (I) (Claim 10, A pharmaceutical composition comprising: a compound according to claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.; (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

Patents

JP2010504344A



Thiazolepyrazolopyrimidines as CRF1 receptor antagonists

Abstract

translated from Japanese

The present invention relates to compounds of formula I, pharmaceutical compositions thereof and their use as corticotropin releasing factor 1 (CRF1) receptor antagonists in the treatment of psychiatric and neuroendocrine disorders, nervous system diseases and metabolic syndrome.

Classifications

C07D487/04 Ortho-condensed systems

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External links: Espacenet, Global Dossier, Discuss

Claims (17)

Hide Dependent
translated from Japanese

Compound of formula I
(Where

R¹ and R² are independently ethyl or n-propyl;

R³ is hydrogen, Cl, Br, methyl, trifluoromethyl or methoxy;

R⁴ is hydrogen, Br, R^a R^b N-, methoxymethyl, n-butyl, acetamide, pyridin-4-yl, morpholin-4-yl,

And

R^a and R^b are independently hydrogen, C₁-C₃ alkyl, H₂NCH₂CH₂-, (CH₃)₃COC(O)NHCH₂CH₂-, or CH₃CH₂CH₂NHCH₂CH₂-)

Or a pharmaceutically acceptable salt thereof. R³ is Cl, Br, or trifluoromethyl, a compound or a pharmaceutically acceptable salt thereof according to claim 1. The compound or a pharmaceutically acceptable salt thereof according to claim 1 or 2, wherein R³ is Cl or Br. R⁴ is R^a R^b N-, pyridin-4-yl, morpholin-4-yl, or

The compound according to any one of claims 1 to 3 or a pharmaceutically acceptable salt thereof. R⁴ is morpholin-4-yl or

The compound according to any one of claims 1 to 4 or a pharmacologically acceptable salt thereof. The compound according to any one of claims 1 to 4, or a pharmacological

thereof, wherein R⁴ is R^a R^b N-, and R^a and R^b are independently C₁-C₃ alkyl. Acceptable salt. 3- [4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl] -2,5-dimethyl-7- (1-propyl)- The compound or a pharmaceutically acceptable salt thereof according to claim 1, which is (butyl)-pyrazolo [1,5-a] pyrimidine. 3- (4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl) -7- (1-ethyl-propyl) -2,5 The compound according to claim 1, which is -dimethyl-pyrazolo [1,5-a] pyrimidine, or a pharmaceutically acceptable salt thereof. 3- (4-Chloro-2-morpholin-4-yl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine, The compound according to claim 1 or a pharmaceutically acceptable salt thereof.

A pharmaceutical composition comprising the compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, and a pharmacologically acceptable excipient. Patient depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders,

allergies, migraine, premenstrual syndrome, infertility, sexual dysfunction, Congenital adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis. A method of treatment comprising administering to a patient in need thereof a therapeutically effective amount of a compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof. Method. A method for treating anxiety or depression in a patient, wherein the patient in need thereof has a therapeutically effective amount of the compound of any one of claims 1 to 9 or a pharmacologically acceptable salt thereof. Administering a possible salt. A method of treating a patient's alcohol or drug abuse, wherein the patient in need thereof has a therapeutically effective amount of the compound of any one of claims 1 to 9 or a pharmacologically acceptable salt thereof. Administering a possible salt. 10. A compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof for use in therapy. Depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders, allergies, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital in the treatment of adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic pituitary tumor, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis. The compound or pharmacologically acceptable salt thereof according to any one of claims 1 to 9, for use. 10. A compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof for use in the treatment of anxiety or depression. 10. A compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof for use in the treatment of alcohol abuse or drug abuse.

Description

translated from Japanese

The present invention relates to novel thiazolepyrazolopyrimidine compounds, pharmaceutical compositions thereof and their use as CRF1 receptor antagonists in the treatment of psychiatric and neuroendocrine disorders, nervous system diseases and metabolic syndrome.

Corticotropin releasing factor (CRF) is a 41 amino acid peptide that is a major physiological regulator of proopiomelanocortin (POMC)-derived peptide secretion from the anterior pituitary gland. In addition to its endocrine role in the pituitary gland, due to the immunohistochemical localization of CRF, this hormone has a broad extrahypothalamic distribution in the central nervous system and as a neurotransmitter or neuromodulator in the brain. Proven to produce a wide range of autonomic effects, electronic physiological effects and effects on action consistent with the role. There is also evidence that CRF plays an important role in integrating responses to physiological, psychological and immunological stressors in the immune system.

CRF is a psychiatric and nervous system disease, including depression and neuroanxiety, and Alzheimer's disease, Huntington's disease, progressive supranuclear palsy, amyotrophic lateral sclerosis, Parkinson's disease, epilepsy, migraine, alcohol abuse and drug abuse and related withdrawal symptoms, obesity, metabolic syndrome, congenital adrenal hyperplasia, Cushing disease, hypertension, stroke, irritable bowel syndrome, stress-induced gastric ulcer, premenstrual syndrome, sexual dysfunction, premature birth, it has been associated with inflammatory disorders, allergies, multiple sclerosis, visceral pain, sleep disorders, pituitary or ectopic pituitary tumors, chronic fatigue syndrome and fibromyalgia.

CRF receptor subtypes (CRF1 and CRF2) have been identified and these are heterogeneously distributed in the brain, suggesting a possible functional diversity. For example, widely distributed brain CRF1 receptors are strongly associated with the emotion associated with exposure to environmental stressors. Importantly, the CRF1 receptor, but not the CRF2 receptor, appears to mediate the selection of anxiety-like behavior. The more discrete septal / hypothalamic distribution and availability of another endogenous ligand suggests a different functional role for the CRF2 receptor. For example, a novel CRF family of neuropeptides with a preferential affinity for the CRF2 receptor over the CRF1 receptor may cause an appetite without producing the behavior-activated appearance observed with selective CRF1 agonism. It has been reported to suppress. In other cases, CRF2 agonism produces effects similar to those reported for CRF1 antagonists or CRF1 gene deletions. For example, although CRF2 agonists have been proposed as anti-obesity agents, CRF1 antagonists may also be important treatments for obesity.

Certain pyrrole [2,3-d] pyrimidines, pyrrole [3,2-d] pyrimidines, pyrazolo [1,5-a] pyrimidines, 1,2,3-triazolo [4,5-b] useful as CRF antagonists. Pyridine and pyrazolo [1,5-e] -1,3,5-triazine are described in Patent Document 1, Patent Document 2, Patent Document 3, and Patent Document 4.

International Publication No. 94/13575 Pamphlet International Publication No. 97/29109 Pamphlet International Publication No. 98/08847 Pamphlet International Publication No. 98/03510 Pamphlet

The present invention provides novel thiazolepyrazolopyrimidines useful as CRF1 receptor antagonists. In view of the above, it would be desirable to discover new effective and selective antagonists of CRF1 as potentially beneficial therapeutics for the treatment of psychiatric and neuroendocrine disorders, nervous system diseases and metabolic syndrome. In addition, novel compounds with preferred bioavailability and pharmacokinetic profiles over known CRF antagonists (eg CP154525 and NBI30775) because the majority of commercially available CNS drugs and cardiovascular drugs exhibit unfavorable bioavailability and pharmacokinetic profiles. It is also desirable to discover.

In one embodiment, the invention provides a compound of formula I

wherein R¹ and R² are independently ethyl or n-propyl, R³ is hydrogen, Cl, Br, methyl, trifluoromethyl or methoxy, R⁴ is hydrogen, Br, R^a, R^b, N-, methoxymethyl, n-butyl, acetamide, pyridin-4-yl, morpholin-4-yl.

R^a and R^b are independently hydrogen, C₁-C₃ alkyl, H₂NCH₂CH₂-, (CH₂)₃-COO(O)NHCH₂CH₂-, or CH₃CH₂CH₂NHCH₂CH₂-, in which] or to provide a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention provides a pharmaceutical composition comprising a compound of formula I or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient.

In another embodiment, the invention relates to patient depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders, allergies, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic pituitary tumor, chronic fatigue syndrome, fibromuscular A method of treating pain, visceral pain or multiple sclerosis comprising administering to a patient in need thereof a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof. About.

In another embodiment, the present invention relates to depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders, allergies, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic pituitary tumor, chronic fatigue syndrome, fibromyalgia, it relates to the use of a compound of formula I or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of visceral pain or multiple sclerosis.

In another embodiment, the present invention relates to depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders, allergies, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic pituitary tumor, chronic fatigue syndrome, fibromyalgia, it relates to a compound of formula I or a pharmaceutically acceptable salt thereof for use in the treatment of visceral pain or multiple sclerosis.

In another embodiment, the present invention relates to a therapeutic compound of formula I or a pharmaceutically acceptable salt thereof.

As used above and throughout the description of the present invention, the following terms shall be understood to have the following meanings unless otherwise indicated.

"Alkyl" means a saturated aliphatic hydrocarbon group which may be straight or branched having 1 to 6 carbon atoms in the chain.

"Pharmaceutically acceptable excipient" refers to a pharmacologically acceptable pharmaceutical carrier, solution, or additive to enhance pharmaceutical properties. Such excipients must be compatible with the other ingredients of the formulation and not deleterious to the user. Such excipients are well known to those skilled in the art (see, eg. Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, 1995).

"Pharmaceutically acceptable salt" refers to the relatively non-toxic, inorganic and organic acid addition salts and base addition salts of the compounds of the present invention. These salts can be prepared in situ in the final isolation and purification steps of the compound. In particular, acid addition salts can be prepared by reacting the purified compound in the free base form separately with a suitable organic or inorganic acid and isolating the resulting salt (see, eg. Remington's Pharmaceutical Sciences, No. 1), 19th edition, see Mack Publishing Company, 1995).

A "therapeutically effective amount" or "effective amount" is the biological or medical response of a tissue, system, animal or human being sought by a researcher, veterinarian, physician or other clinician, or the desired response thereto. It means the amount of a compound of formula I of the invention or a pharmaceutical composition containing a compound of formula I of the invention that causes a therapeutic effect.

Terms such as "treatment", "treat", "treating" and the like are intended to include both slowing the progression of the disorder and directing recovery. These terms also refer to one or more symptoms of the disorder or condition, even if the disorder or condition is not actually resolved, and the progression of the disorder or condition is not slowed by itself and is not likely to recover. Also includes mitigating, improving, mitigating, eliminating or reducing. The term "treatment" and similar terms also include preventive (eg, prophylactic) treatment and treatment for pain relief. Prevention of a disease results in prolonging or delaying the onset of symptoms of the disease.

Symbols in molecular structure

Indicates the bonding position for the specific substituent.

When any variable occurs more than one time in any constituent or in formula I, its definition when each appears is independent of its definition when it appears elsewhere. Also, combinations of substituents and / or variables are acceptable only if such combinations result in stable compounds. In selecting the compounds of the present invention, those skilled in the art will recognize that various substituents, ie R¹, R², etc., should be selected in accordance with the well-known principles of chemical structure connectivity. I will.

Under standard nomenclature used throughout this disclosure, the terminal portion of the designated side chain is described first, followed by the adjacent functionality toward the point of attachment. For example, an arylcarbonylaminoalkyl substituent is equivalent to aryl-C(=O)-NH-alkyl.

The present invention contemplates the following embodiments, which can be further combined differently:

(A) a compound of formula I wherein R¹ and R² are ethyl;

(B) a compound of formula I wherein R¹ and R² are n-propyl;

(C) R³ is Cl, Br; compound of formula I is methyl or trifluoromethyl;

(D) the compound of formula I, wherein R³ is Cl;

(E) the compound of formula I, wherein R³ is Br;

(F) R⁴ is R⁵ R⁶ N-, pyridin-4-yl, morpholin-4-yl, or

A compound of formula I which is

(G) the compound of formula I, wherein R⁴ is morpholin-4-yl;

(H) R⁴ is

A compound of formula I which is

(I) the compound of formula I, wherein R⁵ and R⁶ are independently C₁-C₃ alkyl;

(J) the compound of formula I, wherein R¹ and R² are ethyl, R³ is Cl, and R⁴ is morpholin-4-yl;

(K) the compound of formula I, wherein R¹ and R² are n-propyl, R³ is Cl, and R⁴ is morpholin-4-yl;

(L) a compound of formula I wherein R¹ and R² are ethyl, R³ is Br and R⁴ is morpholin-4-yl;

(M) R¹ and R² are n-propyl, R³ is Br; compound of formula I R⁴ is morpholin-4-yl;

(N) R¹ and R² are ethyl, R³ is Cl, R⁴ is

A compound of formula I which is

(O) R¹ and R² are n-propyl, R³ is Cl, and R⁴ is

A compound of formula I which is

(P) R¹ and R² are ethyl, R³ is Br, and R⁴ is

A compound of formula I which is

(Q) R¹ and R² are n-propyl, R³ is Br, and R⁴ is

A compound of formula I which is

(R) the use of a compound of formula I or a pharmaceutically acceptable salt thereof for the treatment of depression or neuroanxiety;

(S) the use of a compound of formula I or a pharmaceutically acceptable salt thereof for the treatment of alcohol abuse or drug abuse;

(T) the use of a compound of formula I or a pharmaceutically acceptable salt thereof for the treatment of alcohol or drug abuse and related withdrawal symptoms;

(U) a compound of formula I or a pharmaceutically acceptable salt thereof showing a Ki value of 500 nM or less for CRF1 binding;

(V) a compound of formula I or a pharmaceutically acceptable salt thereof showing a Ki value of 50 nM or less for CRF1 binding;

(W) a compound of formula I or a pharmaceutically acceptable salt thereof showing a Ki value of 5 nM or less for CRF1 binding;

(X) a compound of formula I or a pharmaceutically acceptable salt thereof that exhibits a Ki value of 500 nM or less for CRF1 binding and selectively binds to CRF1 over CRF2 (ie, a smaller Ki);

(Y) a compound of formula I or a pharmaceutically acceptable salt thereof that exhibits a Ki value of 50 nM or less for CRF1 binding and selectively binds to CRF1 over CRF2 (ie, a smaller Ki);

(Z) a compound of formula I or a pharmaceutically acceptable salt thereof that exhibits a Ki value of 5 nM or less for CRF1 binding and selectively binds to CRF1 over

CRF2 (ie, a smaller Ki); and / or Alternatively (aa) certain exemplified compounds (such as Example 15) that have better bioavailability and pharmacokinetic profiles than some known CRF antagonists (eg, CP154525 and NBQX775).

The compounds of the present invention are preferably formulated as pharmaceutical compositions that are administered by a variety of routes. Such compositions are preferably for oral administration. Such pharmaceutical compositions and processes for preparing such pharmaceutical compositions are well known in the art (see, eg, Remington: The Science and Practice of Pharmacy, A. Gennaro et al., 19th Edition, Mack Publishing Co., 1995).

The compounds of formula I are generally effective over a wide dosage range. For example, daily dosages typically range from about 0.0001 to about 30 mg / kg body weight. In some cases, dosage levels below the lower limit of the aforementioned range may be sufficient, while in some cases even higher doses may be used without causing any harmful side effects, and therefore The above dosage ranges are not intended to limit the scope of the invention in any way. The amount of the compound actually administered will depend on the condition being treated, the route of administration selected, the actual compound (s) being administered, the age, weight and response of the individual patient, it should be understood that it will be determined by the physician in view of the relevant circumstances, including the severity of the patient's symptoms.

The compounds of formula I are CRF-1 antagonists and are therefore useful for the treatment of medical conditions that can be treated by reducing CRF1 receptor tone or stimulation. Corticotropin releasing factor (CRF), a 41 amino acid peptide that is a major physiological regulator of peptide secretion from proopiomelanocortin (POMC) from the anterior pituitary gland [J. Rivier et al., Proc. Natl. Acad. Sci. (USA) 80: 4851 (1983); Vale et al., Science 213: 1394 (1981)] has been associated with a number of medical conditions. For example, in addition to its endocrine role in the pituitary gland, because of the immunohistochemical localization of CRF this hormone has a broad extra-halamic distribution of the central nervous system and is a neurotransmitter or neuromodulator in the brain It has been demonstrated to produce a wide range of autonomic effects, electrophysiological effects and action effects consistent with its role as [W. Vale et al., Rec. Prog. Horm. Res. 39: 245 (1983); F. Koob, Persp. Behav. Med. 2:39 (1985); B. De Souza et al., J. Mol. Neurosci. 5: 3189 (1995)]. There is also evidence that CRF plays an important role in integrating responses to physiological, psychological and immunological stressors in the immune system [see, for example, J. et al. E. Black. Physiological Reviews 69: 1 (1989); E. Morley, Life Sci. 41: 527 (1987)].

CRF is associated with psychiatric disorders and nervous system disorders, including depression and neuroanxiety [D. M.M. Nielsen, Life Sci. 78: 909-919; E. Kunzel et al. Psychiatr. Res. 37: 525-533; R. Gellert et al., Eur. J. et al. Pharmacol. 509: 145-153]. The role of CRF has also been postulated in the pathogenesis and pathophysiology of Alzheimer's disease, Huntington's disease, progressive supranuclear palsy and amyotrophic lateral sclerosis. Because they are related to dysfunction of CRF neurons in the central nervous system [for review, see E.C.B. De Souza, Hosp. Practice 23:59 (1988)]. Chronic administration of CRF has been shown to produce dysfunction of the dopamine system, suggesting a role in Parkinson's disease [E. Izso et al., Pharmacol. Biochem. Behav. 81: 701-708 (2005)]. Other neurological disorders involving CRF include sputum [Y. Z. Baram et al., Brain Res. 779: 89-95 (1997)] and migraine [T. C. Theodorides et al., Endocrinology 136: 5745-5750 (1995)]. CRF has been associated with alcohol and drug abuse and related withdrawal symptoms [D. H. Overstreet et al., Pharmacol. Biochem. Behav. 77: 405-413; Shaham et al., Psychopharmacology (Ber) 137: 184-190]. In addition, CRF is associated with various endocrine disorders and cardiovascular diseases such as obesity [E. Timofeyeva and D. Richard, Neuroendocrinology 66: 327-340 (1997)], metabolic syndrome [AM Ward et al., Metabolism 53: 720 -726 (2004)], congenital adrenal hyperplasia [GP Merke and GG Cutler Jr., Endocrinol. Metab. Clin. North Am. 30: 121-135 (2001)], Cushing's disease [M. Labeur et al., Curr. Drug Targets Immunod. Endocr. Metabol. Disord. 4: 335-342 (2004)], hypertension [RJ Biscoe et al., Br. Res. 561: 204-207 (2000)], and seizures [S.L. Stevens et al., J. Cereb. Blood Flow Metab. 23: 1151-1159 play a role in (2003)]; there is evidence that. Gastric disorders (eg, irritable bowel syndrome [Y. Tache et al., Eur. J. Surg. Suppl. 16-22 (2002)] and stress-induced gastric ulcer [KE Gabry et al., Mol. Psychiatry 7: 474]-483,433 (2002)] has been shown to be related to CRF. In addition, CRF has been identified in various areas of human female health, such as premenstrual syndrome [E. Facchinetti et al., Psychosom. Med. 56: 418-422 (1994)], infertility [L. Ghizzoni et al., Endocrinology 138: 4805-4811 (1997)], sexual dysfunction [J. E. Jones et al., Am. J. et al. Physiol. Regul. Integr. Comp. Physiol. 283: R591-597 (2002)], and preterm birth [P. D. Wadhwa et al., Am. J. et al. Obstet. Gynecol. 191: 1063-1069 (2004)]. There is also evidence that CRF plays an important role in the immune system. This is because inflammatory disorders [A. Gravanis and A.M. N. Margioris, Curr. Med. Chem. 12: 1503-1512 (2005)], allergies [L. K. Singh et al., Brain Behav. Immun. 13: 225-239 (1999)], multiple sclerosis and other autoimmune disorders [C. Benou et al., Immunol. 174: 5407-5413 (2005)]. In addition to the above, CRF is a visceral pain [M. Nijssen et al., Neurogastroenterol. Motil. 17: 423-432 (2005)], sleep disorders [T. M.M. Buckley and A.M. F. Schattberg, J.A. Clin. Endocrinol. Metab. 90: 3106-3114 (2005)], pituitary tumors or ectopic pituitary tumors [K. D. Dieterich et al., J. Mol. Clin. Endocrinol. Metab. 83: 3327-3331 (1998)], chronic fatigue syndrome and fibromyalgia [G. Neeck and L.M. J. et al. Croford, Rheum. Dis. Clin. North Am. 26: 989-1002 (2000)].

CRF receptor subtypes (CRF1 and CRF2) have been identified and these are heterogeneously distributed in the brain [D. T.A. Chalmers et al., TIPS 17: 166-72 (1996)], suggesting the possibility of functional diversity [S. C. Heinrichs et al., Regul. Peptides 71:15 (1997)]. For example, widely distributed brain CRF1 receptors are strongly associated with the emotionality associated with exposure to environmental stressors [G. Liebisch et al., Regul. Peptides 59: 229-39 (1995); W. Schulz, PNAS 93: 10477-82 (1996)]. Importantly, the CRF1 receptor, but not the CRF2 receptor, appears to mediate selection of anxiety-like behavior [Heinrichs et al., 1997]. A more discrete septum / hypothalamus distribution [D. T.A. Chalmers et al., Neurosci. 15 (10): 5340-50 (1995)] and the availability of other endogenous ligands [J. Vaughan et al., Nature 378: 287-92 (1995)] suggests a different functional role for the CRF2 receptor [Heinrichs et al., 1997]. For example, a novel CRF family of neuropeptides with a preferential affinity for the CRF2 receptor over the CRF1 receptor may cause an appetite without producing the behavior-activated appearance observed with selective CRF1 agonism. [H. Tezval et al., PNAS 101 (25): 9458-9473 (2004)]. In some cases, CRF2 agonism produces effects similar to those reported for CRF1 antagonists or CRF1 gene deletion [S. C. Heinrichs, Trends in Pharmacological Sciences 20 (8): 311-5 (1999)]. For example, CRF2 agonists have been proposed as anti-obesity agents, but CRF1 antagonists may also be important treatments for obesity [C. Contoreggi et al., Neuroendocrinology 80 (2): 111-23 (2004)].

(Preparation of compounds of the invention)

All of the compounds of the present invention can be chemically prepared, for example, according to the synthetic routes shown below. However, the following discussion is in no way intended to limit the scope of the present invention. For example, the specific synthetic steps for each of the described routes may be combined in different ways to prepare additional compounds of formula I or may be combined in conjunction with steps from different schemes. . The product of each step can be recovered by conventional methods including extraction, evaporation, precipitation, chromatography, filtration, powdering, crystallization and the like. In the schemes below, all substituents are as previously defined unless otherwise stated, and suitable reagents are well known and recognized in the art.

Formation of compounds of formula (6a, b), formula (7), or formula 8 (ac) can be carried out according to the reaction shown in Scheme 1. Suitable compounds of formula (6a, b), formula (7), or formula (8a-c) are those where R¹ and R² are as defined for formula I and R^{3a} = H or CH₃, R^{3b} = Br or CH₃ and R^{3c} = H, CH₃, or Br.

In Scheme 1, Step 1, pyrazolo [1,5-a] pyrimidin-7-one of formula (1) uses phosphorus oxychloride and dimethylaniline in an inert solvent (such as toluene) at the reflux temperature of the solvent. To 7-chloro-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine.

In Step 2, the Grignard reagent of formula (3) (X = Cl or Br) is reacted with the chloride of formula (2) at reflux temperature in an inert solvent (such as toluene) to yield the 7-alkyl of formula (4). Give pyrazolopyrimidine.

Alternatively, the 7-alkylpyrazolopyrimidine of formula (4) can be obtained as shown in Step 3, Step 4, and Step 5. In Step 3, ethyl acetosuccinate is acylated with an acid halide of formula (4a) in the presence of magnesium chloride to give a diketo ester of formula (4b). The diketo-ester of formula (4b) is decarboxylated under Krapcho conditions to give the diketone of formula (4c). For example, (4b) is heated in dimethyl sulfoxide at a temperature of about 130-170 ° C. to effect this decarboxylation. In

step 5, the diketone of formula (4c) is cyclized with 3-amino-5-methylpyrazole in a protic solvent (such as methanol, ethanol, or acetic acid) to give a 7-alkylpyrazolopyrimidine of formula (4). give. In preferred conditions, acetic acid is used at a temperature of about 0-60 ° C.

The pyrazolopyrimidine of formula (4) is functionalized in step 6 to an iodopyrazolopyrimidine of formula (5) using an excess of N-iodosuccinimide in acetonitrile.

In Scheme 1, Step 7 or Step 8, iodopyrazolopyrimidine of formula (5) is reacted with thiazole zinc halide in a Negishi cross-coupling reaction to form thiazolopyrazolopyrimidine of formula (6a) or formula (6b) (Jensen, J., Skjaerbaek, N., Vedso, P. Synthesis 2001, 128). The thiazole zinc halide is produced using methods well known to those skilled in the art. For example, in step 7, 2-trimethylsilylthiazole is treated with n-butyllithium, sec-butyllithium or tert-butyllithium, and then lithium ZnCl₂ - zinc replacement is made. The organozinc reagent is present in an inert solvent (such as THF) in the presence of a palladium catalyst such as dichloro [1,1'-bis (diphenyl-phosphino) ferrocene] palladium (II) dichloromethane at reflux temperature for about 12-36 hours. Below 'coupled with iodopyrazolopyrimidines of formula (5)' to give thiazole-pyrazolopyrimidines of formula (6a).

Alternatively, in step 8, thiazole zinc bromide is generated using 5-bromo-4-methylthiazole and zinc metal and is used for Negishi cross coupling essentially as described for step 7 to obtain the formula (6b) Thiazolopyrazolopyrimidine.

In Scheme 1, Step 9, the thiazole of formula (6a, b) is brominated to give the bromothiazole or dibromothiazole of formula (7) where R^{3b} = Br or CH₃. This thiazole is brominated with 1 or 2 equivalents of N-bromosuccinimide, depending on whether R^{3a} is CH₃ or H, respectively.

The alkyl thiazole of formula (8a-c) is obtained in step 11 from the thiazole of formula (6a, b) or in step 10 from the formula (7) bromothiazole. In step 10, halogen-lithium exchange with n-butyllithium, sec-butyllithium, or t-butyllithium provides a thiazole lithium reagent, which is then subjected to an electrophile (an alkyl such as iodomethyl methyl ether or iodobutane). Reaction with halide). In step 11, the thiazole lithium reagent is generated by deprotonation using n-butyllithium, sec-butyllithium, or tert-butyllithium, followed by an electrophile such as iodomethyl methyl ether or iodobutane. React with.

The thiazole ring system is easily functionalized and that thiazole intermediates such as 2-trimethylsilylthiazole (Dondoni, A. et al., J. Org. Chem. 1988, 53, 1748) can be easily prepared. Those skilled in the art will understand. 5-Bromo-4-methylthiazole is obtained by bromination of 4-methylthiazole with bromine in acetic acid (Collins, J. et al., WO200309522, Nov. 13, 2003). 2,5-Dimethyl-4H-pyrazolo [1,5-a] pyrimidin-7-one (1) is obtained by condensation of ethyl acetoacetate and 5-methyl-2H-pyrazol-3-ylamine in refluxing acetic acid. Easy to prepare.

Formation of the compound of formula (9), formula (10), or formula (11) is carried out according to the reaction shown in Scheme 2. Suitable compounds of formula (9), formula (10), or formula (11) are as R¹, R², R³, and R⁴ are as defined for formula 1, and R^{3b} = Br or CH₃, R^{4a} = -NR^aR^b or -N-morpholinyl, a compound defined as "het".

In Step 1, the bromothiazole of formula (7) is coupled with a heterocyclic zinc reagent in a Negishi cross-coupling reaction to give a thiazole heterocycle of formula (9). For example, 1-methyl-1,2,4-thiazole is treated with n-butyllithium, sec-butyllithium, or tert-butyllithium at about -80 to -65 ° C, and then with zinc chloride. React with bromothiazole of formula (7) in situ. This reaction is carried out in an inert solvent (such as THF) with a palladium catalyst (such as dichloro [1,1'-bis (diphenyl-phosphino) ferrocene] palladium (II) dichloromethane or tetrakis (triphenylphosphine) palladium (0)). Is preferably carried out in the presence of The reaction is warmed to reflux temperature. Alternatively, the heterocyclic zinc reagent is formed from a haloheterocycle (such as 4-iodopyridine) and zinc metal.

In Scheme 2, Step 2, the intermediate 2-formylthiazole is formed by halogen-lithium exchange using n-butyllithium, sec-butyllithium, or t-butyllithium, followed by reaction with N-formylmorpholine. This formylthiazole is subjected to reductive amination in the presence of an organic amine (such as morpholine) to give the morpholinylmethylthiazole of formula (10). Reductive amination is well known in the art and is usually performed using an inorganic borohydride reagent (such as sodium borohydride or sodium cyanoborohydride). Preferred conditions use sodium triacetoxyborohydride in an inert solvent such as dichloromethane or THF.

In Step 3, the bromothiazole of formula (7) undergoes a substitution reaction with an amine or morpholine of formula -NR^aR^b to give an aminothiazole of formula (11). This reaction is carried out at 70-110 ° C using an inorganic base (such as cesium carbonate) in an inert solvent (such as THF or dioxane).

Formation of the compound of formula (13), formula (14), or formula (15) can be carried out according to the reaction shown in Scheme 3. Suitable compounds of formula (13), formula (14), or formula (15) are those where R¹, R², and R⁴ are as defined for formula 1.

One skilled in the art will recognize that 4-bromothiazole (such as that of formula (12)) is readily manipulated for other functionalizations. For example, in Step 1, the bromide can be dehalogenated with copper (I) chloride to give the thiazole of formula (13), which is then chlorinated with N-chlorosuccinimide to obtain the formula 4-chlorothiazole of (14) is obtained.

In Scheme 3, Step 3, 4-bromothiazole of formula (12) is dissolved in sodium methoxide in the presence of copper (I) iodide in methanol at about 100-120 ° C, in an inert solvent (such as dimethylformamide). To give 4-methoxythiazole of formula (15).

Formation of the compound of formula (17), formula (18), formula (19), formula (20), formula (20a), or formula (21) can be carried out according to the reaction shown in Scheme 4. Suitable compounds of formula (17), formula (18), formula (19), formula (20), formula (20a), or formula (21) are those wherein R¹, R², R³, and R⁴ are of formula A compound as defined for i, wherein R^{4a} is -NR^aR^b or -N-morpholinyl and "het" is indicated.

In Scheme 4, Step 1, iodopyrazolopyrimidine of formula (5) and 5-bromothiazole of formula (16) undergo Negishi cross-coupling to produce dimethylpyrrolylthiazole of formula (17). For example, 5-bromothiazole of formula (16) is treated with n-butyllithium, sec-butyllithium, or tert-butyllithium at about -80 to -65 ° C, and then with zinc chloride. This organozinc reagent is reacted in situ with the iodopyrazolopyrimidine of formula (5). This coupling reaction is preferably carried out in an inert solvent (such as THF) at the reflux temperature in the presence of a palladium catalyst (such as bis (tri-t-butylphosphine) palladium (0)).

In Step 2, the dimethylpyrrolylthiazole of formula (17) is deprotected to give the aminothiazole of formula (18). The dimethylpyrrole is treated with hydroxylamine in acetic acid at a temperature of about 50-100 ° C. for about 4-8 hours, preferably about 5 hours. In step 3, the same conditions as in step 2 are used except that the reaction is continued for about 72 hours to produce thiazoleacetamide of formula (18).

In Scheme 4, Step 4, 2-aminothiazole of formula (19) is converted to 2-bromothiazole of formula (20) using a modified Sandmeyer reaction. Preferred conditions use alkyl nitrite (such as t-butyl nitrite) and copper (II) bromide in acetonitrile at a temperature of about 60-80 ° C.

In Step 5, 2-bromothiazole of formula (20) undergoes a substitution reaction with an amine or morpholine of formula -NR^aR^b to give an aminothiazole of formula (21). The reaction is carried out in an inert solvent (such as methanol, THF, or dioxane) or treated neat with an excess of amine at a temperature of about 70-110 ° C. Alternatively, this reaction is carried out using an amine as a reactant and an excess of triethylamine or an inorganic base (such as cesium carbonate). Various deprotection steps are also envisaged in the synthesis of the aminothiazole of formula (21). For example, if -NR^aR^b possesses additional amine functionality as defined

in Formula I, if necessary or beneficial to carry out the above reaction, tert-butyl ester carbamic acid (BOC) is removed. The selection and use of suitable protecting groups is well known and recognized in the art (see, eg, *Protection Groups in Organic Synthesis*, Theodora Greene (Wiley-Interscience)).

In Scheme 4, Step 6, the 2-bromothiazole of formula (20) is coupled with a heterocyclic zinc reagent in a Negishi cross-coupling reaction and is analogous to that described in Scheme 2, Step 1 (20a). Thiazole heterocycle.

One skilled in the art will recognize that the functionalized thiazole of formula (16) can be prepared by means known in the art. For example, cyclization of thiourea with bromoketones (such as 3-bromo-1,1,1-trifluoropropan-2-one) provides 4-trifluoromethyl-thiazol-2-ylamine. Subsequent bromination and protection of the amine with hexane 2,5 dione gives (16).

Formation of the compound of formula (23) can be carried out according to the reaction shown in Scheme 5. Suitable compounds of formula (23) are those compounds in which R¹ and R² are as defined for formula I and R^{4b} is indicated.

An iodopyrazolopyrimidine of formula (5) and for example 4-chloro-2-morpholino-thiazole of formula (22) undergo cross-coupling to produce a pyrazolopyrimidine thiazole of formula (23). For example, these reactants are coupled with a base (such as cesium carbonate) in the presence of copper iodide, palladium acetate, and triphenylphosphine. This coupling reaction is preferably carried out in an inert solvent (such as DMF) at about 100 to 150 °C. for 4 to 24 hours.

One skilled in the art will recognize that the functionalized thiazole of formula (22) can be prepared by means known in the art. For example, 2-morpholino-thiazole of formula (22) can be obtained by reacting 2,4-dichlorothiazole with morpholine. In addition, 2,5-dichlorothiazole can be all brominated to obtain 2,5-dibromo-4-chlorothiazole. Subsequent bromine-lithium exchange with n-butyllithium and quench with water at -90 °C. in THF yields 2-bromo-4-chlorothiazole [J. Chem. Soc. Perkin Trans I: Org. Bioorg. Chem. (1972-1999), (2): 215-219 (1992)]. 2-Bromo-4-chlorothiazole can also undergo a Negishi cross-coupling reaction with a heterocyclic zinc reagent to give thiazol-2-ylthiazole or thiazol-2-ylpyridine.

Formation of the compound of formula (27) can be carried out according to the reaction shown in Scheme 6. Suitable compounds of formula (27) are those wherein R¹ and R² are as defined for formula I and "het" is defined as indicated for step 1 or step 3, respectively.

In Scheme 6, Step 1, for example, a heterocyclic thiazole of formula (25) is obtained by reaction of iodopyrazolopyrimidine of formula (5) with bromothiazole of formula (24). This reaction is carried out using palladium catalyst (such as palladium acetate) and tris (2,4-di-tert-butyl-phenyl)-phosphane in the presence of N-butylammonium bromide and base (such as potassium acetate). It is carried out in an active solvent (such as N-methylpyrrolidinone) at a temperature of about 100-150 °C.

Alternatively, in step 2, the thiazolyl pyrazolopyrimidine of formula (5a) is iodinated to give 2-iodothiazole of formula (26). The thiazole is treated with lithium diisopropylamide for about 1 hour at a temperature of -70 to -80 °C in an inert solvent (such as THF) and then with N-iodosuccinimide at about the same temperature. This is followed by step 3, in which thiazolylthiazole or 4-pyridylthiazole of formula (25) is formed using Negishi cross-coupling conditions similar to those described for Scheme 2, Step 1.

In Scheme 6, Step 4, the thiazolylpyrazolopyrimidine of formula (25) is brominated to give the bromothiazole of formula (27). This bromination is performed using N-bromosuccinimide in the presence of a small amount of acetic acid in an inert solvent (such as acetonitrile).

The compound of formula (27), where het = 2,5-dimethyl-pyrrol-1-yl, is further converted as described in Scheme 4, Step 2, Step 3, Step 4, and Step 5. A compound can be obtained.

One skilled in the art will recognize that the heterocyclic thiazole of formula (24) can be readily prepared by means known in the art. For example, thiazole-2-carboxylic acid amide can be cyclized with 1,1-dimethoxy-N, N-dimethyl-methanamine to form a thiazole, which is then treated with N-methylhydrazine to give a thiazolyl. Luthiazole is provided, which can then be brominated with N-bromosuccinimide. Literature procedures give 4-thiazol-2-yl pyridine, which can be brominated to give 4-(5-bromo-thiazol-2-yl)-pyridine. 5-Bromo-2-(2,5-dimethyl-pyrrol-1-yl)-thiazole can be easily obtained by reacting 2-amino-5-bromothiazole with hexane-2,5-dione.

As used herein, "TLC" refers to thin layer chromatography, "HPLC" refers to high performance liquid chromatography, "LC / MS" refers to liquid chromatography / mass spectrometry, and "GC / MS". Refers to gas chromatography / mass spectrometry, "HR-ToF" refers to high-resolution time of flight, "APCI" refers to atmospheric pressure chemical ionization, and "δ" refers to the transition from tetramethylsilane to a low magnetic field. ppm, "THF" refers to tetrahydrofuran, "EtOAc" refers to ethyl acetate, "MeOH" refers to methanol, "EtOH" refers to ethanol, and "DMF" refers to dimethylformamide.

Without further elaboration, it is believed that one skilled in the art can fully practice the invention using the foregoing description. The following preparations and examples are provided to further illustrate the present invention. They are intended to illustrate the invention and are not intended to limit the invention in any way. Reagents and starting materials are readily available to those skilled in the art or can be readily synthesized. Examples 1 to 35 provide representative compounds and illustrate their preparation. Examples A-D illustrate various biological tests that can be used to determine the biological properties of the compounds of the present invention. Those skilled in the art will readily recognize appropriate variations from the procedures described in the examples. The names of the compounds of the present invention are those provided by ChemDraw Ultra® version 7.0.1. The salt was named by adding the conjugate acid to the free base.

(Preparation Example 1)

2,5-Dimethyl-4H-pyrazolo [1,5-a] pyrimidin-7-one While maintaining the temperature at 25-28 °C., ethyl acetate (128 g, 0.98 mol) was replaced with 5-methyl-2H-pyrazole. The solution was added dropwise to an acetic acid solution (500 mL) of 3-ylamine (100 g, 0.95 mol). The mixture was heated at reflux for 10 hours and cooled to room temperature. The reaction was added to tert-butyl methyl ether (5 L) cooled to 5 °C. keeping the temperature below 10 °C. Stir at 5 °C. for 1 hour and filter. The resulting material was dried in vacuo overnight to give a white solid (158 g, 96%).

(Preparation Example 2)

7-chloro-2,5-dimethyl pyrazolo [1,5-a] pyrimidine 2,5-dimethyl-4H-pyrazolo [1,5-a] pyrimidin-7-one (10.0 g) in toluene (160 mL), 61.3 mmol) was added N, N-dimethylaniline (9.7 mL, 76.7 mmol). Phosphorus oxychloride (11.2 mL, 122.6 mmol) was added dropwise to the white suspension. Refluxed under an inert atmosphere for 3 hours, cooled to room temperature, and concentrated the reaction to a brown oil using reduced pressure. This oil was dissolved in ethyl acetate (250 mL) and made basic with 1.0 N NaOH. The organic phase was separated and the basic aqueous phase was extracted with ethyl acetate (2 x 100 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to give a brown solid. Use flash chromatography to elute from 80% hexane / 20% (30% THF / hexane) to 0% hexane / 100% (30% THF / hexane) with a step gradient in 20% increments. Was purified to give a pale green solid (6.65 g, 50%). ES / MS m/z (35 O) 182.3 (M + 1) +.

(Alternative procedure)

2,5-Dimethylpyrazolo [1,5-a] pyrimidin-7-one (20 g, 122 mmol) was added to 1,4-dioxane (60 mL). The mixture was stirred at 22 °C. for 10 minutes and then N, N-diethylaniline (20.8 mL, 128 mmol) was added. Stir for an additional 5 minutes and then add phosphorus oxychloride (11.7 mL, 125 mmol) over 15 minutes. The mixture was stirred at 22 °C. for 15 minutes and then heated to 80-85 °C. over 35 minutes and the reaction was held at this temperature for 1.5 hours. The cooled reaction mixture was slowly added to an aqueous solution (325 mL) of dipotassium hydrogen phosphate (106.7 g, 612.82 mmol) cooled to 0-5 °C., keeping the temperature below

5 ° C. during the addition. The mixture was stirred at 22 ° C. and methyl-*t*-butyl ether (150 mL) was added. The organic layer was separated and the aqueous layer was extracted with methyl-*t*-butyl ether (2 × 100 mL). The organic portions were combined, dried over sodium sulfate, filtered and the solvent was evaporated. Purification by silica gel chromatography eluting with hexane / ethyl acetate (2/1) gave the title compound as a yellow solid (20.7 g, 88%). ES / MS *m/z* 182 (*M* + 1)⁺.

(Preparation Example 3)

7-(1-propyl-butyl)-2,5-dimethyl-pyrazolo [1,5-*a*] pyrimidine

An oven-dried flask equipped with a reflux condenser was charged with anhydrous THF (40 mL), iodine (catalytic amount), magnesium ribbon (1.92 g, 78.9 mmol) and 4-bromoheptane (9.4 mL, 52 mmol). The reaction solution was heated to reflux in an oil bath. The temperature of this reaction solution rose rapidly when the Grignard reaction started. The reaction was stirred at 90 ° C. for a further 4 hours and cooled to room temperature. Magnesium metal is allowed to settle (centrifuged) and the Grignard reagent is added to 7-chloro-2,5-dimethylpyrazolo [1,5-*a*] pyrimidine (in anhydrous toluene (20 mL) with positive argon pressure). The cannula was transferred to a flask containing 4.80 g, 26.3 mmol. The reaction was refluxed overnight under an inert atmosphere. The reaction was cooled to room temperature and quenched with water. Dilute with ethyl acetate (150 mL) and add water (150 mL) and saturated ammonium chloride (50 mL). The layers were separated and the aqueous phase was extracted with dichloromethane (75 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. Using flash chromatography, the residue produced using 80% hexane / 20% (20% ethyl acetate / hexane) to 0% hexane / 100% (20% ethyl acetate / hexane) with a step gradient in 20% increments. Elution was carried out to obtain yellow crystals (3.08 g, 48%). ES / MS *m/z* 245.3 (*M* + 1)⁺.

(Alternative procedure)

A mixture of magnesium shavings (3.5 g, 144 mmol) and catalytic amount of iodine (100 mg) in THF (100 mL) was heated to 65 ° C. under a nitrogen atmosphere. A few drops of neat 4-bromoheptane was added and the mixture was heated until the reaction started. A solution of 4-bromoheptane (17.6 mL, 94.9 mmol) in THF (42 mL) was then added over 2 hours keeping the temperature at 65-70 ° C. The mixture was refluxed for an additional hour and the reaction was cooled to 22 ° C. Prepared Grignard reagent was added to a solution of 7-chloro-2,5-dimethyl-pyrazolo [1,5-*a*] pyrimidine (19.2 g, 55.3 mmol) in THF (60 mL) cooled to 0 ° C. under a nitrogen atmosphere. Added below. The magnesium reagent solution was added using a cannula over 45 minutes keeping the temperature below 10 ° C. The mixture was stirred at 5 ° C. for an additional 30 minutes. To this mixture was added 10% aqueous ammonium chloride (w / w) (125 mL), and the mixture was stirred at 22 ° C. for 30 min. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 × 25 mL). The organic layers were combined and dried over sodium sulfate. The mixture was filtered and the solvent was evaporated. The crude product was purified by flash chromatography on silica gel using an eluent of hexane / ethyl acetate (5/1) to give the title compound (8 g, 62%). ES / MS *m/z* 246 (*M* + 1)⁺.

(Preparation Example 3a)

7-(1-propyl-butyl)-2,5-dimethyl-pyrazolo [1,5-*a*] pyrimidine (alternative route)

Step 1: 3-Acetyl-3-oxo-4-propyl-heptanoic acid ethyl ester Magnesium chloride (14.63 g, 153.70 mmol) was added to dichloromethane (500 mL), followed by ethyl acetoacetate (19.55 mL, 100.00 g), 153.79 mmol) were added all at once and stirred at room temperature for 1 hour. The mixture was cooled in an ice-water bath and pyridine (24.86 mL, 24.32 g, 307.39 mmol) was added dropwise. Di-*n*-propylacetyl chloride (25.06 g, 153.70 mmol) was added dropwise to this white slurry at 0 ° C. under nitrogen. After the addition was complete, the cooling bath was removed, warmed to ambient temperature and stirred for 16 hours. The reaction was quenched with 1N HCl (400 mL) and the bottom layer was separated. The organic portion was dried over magnesium sulfate, filtered and concentrated in vacuo to give a yellow oil (34 g, 86%). This material was used directly in the next step without further purification.

Step 2: 5-propyl-octane-2,4-dione 3-acetyl-3-oxo-4-propyl-heptanoic acid ethyl ester (32.4 g, 126.39 mmol) in dimethyl sulfoxide (100 mL) and water (5 mL) dissolved in. The solution was heated at 150 ° C. for 8-8 hours or the reaction was followed by GC / MS. The reaction was cooled and extracted with heptane (3 × 100 mL). The combined organic portions were washed with water (100 mL) and brine (100 mL). Concentration in vacuo at 50 ° C. removed most of the heptane. 23.29 g of an oil was obtained and used directly in the next step.

Step 3: 7-(1-propyl-butyl)-2,5-dimethyl-pyrazolo [1,5-*a*] pyrimidine 5-propyl-octane-2,4-dione (15 g, 81.40 mmol) was added to acetic acid (15 mL).) And cooled in an ice bath. 5-Amino-3-methylpyrazole (7.91 g, 81.40 mmol) was added in portions and stirred at ambient temperature. After 3 hours, the completion of the reaction was checked by GC / MS. GC showed correct regioisomer production by comparison with the reference sample. Excess acetic acid was distilled off. Water (50 mL) was added and extracted with heptane (50 mL). The heptane was washed with brine (50 mL). The organic layer was dried over MgSO₄, filtered and concentrated in vacuo to give a crude oil (15.8 g, 79%). ¹H NMR (CDCl₃) : 6.39 (s, 1H); 6.31 (s, 1H); 3.75 (m, 1H); 2.56 (s, 3H); 2.45 (s, 3H); 1.71 (q, 4H); 1.23 (m, 4H); 0.85 (t, 6H).

The following compounds were prepared using either procedure, essentially as described in Preparative Example 3. Grignard reagent was prepared using 3-bromopentane.

(Preparation Example 5)

7-(1-propyl-butyl)-3-iodo-2,5-dimethyl-pyrazolo [1,5-*a*] pyrimidine 7-(1-propyl-butyl)-2,5-dimethyl-pyrazolo [1,5-*a*] Pyrimidine (3.08 g, 12.5 mmol) was dissolved in anhydrous acetonitrile (25 mL), and *N*-iodosuccinimide (4.2 g, 18.7 mmol) was added 6 times at intervals of 10 minutes (0.70 g each time).) And added. Stir at room temperature over the weekend. Acetonitrile was distilled off and the oil was diluted with dichloromethane (100 mL). Wash with saturated ammonium chloride solution (2 × 50 mL). The organic phase was collected, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to give a dark red oil. Use flash chromatography to elute from 100% hexane / 0% (20% ethyl acetate / hexane) to 0% hexane / 100% (20% ethyl acetate / hexane) with a step gradient in 50% increments. The oil was purified to give an orange oil (10.97 g, 87%). ¹H NMR (400 MHz, CDCl₃) : 6.42 (s, 1H), 3.74-3.70 (m, 1H), 2.58 (s, 3H), 2.45 (s, 3H), 1.74-1.68 (m, 4H), 1.28-1.14 (m, 4H), 0.84 (t, J = 7.0 Hz, 6H).

The following compounds were prepared essentially as described in Preparation Example 5.

(Alternative procedure for Preparation 5)

Acetic acid (1 mL) and *N*-iodosuccinimide (6.7 g, 29.9 mmol) were added to 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-*a*] in acetonitrile (60 mL). To a solution of pyrimidine (6 g, 27.5 mmol) was added in one portion. The mixture was stirred at 22 ° C. for 2 hours. The solvent was evaporated and the residue was taken up in water (50 mL) and methyl-*t*-butyl ether (100 mL). The organic portion was separated, dried over sodium sulfate, filtered and the solvent was evaporated to give the title compound (9.2 g, 96%). ES / MS *m/z* 344 (*M* + 1)⁺.

(Preparation Example 7)

2-Trimethylsilyl-thiazole in a three-necked flask equipped with a dropping funnel and a thermometer, *n*-butyllithium (20.4 mL, 51.0 mmol, 2.5 M hexane solution) was mixed with diethyl ether (50 mL). . Cool to -78 ° C. and add dropwise a solution of thiazole (4.25 g, 50.0 mmol) in diethyl ether (50 mL). After the addition was complete, the reaction mixture was stirred at -78 ° C. for 30 minutes and then chlorotrimethylsilane (5.4 g, 50.0 mmol) was added. Stir at -78 ° C. for 1 hour and then warm to room temperature. The reaction was quenched by adding saturated sodium bicarbonate. The aqueous layer was extracted with diethyl ether. The combined organic portions were washed with brine and dried over sodium sulfate. Filtration and concentration under reduced pressure gave a residue. Purification by distillation gave 8.33 g (52-56 ° C./15 mmHg) of the title compound. ¹H NMR (400 MHz, CDCl₃) : 8.13 (d, 1H, J = 2.6 Hz), 7.54 (d, 1H, J = 2.6 Hz), 0.43 (s, 9H).

(Preparation Example 8)

5-Bromo-4-methylthiazole Bromine (9.27 mL, 182 mmol) was added to a solution of 4-methylthiazole (15.0 g, 152 mmol) in acetic acid (50 mL) at 0 °C. The reaction mixture was slowly warmed to room temperature and stirred overnight. Diluted with dichloromethane and washed with 1N NaOH and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under vacuum. The crude product was purified by silica gel column chromatography eluting with hexane / ethyl acetate (5/1) to give the title compound (9.94 g, 37%). ¹H NMR (400 MHz, CDCl₃): δ 8.69 (s, 1H), 2.43 (s, 3H).

(Preparation Example 9)

Thiazole-2-carboxylic acid ethyl ester To a mixture of 2-trimethylsilylthiazole (135 g, 858.1 mmol) in toluene (1350 mL) was added a solution of ethyl chloroformate (93.4 mL, 1.03 mol) in toluene (1350 mL). Added over 15 minutes. The reaction was stirred at 22 °C for 2 hours. This solution was added onto a 25% (w / w) aqueous solution of sodium carbonate (5 L) and stirred for 30 minutes. The organic layer was separated and the aqueous layer was re-extracted with methylene chloride (2 × 1 L). The organic layers were combined and the solvent was evaporated to give the title compound (134 g, 99%). ES / MS m / z 158 (M + 1) ⁺.

(Preparation Example 10)

Thiazole-2-carboxylic acid amide Thiazole-2-carboxylic acid ethyl ester (150 g, 0.9 mmol) was added to a mixture of methanol (75 mL) and 30% aqueous ammonium hydroxide (750 mL) and the mixture was refluxed for 1 hour. Heated. Cool to 22 °C, and evaporate the methanol under vacuum. The mixture was stirred at room temperature for 30 minutes and the solid was filtered. The isolated solid was dried under vacuum to give the title compound (98 g, 85%). ES / MS m / z 129 (M + 1) ⁺.

(Preparation Example 11)

1-methyl-5-thiazol-2-yl-1H-[1,2,4] triazole 1,1-dimethoxy-N,N-dimethyl-methanamine (240 mL) was cooled to 10 °C, and thiazole-2-carboxylic acid Acid amide (60 g, 421 mmol) was added in three portions. The mixture was stirred at 10 °C for 30 minutes. The mixture was heated to reflux gradually over 45 minutes. The produced methanol was distilled off and the reaction solution was heated at 100 °C for 1.5 hours. The mixture was cooled to 60 °C, and excess 1,1-dimethoxy-N,N-dimethyl-methanamine was removed by vacuum distillation. The residue was cooled to 22 °C, and hexane (200 mL) was added. This mixture was pulverized for 15 minutes, filtered and the solid was dried to constant weight before being used in the next step.

The solid isolated above (58 g) was added to acetic acid (630 mL) and the mixture was cooled to 10 °C. N-methyl-hydrazine (27 mL, 559 mmol) was added at a rate to keep the temperature below 15 °C. The mixture was warmed to 20 °C, over 30 minutes and then gradually heated to 90 °C. The mixture was stirred at 90 °C for 30 minutes and cooled to 22 °C. Acetic acid was removed by vacuum distillation. The residue was added to water and adjusted to pH = 8 by adding 25% aqueous sodium hydroxide. The aqueous layer was extracted with methyl-t-butyl ether (3 × 600 mL). The organic layers were combined and the solvent was evaporated. The resulting residue was purified by silica gel chromatography using an eluent of hexane / isopropanol (9/1) to give the title compound (49 g, 70%). ES / MS m / z 167 (M + 1) ⁺.

(Preparation Example 12)

5-(5-Bromo-thiazol-2-yl)-1-methyl-1H-[1,2,4] triazole Methyl 5-thiazol-2-yl-1H-[1,2,4] triazole (6.055 g; 39.4 mmol) and dimethylformamide (32 mL) were added. N-bromosuccinimide (14 g, 76.8 mmol) in three portions over 1 hour. The mixture was stirred at 22 °C for 18 hours and then added to water (300 mL) cooled to 0-5 °C. The aqueous layer was separated and extracted with methyl-t-butyl ether (2 × 200 mL). The organic layers were combined, washed with 7% aqueous sodium hydrogen carbonate solution (100 mL), and dried over sodium sulfate. Filtration and evaporation of the solvent gave the title compound (9.5 g, 93%). ES / MS m / z 178 / 181 (M + 1) ⁺.

(Preparation Example 13)

2,5-Dimethyl-3-[2-(2-methyl-2H-[1,2,4] triazol-3-yl)-thiazol-5-yl]-7-(1-propyl-butyl)-pyrazolo [1,5-a] pyrimidine 5-(5-bromo-thiazol-2-yl)-1-methyl-1H-[1,2,4] triazole (6.5 g, 23.8 mmol) and 2,5-Dimethyl-7-(1-propyl-butyl)-pyrazolo [1,5-a] pyrimidine (6 g, 24.5 mmol) is mixed in N-methylpyrrolidinone (58 mL) and stirred under nitrogen for complete dissolution. Tetra-N-butylammonium bromide (5.47 g, 16.7 mmol) and potassium acetate (11.8 g, 119 mmol) were added and the mixture was heated to 100 °C under a nitrogen atmosphere. The hot mixture was degassed by 3 cycles of vacuum / nitrogen purge. Palladium acetate (216 mg, 0.94 mmol) and tris (2,4-di-tert-butyl-phenyl)-phosphane (787 mg, 1.2 mmol) were added. The mixture was heated at 125 °C for 4 hours under nitrogen. The mixture was cooled to 22 °C, and added to water (750 mL). The aqueous layer was extracted with methyl-t-butyl ether (3 × 200 mL), the organic portions were combined and evaporated. The residue was purified by filtration through a silica gel pad eluting with hexane / ethyl acetate (4/1). Fractions containing the product were combined and the solvent was evaporated to give the title compound (7 g, 72%). ES / MS m / z 410 (M + 1) ⁺.

(Preparation Example 14)

2,5-Dimethyl-7-(1-propyl-butyl)-3-thiazol-5-yl-pyrazolo [1,5-a] pyrimidine 2-Triethylsilylthiazole (1.1) dissolved in anhydrous THF (30 mL), 765 g, 11.24 mmol) was placed in an oven-dried flask and cooled to -78 °C under an inert atmosphere. n-Butyllithium (2.5 M hexane solution, 4.5 mL, 11.24 mmol) was slowly added, and the mixture was stirred at -78 °C for 30 minutes. Anhydrous zinc chloride (2.26 g, 16.58 mmol) was added in one portion and stirred at -78 °C for 30 minutes. The reaction was warmed to room temperature, stirred for 30 minutes, and 7-(1-propyl-butyl)-3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (1.624 g, 5.18 mmol) and dichloro [1,1'-bis (diphenylphosphino) ferrocene] palladium (II) dichloromethane (0.423 g, 0.518 mmol) were added. Reflux overnight in an oil bath (90 °C) under an inert atmosphere. The reaction was cooled to room temperature, quenched with saturated sodium bicarbonate, and diluted with ethyl acetate (150 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (75 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. This product was generated using flash chromatography eluting with 100% hexane / 0% (30% THF / hexane) to 0% hexane / 100% (30% THF / hexane) with a step gradient in 10% increments. The residue was purified to give a white solid (0.720 g, 42%). ES / MS m / z 329.0 (M + 1) ⁺.

The following compounds were prepared essentially as described in Preparation Example 14.

(Alternative procedure for Preparation 15)

n-Butyllithium (76.5 mL, 191 mmol, 2.5 M hexane solution) was added to a solution of 2-trimethylsilylthiazole (30 g, 191 mmol) in THF (450 mL) at -78 °C under nitrogen. The temperature was kept below -74 °C during the addition. The mixture was stirred at -78 °C for 30 minutes, then zinc chloride, dry powder (39.9 g, 285 mmol) was added in one portion and the mixture was warmed to 22 °C over 1 hour. 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (30 g, 87 mmol) and 1,1'-bis (diphenylphosphino) ferrocene palladium (II) chloride (6.5 g, 8 mmol) was added and the mixture was heated at reflux under nitrogen for 8 hours. The mixture was cooled to 22 °C, and 10% aqueous ammonium chloride (450 mL) was added. The organic layer was separated and the aqueous layer was washed with methyl-t-butyl ether (2 × 100 mL). The organic portions were combined, dried over sodium sulfate, filtered, and the solvent was evaporated to give the title compound (20.4 g, 78%). ES / MS m / z 391 (M + 1) ⁺.

(Preparation Example 16)

7-(1-ethyl-propyl)-3-(2-iodo-thiazol-5-yl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine lithium di-tert-butylamide (150 mL, 62.4 mmol, 0.6 M THF solution) was prepared as 7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo [1,5-a] pyrimidine (17.8 g, 62.4 mmol) was added at -78 °C under a nitrogen atmosphere. The temperature was kept below -74 °C during the addition. The mixture was stirred at -78 °C for 1 hour and then a solution of N-iodosuccinimide (15 g, 63 mmol) in THF (100 mL) was added keeping the temperature below -74 °C. The reaction was gradually warmed to 22 °C, and then a 10% aqueous solution of ammonium chloride (300 mL) was added. The organic layer was separated and the aqueous layer was washed with methyl-t-butyl ether (2 × 200 mL). The organic layers were combined, dried over

sodium sulfate, filtered and the solvent was evaporated. The resulting residue was purified by silica gel chromatography eluting with hexane / acetone (5/1) to give the title compound (15 g, 60%). ES / MS m/z 427 ($M+1$)⁺.

Example 1

7- (1-ethyl-propyl)-2,5-dimethyl-3- (4-methyl-thiazol-5-yl)-pyrazolo [1,5-a] pyrimidine Rleke® zinc (in 100 mL THF) 10 g, 13.2 mL, 18.48 mmol) was added to 5-bromo-4-methylthiazole (2.13 g, 18.48 mmol) and heated at reflux for 2 hours. The mixture was cooled to room temperature and the zinc was precipitated by centrifugation. Nitrogen gas was blown into 7- (1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (900 mg, 2.62 mmol) in anhydrous tetrahydrofuran (10 mL). The organozinc bromide solution was added followed by [1,1'-bis (diphenylphosphino) ferrocene]-dichloropalladium (II) (106 mg, 0.13 mmol). The reaction mixture was stirred at reflux overnight and cooled to room temperature. Ammonium chloride solution was added to the reaction mixture and extracted with dichloromethane. The organic portion was dried over sodium sulfate, filtered and the solvent removed in vacuo. The crude product was purified by silica gel column chromatography eluting with hexane / ethyl acetate (5/1) to give the title compound (552 mg, 79%). ¹H NMR (400 MHz, CDCl₃): 6.8.80 (s, 1H), 6.48 (s, 1H), 3.63 (m, 1H), 2.57 (s, 3H), 2.43 (s, 3H), 2.40 (s, 3H), 1.85 (m, 4H), 0.90 (t, 6H, J = 7.3 Hz). ES / MS m/z 315 ($M+1$)⁺.

(Preparation Example 17)

3- (2,4-Dibromo-thiazol-5-yl)-2,5-dimethyl-7- (1-propyl-butyl)-pyrazolo [1,5-a] pyrimidine 2,5-dimethyl-7- (1-propyl-butyl)-3-thiazol-5-yl-pyrazolo [1,5-a] pyrimidine (3.15 g, 9.59 mmol) was dissolved in acetonitrile (100 mL) and N-bromosuccinimide (4.27 g), 24.0 mmol) was added in one portion. The mixture was stirred overnight under an inert atmosphere, and the reaction was confirmed to be complete using TLC. Concentrated under reduced pressure and the oil was diluted with dichloromethane (150 mL) and washed with water (75 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. Step gradient (0-10-15-20-25-30-35) from 100% hexane / 0% (30% THF / hexane) to 0% hexane / 100% (30% THF / hexane) using flash chromatography. The resulting oil was purified by eluting with 35-40-45-50-100% 30% THF / hexanes to give yellow crystals (3.70 g, 75%). ES / MS m/z (79 Br / 81 Br) 486.7 ($M+1$)⁺.

The following compounds were prepared essentially as described in Preparation Example 17 except that dichloromethane was used as the solvent.

* 1.1 equivalents of NBS was used and stirred for 3 days.

Example 4

3- (4-Bromo-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-7- (1-propyl-butyl)-pyrazolo [1,5-a] pyrimidine

An oven-dried flask was charged with 3- (2,4-dibromothiazol-5-yl)-2,5-dimethyl-7- (1-propyl-butyl)-pyrazolo [1,5-a] pyrimidine (0.973 g, 2.00 mmol), anhydrous dioxane (20 mL), morpholine (0.872 g, 10.0 mmol), and cesium carbonate (1.95 g, 5.00 mmol). Reflux overnight in an oil bath (105 °C) under an inert atmosphere. LC / MS was used to confirm that the reaction was complete. Diluted with ethyl acetate (100 mL), washed with water (50 mL), and the aqueous layer was back extracted with ethyl acetate (50 mL). The organic phases were combined, dried over magnesium sulfate, filtered and concentrated under reduced pressure. Using flash chromatography with a step gradient in 10% increments from 100% hexane / 0% (40% dichloromethane / 20% ethyl acetate / 2% 7N ammonia in methanol / 38% hexane) to 0% hexane / 100% (The resulting residue was purified eluting with 40% dichloromethane / 20% ethyl acetate / 2% 7N ammonia in methanol / 38% hexane) to give an off-white solid (0.678 g, 89%). ES / MS m/z (79 Br) 491.7 ($M+1$)⁺.

The following examples were prepared essentially as described in Example 4, using either 2,0 dimethylamine / THF or morpholine as the amine and THF or dioxane as the solvent. The reaction was carried out in a sealed tube or a Schlenk tube.

* 4 equivalents of cesium carbonate were used.

(Example 10)

3- [4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7- (1-propyl-Butyl)-pyrazolo [1,5-a] pyrimidine

An oven-dried flask was charged with 1-methyl-1,2,4-triazole (0.498, 5.00 mmol) and anhydrous THF (20 mL) and cooled to -78 °C under an inert atmosphere. n-Butyllithium (2.5 M hexane solution, 2.4 mL, 6.0 mmol) was slowly added and stirred for 30 minutes. Anhydrous zinc chloride (1.36 g, 10.0 mmol) was added in one portion and stirred at -78 °C for 30 minutes. The reaction was warmed to room temperature, stirred for 30 minutes, and 3- (2,4-dibromothiazol-5-yl)-2,5-dimethyl-7- (1-propyl-butyl)-pyrazolo [1,5-a] pyrimidine (0.973 g, 2.00 mmol) and dichloro [1,1'-bis (diphenylphosphino) ferrocene] palladium (II) dichloromethane (3.163 g, 0.200 mmol) were added. The reaction was refluxed overnight in an oil bath (90 °C) under an inert atmosphere. The reaction was cooled to room temperature, quenched with water and diluted with ethyl acetate (100 mL). The layers were separated and the aqueous layer portion was extracted with dichloromethane (50 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. Using flash chromatography with a step gradient in 10% increments from 100% hexane / 0% (10% acetonitrile / 40% THF / 50% hexane) to 0% hexane / 100% (10% acetonitrile / 40% THF / 50% hexane) The resulting residue was purified by elution with hexane to give a white solid (0.090 g, 9%). ES / MS m/z (79 Br) 487.7 ($M+1$)⁺.

(Alternative preparation example)

2,5-Dimethyl-3- [2- (2-methyl-2H- [1,2,4] triazol-3-yl)-thiazol-5-yl]-7- (1- To a solution of propyl-butyl)-pyrazolo [1,5-a] pyrimidine (6 g, 14.65 mmol), N-bromosuccinimide (2.74 g, 15.4 mmol) was added in one portion and stirred at 22 °C for 10 hours. The solvent was evaporated and the residue was dissolved in a mixture of water (50 mL) and methyl-t-butyl ether (100 mL). The organic layer was separated and the aqueous layer was extracted with additional methyl-t-butyl ether (2 x 50 mL). The organic portions were combined and the solvent was evaporated. The resulting material was purified by filtration through a silica gel pad eluting with hexane / ethyl acetate (3/1). Fractions containing product were combined and the solvent was evaporated. Heptane (25 mL) was added to powder this solid. The solid was filtered and dried under vacuum to give the title compound (5.5 g, 77%). ES / MS m/z (79 Br / 81 Br) 488/490 ($M+1$)⁺.

Example 10a

3- [4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7- (1-propyl-Butyl)-pyrazolo [1,5-a] pyrimidine, hydrochloride

3- [4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7- (1-propyl-Butyl)-pyrazolo [1,5-a] pyrimidine (756 mg, 1.54 mmol) was dissolved in acetone (5 mL) and 1M HCl diethyl ether solution (1.84 mL, 1.84 mmol) was added. The mixture was stirred at room temperature for 3 hours and concentrated in vacuo. The residue was dissolved in diethyl ether / hexane = 1/1 (5 mL) to crystallize the desired HCl salt (526 mg, 65%). ES / MS m/z (79 Br) 490 ($M+1$)⁺; ¹H-NMR (CDCl₃): 8.20 (s, 1H), 6.82 (s, 1H), 4.21 (s, 3H), 3.64 (m, 1H), 2.49 (m, 3H), 2.44 (s, 3H), 1.75 (m, 4H), 1.96 (m, 4H), 0.81 (M, 6H).

The following compounds were prepared essentially as described in Example 10.

* 0.5M zinc chloride in THF was used instead of anhydrous zinc chloride. Stir at 80 °C for 3 days.

(Example 12)

3- (4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl)-thiazol-5-yl)-7- (1-ethyl-propyl)-2,5-Dimethyl-pyrazolo [1,5-a] pyrimidine

Under a nitrogen atmosphere, n-butyllithium (2.5 M hexane solution, 0.6 mL, 1.5 mmol) was added to 1-methyl-1,2,4-triazole (124.5 mg, 1.5 mmol) in THF (3 mL). At -78

¹ C. and stirred for 30 minutes. Anhydrous zinc chloride (409 mg, 3.0 mmol) was added and stirring was continued for 30 minutes, warmed to room temperature and stirred for 2 hours. Add 3-(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (229 mg, 0.5 mmol). Then tetrakis (triphenylphosphine) palladium (58 mg, 0.05 mmol) was added and refluxed overnight. Cool to room temperature, dilute with ethyl acetate and wash with saturated ammonium chloride. The organic portion was dried over sodium sulfate, filtered and concentrated to give a residue. The crude product was purified by flash chromatography eluting with hexane: ethyl acetate (10: 2.5) to give the title compound as a yellow foam (77 mg). ^{MS (APCI) m/z} (79 Br) 460.4 (M + 1) +

(Alternative preparation from Example 21)

Acetic acid (1 mL) and N-bromosuccinimide (4.1 g, 22 mmol) were added to 7-(1-ethyl-propyl)-2,5-dimethyl-3-[2-(2-methyl)-1,2,4-triazol-3-yl]-thiazol-5-yl-pyrazolo [1,5-a] pyrimidine (8 g, 21 mmol) was added. The mixture was stirred at 22 ° C. for 2 hours. The solvent was evaporated and water (50 mL) and methyl-t-butyl ether (100 mL) were added to the resulting residue. The organic portion was separated, dried over sodium sulfate, filtered and the solvent was evaporated.

The resulting residue was recrystallized from isopropyl alcohol to give the title compound (8.7 g, 90%). ¹⁵ / ^{MS (m/z)} (79 Br / 81 Br) 460/462 (M + 1) +.

Example 12a

3-(4-Bromo-2-(2-methyl-2H-[1,2,4] triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine, p-toluenesulfonic acid 3-(4-bromo-2-(2-methyl-2H-[1,2,4] triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (50 mg, 0.109 mmol) was dissolved in acetone (3 mL). A 0.25M aqueous solution of p-toluenesulfonic acid (434.4 μL, 0.109 mmol) was added and the resulting mixture was evaporated to dryness. Ethyl acetate (12 mL) was added to partially dissolve this solid. Methanol (1 mL) was added to give a clear solution. The solution was concentrated by gentle evaporation until crystals were observed. The crystals were isolated by filtration and dried under vacuum at 25 ° C. to give 50 mg of the title compound.

The stoichiometry of this salt was determined by ion chromatography using the following HPLC conditions: Column: Phenomenex Phenosphere SAX at 30 ° C., 4.6 × 150 mm; Mobile phase: 50% acetonitrile / 50% 0.025M phosphorus Sodium acid buffer (pH = 4.5); flow rate = 1.5 mL / min; detection: UV at 255 nm; injection volume = 5 μL; measurement time = 3 minutes. Calculated theoretical amount: 27.2% tosylate; Found: 28.4% tosylate (average of 3 HPLC measurements).

(Example 13)

{4-Chloro-5-[2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo [1,5-a] pyrimidin-3-yl]-thiazol-2-yl}-dimethylamine oven dried in a prepared flask was {4-bromo-5-[2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo [1,5-a] pyrimidin-3-yl]-thiazol-2-yl}-dimethylamine. (0.20, 0.44 mmol) and anhydrous THF (3.0 mL) were charged and cooled to -78 ° C. under inert atmosphere. n-Butyllithium (1.6M hexane solution, 0.42 mL, 0.67 mmol) was added slowly and stirred for 30 minutes. N-chlorosuccinimide (0.120 g, 0.889 mmol) was added in one portion and stirred at -78 ° C. for 30 minutes. The reaction was warmed to room temperature and stirred for 5 hours and the progress was checked using LC / MS. Dilute with ethyl acetate (100 mL), wash with saturated ammonium chloride (50 mL), and back-extract the aqueous layer with dichloromethane (50 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. This was generated using flash chromatography eluting with 100% hexane / 0% (25% THF / hexane) to 0% hexane / 100% (25% THF / hexane) with a step gradient in 10% increments. The residue was purified to give a white solid (0.087 g, 48%). ^{ES / MS m/z} (35 Cl) 406.0 (M + 1) +.

The following examples were prepared essentially as described in Example 13, using the appropriate bromothiazole prepared above.

(Preparation Example 18)

A mixture of thiazolidine-2,4-dione (50 g, 0.43 mol) in 2,4-dichlorothiazole phosphorus oxychloride (240 mL) was cooled to 5 ° C. and pyridine (34 mL, 0.43 mol) was added over 15 minutes, added. The mixture was heated to 125 ° C. for 4 hours and cooled to 22 ° C. Excess phosphorus oxychloride was removed by vacuum distillation and the residue was added to water (1 L) cooled to 5 ° C. This mixture was extracted with methylene chloride (3 × 400 mL). The organic portions were combined and the solvent was evaporated to give the title compound (50 g, 76%). ^{ES / MS m/z} (35 Cl) 35 Cl / 35 Cl 37 Cl / 37 Cl 37 Cl 153/155/157 (M + 1) +

(Preparation Example 19)

4-Chloro-2-morpholino-thiazole To a mixture of 2,4-dichlorothiazole (34 g, 0.22 mol) in acetonitrile (425 mL) was added potassium carbonate (60.9 g, 0.44 mol), followed by morpholine (21.2 mL, 0.225 mol) was added dropwise over 30 minutes. The mixture was refluxed at 40 ° C. and then cooled to 22 ° C. The mixture was filtered and the filtrate was evaporated. The residue was triturated with i-propyl alcohol (50 mL) at 22 ° C. for 1 hour. The solid was filtered and dried under vacuum to constant weight to give the title compound (14.5 g, 76%). ^{ES / MS m/z} (35 Cl) 205 (M + 1) +.

(Example 16)

3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine
Under a nitrogen atmosphere, 3-(4-bromo-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] Pyrimidine (116 mg, 0.25 mmol) was dissolved in THF (1.5 mL) and cooled to -78 ° C. n-Butyllithium (0.1 mL, 2.5 M hexane solution, 0.25 mmol) was added, and the mixture was stirred at -78 ° C. for 30 minutes. N-chlorosuccinimide (33.4 mg, 0.25 mmol) was added, stirred for an additional 30 minutes and slowly warmed to room temperature. After stirring overnight, the reaction was quenched by adding a solution of saturated ammonium chloride and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated to give a residue. The crude product was purified by flash chromatography eluting with hexane: dichloromethane: ethyl acetate (5: 5: 2) to give the title compound (54 mg). ^{MS (APCI) m/z} (35 Cl) 420.6 (M + 1) +; ¹ H NMR (400MHz, CDCl₃) 6.44 (s, 1H), 3.79 (t, 4H, J = 4.8 Hz), 3.63-3.56 (m, 1H), 3.47 (t, 4H, J = 4.8 Hz), 2.55 (s, 3H), 2.45 (s, 3H), 1.88-1.75 (m, 4H), 0.87 (t, 6H, J = 7.5 Hz).

(Alternative preparation example from Preparation Example 6)

7-(1-Ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine, (9 g, 26.2 mmol) and 4-chloro-2-morpholino-thiazole (7.5 g, 36.7 mmol) was mixed in dimethylformamide (90 mL) that had previously been degassed with nitrogen. Cesium carbonate (17.8 g, 55 mmol), copper iodide (250 mg, 1.31 mmol), triphenylphosphine (550 mg, 2.09 mmol) and palladium acetate (117 mg, 0.52 mmol) were added. The mixture was heated to 125 ° C. for 15 hours and cooled to 22 ° C. Water (300 mL) was added and extracted with methyl-t-butyl ether (3 × 200 mL). The organic portions were combined and the solvent was evaporated. Purification by silica gel chromatography eluting with hexane / ethyl acetate (4/1) gave the title compound (6.4 g, 62%). ^{ES / MS m/z} (35 Cl) 420 (M + 1) +.

Example 16a

3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine, hydrochloride 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (1.49 g, 3.33 mmol) was dissolved in acetone (10 mL) at 50 ° C. and cooled to room temperature. Hydrogen chloride (2M diethyl ether solution, 2.0 mL, 4.0 mmol) was added and stirred well in the sonicator. The solution was concentrated slightly and a minimum amount of diethyl ether was added to crystallize the HCl salt. The mixture was cooled in the refrigerator overnight. Additional hydrogen chloride (2M diethyl ether solution, 2.0 mL, 4.0 mmol) was added and cooled in the refrigerator. The crystalline material was filtered and dried to give the title compound (1.15 g, 75%). ^{ES / MS m/z} (35 Cl) 420 (M + 1) +; ¹ H NMR (CDCl₃) 9.18 (br, 1H), 5.86 (s, 1H), 3.73 (m, 4H), 3.49 (m, 1H), 3.39 (m, 4H), 2.48 (s, 3H), 2.38 (s, 3H), 1.79 (m, 4H), 0.79 (m, 6H).

(Example 17)

3-(4-Bromo-2-butyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine n-Butyl lithium (2.5 M hexane solution, 0.2 mL, 0.5 mmol) was added to 3-

(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl) in THF (3 mL). To a solution of 2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (230 mg, 0.5 mmol) was added at -78°C . After 30 minutes, 1-iodobutane (108 mg, 0.75 mmol) was added and stirring was continued for 1 hour. Warmed to room temperature and stirred for 1 hour. The reaction was quenched by adding saturated ammonium chloride solution and extracted with ethyl acetate. The organic portion was dried over sodium sulfate, filtered and concentrated to give a residue. The crude product was purified by flash chromatography eluting with hexane / ethyl acetate (10 / 1.5) to give the title compound as an orange foam (76 mg). ES / MS m/z (B) 437.4 (M + 1) $^{+}$.

The following examples were prepared essentially as described in Example 17.

(Example 19)

7-(1-Ethyl-propyl)-3-(2-methoxymethyl-thiazol-5-yl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine Under nitrogen atmosphere, n-butyllithium (2.5M hexane solution, 0.4 mL, 1.0 mmol) was added to 7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo [1,5-a] pyrimidine (150 mg, 0.5 mmol) in THF (3 mL). To a stirred solution of 5-a] pyrimidine (300 mg, 1.5 mmol) was added at -78°C . Stir for 30 minutes and add 1-iodomethyl methyl ether (205 mg, 1.2 mmol). Stirring was continued for 1 hour, then slowly cooled to room temperature and stirred overnight. The reaction was quenched by adding saturated ammonium chloride solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo to give a residue. The crude product was purified by flash chromatography eluting with hexane / ethyl acetate (10/2) to give the title compound as a yellow foam (184 mg). MS (APCI) m/z 345.3 (M + 1) $^{+}$.

(Example 20)

3-(4-Chloro-2-methoxymethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine n-butyllithium (2.5M hexane solution, 174 mL, 0.43 mmol) was added to 7-(1-ethyl-propyl)-3-(2-methoxymethyl-thiazol-5-yl)-2,5-dimethyl- in THF (3 mL). To a stirred solution of pyrazolo [1,5-a] pyrimidine (150 mg, 0.43 mmol) was added at -78°C . Stir for 30 minutes and add N-chlorosuccinimide (57 mg, 0.653 mmol). Stir for 30 minutes, then warm the reaction slowly to room temperature and allow the reaction to continue overnight. The reaction was quenched by adding saturated ammonium chloride solution, extracted with ethyl acetate, dried over sodium sulfate, filtered and concentrated to give a residue. Purification by flash chromatography eluting with hexane / ethyl acetate (10/2) gave the title compound (7 mg). MS (APCI) m/z (< 35 > C) 379.3 (M + 1) $^{+}$.

(Example 21)

7-(1-Ethyl-propyl)-2,5-dimethyl-3-[2-(2-methyl-2H-[1,2,4] triazol-3-yl)-thiazol-5-yl]-pyrazolo [1,5-a] pyrimidine 3-(4-bromo-2-(2-methyl-2H-[1,2,4] triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (175 mg, 0.38 mmol) and copper (I) chloride (132 mg, 1.33 mmol) were mixed in DMF (5 mL). Heated at 120°C for 24 hours. Cool to room temperature, dilute with ethyl acetate and wash with brine. The organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to a residue. Purification by flash chromatography eluting with hexane then hexane / EtOAc (10 / 1.8) gave a yellow to orange solid (45 mg). ES / MS m/z 382.0 (M + 1) $^{+}$.

(Alternative preparation example)

n-Butyllithium (2.5M hexane solution, 57.5 mL, 144 mmol) was added to a solution of N-methylthiazole (11.95 g, 144 mmol) in THF (600 mL) at -78°C under a nitrogen atmosphere. The temperature was kept below -74°C during the addition. Zinc chloride dry powder (26 g, 192 mmol) was added in one portion and the mixture was warmed to 22°C over 1 hour. 7-(1-Ethyl-propyl)-3-(2-iodo-thiazol-5-yl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (12.5 g, 29 mmol) and tetrakis (tri Phenyl) phosphine palladium catalyst (1.15 g, 0.01 mol) was added in one portion and the mixture was heated at reflux under nitrogen for 8 hours. The mixture was cooled to 22°C and water (300 mL) was added. The organic layer was separated and the aqueous layer was extracted with methyl-t-butyl ether (2 x 200 mL). The organic portions were combined, dried over sodium sulfate, filtered and the solvent was evaporated. Purification using hexane / ethyl acetate (4/1) eluting through a silica gel pad afforded the title compound (8 g, 72%). ES / MS m/z 382 (M + 1) $^{+}$.

(Example 22)

3-(4-Chloro-2-(2-methyl-2H-[1,2,4] triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-Dimethyl-pyrazolo [1,5-a] pyrimidine 7-(1-ethyl-propyl)-2,5-dimethyl-3-[in dichloromethane (0.5 mL) and acetonitrile (0.5 mL) in a vial. 2-(2-Methyl-2H-[1,2,4] triazol-3-yl)-thiazol-5-yl)-pyrazolo [1,5-a] pyrimidine (20 mg, 0.052 mmol) and N-chloro A mixture of succinimide (7.6 mg, 0.0569 mmol) was stirred at room temperature overnight. Concentration gave a residue. Purification by flash chromatography eluting with hexane then hexane / ethyl acetate (10 / 1.5) gave the title compound (16 mg). ES / MS m/z (< 35 C) 416.0 (M + 1) $^{+}$.

(Example 23)

7-(1-Ethyl-propyl)-3-(4-methoxy-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine Sealed 3-(4-bromo-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl- in methanol (3 mL) in a 4 mL vial A mixture of pyrazolo [1,5-a] pyrimidine (162 mg, 0.35 mmol), sodium methoxide (57 mg, 1.05 mmol) and copper (I) iodide (57 mg, 0.35 mmol) was stirred at 120°C for 15 hours. Cool to room temperature, remove the solid by filtration, and concentrate the filtrate in vacuo. The residue was purified by flash chromatography eluting with hexane / THF (10/2). The product was recrystallized from methanol to give the title compound (20 mg). ES / MS m/z 416.0 (M + 1) $^{+}$.

(Preparation Example 20)

4-Bromo-5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl]-thiazole-2-carbaldehyde under nitrogen atmosphere, n-Butyllithium (1.6 M in hexane, 0.312 mL, 0.50 mmol) was added to 3-(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl- A solution of pyrazolo [1,5-a] pyrimidine (230 mg, 0.50 mmol) in THF (2.5 mL) was added at -78°C and stirred for 30 minutes. A solution of N-formylmorpholine (58 mg, 0.50 mmol) in THF (0.5 mL) was added. Stir for 1 hour and store the reaction at -20°C overnight. The reaction was warmed to room temperature, diluted with ether and quenched by the addition of 4N HCl (4 mL). The layers were separated and the organic phase was extracted with 4N HCl (2 x 4 mL). The aqueous layers were combined, treated with solid sodium bicarbonate to pH = 8-9 and extracted with diethyl ether. All organic layers were combined, washed with brine, dried over sodium sulfate, filtered and concentrated to a residue. The crude product was purified by flash chromatography eluting with hexane / dichloromethane / ethyl acetate (5/5/1) to give the title compound (154 mg). MS (APCI) m/z (B) 409.0 (M + 1) $^{+}$.

(Example 24)

3-(4-Bromo-2-morpholin-4-yl-methyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine 4-Bromo-5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl]-thiazole-2-carbaldehyde (150 mg, 0.368 mmol), Morpholine (35 mg, 0.405 mmol) and sodium triacetoxyborohydride (97 mg, 0.46 mmol) were mixed in dichloromethane (3 mL) and methanol (0.5 mL). Stir overnight and add additional morpholine (35 mg, 0.405 mmol) and sodium triacetoxyborohydride (97 mg, 0.46 mmol) and stir for an additional 4 hours. The solvent was removed under vacuum, diluted with dichloromethane and washed with brine. The organic phase was dried over sodium sulfate, filtered and concentrated under vacuum. The resulting material was purified using flash chromatography eluting with dichloromethane: 2M ammonia in methanol (10:1) to give a mixture. The mixture was purified using a reverse phase column eluting with water / acetonitrile (80/20) to water / acetonitrile (10/90) to give the title compound (20 mg). ES / MS m/z (B) 409.0 (M + 1) $^{+}$.

(Example 25)

3-(4-Bromo-2-pyridin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine Under nitrogen atmosphere N-butyllithium (1.6M hexane solution, 0.312 mL, 0.5 mmol) was added to 3-(2,4-dibromo-thiazol-5-yl)-7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (229 mg, 0.5 mmol) was added at -78°C . After stirring for 30 minutes, anhydrous zinc chloride (264 mg, 1.5 mmol) was added and stirring was continued for 30 minutes. The reaction was warmed to room temperature

and stirred for 1 hour. 4-iodopyridine (103 mg, 0.5 mmol) was added, followed by 1,1'-bis (diphenylphosphino) ferrocenepalladium (II) chloride (dichloromethane adduct) (0.403 mg, 0.05 mmol). It was. The reaction was heated at reflux overnight. Cool to room temperature, dilute with ethyl acetate and wash with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated to give a residue. The crude product was purified by flash chromatography eluting with 2N ammonia in dichloromethane: methanol (10: 0.75) to give a mixture. The mixture was purified using a reverse phase column eluting with water: acetonitrile = 80: 20 to water: acetonitrile = 10: 90 to give the title compound (10 mg). ES / MS m / z (⁷⁹ Br) 456.0 (M + 1) ⁺.

(Preparation Example 21)

4-trifluoromethyl-thiazol-2-ylamine thioureas (4.0 g, 52.3 mmol) and 3-bromo-1,1,1-trifluoropropan-2-one (5.5 mL, 10 g, 52.3 mmol) was added to ethanol (100 mL) and heated at 50 ° C. for 2 hours. Cool to room temperature and concentrate to dryness. This residue was dissolved in water and the pH was adjusted to greater than 12 using 2M NaOH. Extracted with diethyl ether (4x). The combined organic extracts were dried over sodium sulfate, filtered and concentrated under vacuum. The resulting material was purified by silica gel chromatography (CH₂ Cl₂) to give the title compound (5.9 g, 79%). ES / MS m / z 159 (M + 1) ⁺.

(Preparation Example 22)

5-Bromo-4-trifluoromethyl-thiazol-2-ylamine, hydrobromide Bromine (2.0 mL, 6.28 g, 39.3 mmol) was added to 4-trifluoromethyl- in diethyl ether (60 mL). Thiazol-2-ylamine (5.0 g, 35.7 mmol) was added dropwise to the solution cooled in an ice bath. Stir for 1 hour after the end of the addition and then warm to room temperature. The solid was collected by filtration and washed with diethyl ether to give the title compound (10.5 g, 90%). ES / MS m / z (⁷⁹ Br / ⁸¹ Br) 247/249 (M + 1) ⁺.

(Preparation Example 23)

5-Bromo-2- (2,5-dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazole hexane-2,5-dione (3.5 mL, 3.4 g, 30.2 mmol) was added to methanol. To a solution of 5-bromo-4-trifluoromethyl-thiazol-2-ylamine hydrobromide (9.0 g, 27.4 mmol) in (60 mL). Stir overnight at room temperature. Phosphate buffer (50 mL, pH = 7) was added. The resulting precipitate was collected by filtration and washed with water. The filter cake was dissolved in CH₂ Cl₂ and dried over sodium sulfate. Filtration and concentration in vacuo gave the title compound (8.2 g, 92%). ¹H NMR (400 MHz, CDCl₃) δ 5.91 (s, 2H), 2.27 (s, 6H).

(Example 26)

3- (2- (2,5-Dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine

A solution of 5-bromo-2- (2,5-dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazole (2.2 g, 5.6 mmol) in THF (25 mL) was placed in a dry ice bath. Cooled down, t-butyllithium (1.7 M pentane solution, 8.5 mL, 14.5 mmol) was added dropwise. Stir for 45 minutes, then add zinc chloride (0.5 M in THF, 14.6 mL, 7.3 mmol) dropwise. Stir for 5 minutes and remove the cooling bath. Stir for 30 minutes, then 7- (1-ethyl-propyl) -3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (1.5 g, 4.4 mmol) and bis (triphenylphosphine) palladium (0) (450 mg, 0.9 mmol) was added. Refluxed for 24 hours. The reaction was cooled and the mixture was poured into diethyl ether and washed with water (2 x). The combined aqueous layer was extracted with diethyl ether. The combined organic portions were dried over sodium sulfate, filtered and concentrated under vacuum until dryness. The resulting residue was purified by silica gel chromatography (75-100% CH₂ Cl₂ in hexanes) to give the title compound (1.47 g, 72%). ¹³C

ES / MS m / z C 20 H 26 F 3 N 5 S + H calcd for ⁺ 462.1939, found: 462.1915.

(Example 27)

5- (7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl) -4-trifluoromethyl-thiazol-2-ylamine hydroxylamine (2 mL, 50% aqueous solution) in 3- (2- (2,5-dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazol-5-yl) -7- (1-ethyl) in acetic acid (10 mL). -Propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (1.1 g, 2.3 mmol) was added. The reaction was heated to 80 ° C. for 6 hours. Cooled to room temperature. Pour into diethyl ether and wash once with 2M NaOH (2 x) then water. The organic portion was dried over sodium sulfate, filtered and concentrated under vacuum until dryness. The resulting residue was purified by silica gel chromatography (40% ethyl acetate in hexane) to give the title compound (0.76 g, 87%). ES / MS m / z 384 (M + 1) ⁺.

(Preparation Example 24)

3- (2-Bromo-4-trifluoromethyl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine in acetonitrile (20 mL) Of copper (II) bromide (540 mg, 2.4 mmol) and t-butyl nitrite (0.36 mL, 310 mg, 3.0 mmol) were heated to 60 ° C. 5- (7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl) -4-trifluoromethyl-thiazol-2-ylamine (755 mg, 2.0 mmol) was added as a solid. The reaction was heated to 80 ° C. for 2 hours. The reaction was cooled, poured into diethyl ether and extracted with water (3x). The organic layer was dried over sodium sulfate, filtered and concentrated in vacuo until dryness. The resulting residue was purified by silica gel chromatography with CH₂ Cl₂ to give the title compound (0.77 g, 87%). ES / MS m / z (⁷⁹ Br / ⁸¹ Br) 447,449 (M + 1) ⁺.

(Example 28)

5- (7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl) -4-trifluoromethyl-thiazol-2-ylamine (2-Bromo-4-trifluoromethyl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] in methanol (4 mL) The sealed reaction tube containing pyrimidine (313 mg, 0.7 mmol) and dimethylamine (2M THF solution, 4 mL, 8 mmol) was heated to 80 ° C. for 2 hours. The reaction was cooled and concentrated under vacuum. The residue produced by silica gel chromatography eluting with CH₂ Cl₂ containing 0-30% ethyl acetate was purified to give the title compound (0.28 g, 97%). ¹³C 19 H 24 F 3 N 5 O S + H calcd 412.1770 for S + H ⁺. Found: 412.1783.

The following examples were prepared essentially as described for Example 28 except that the reaction was refluxed for 8 hours.

(Example 30)

N- (5- (7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl) -4-trifluoromethyl-thiazol-2-yl) -Acetamide hydroxylamine (50% aqueous solution, 5 mL) was added 3- (2- (2,5-dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazol-5-yl) in acetic acid (25 mL). 7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (2.4 g, 5.2 mmol) was added to the solution. The reaction was heated to 80 ° C. for 72 hours. The reaction was cooled to room temperature, poured into diethyl ether, washed once with 2M NaOH (2 x) and then water. The organic portion was dried over sodium sulfate, filtered and concentrated to dryness. The resulting residue was purified by column chromatography eluting with 40% ethyl acetate in hexanes to give the title compound (0.28 g, 13%). ¹³C 19 H 22 F 3 N 5 O S + H calcd for ⁺ 426.1575, Found: 426.1565.

(Example 31)

2- (5- (7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl) -4-trifluoromethyl-thiazol-2-ylamino) -Ethyl) -carbamate acid tert-butyl ester (2-Amino-ethyl) -carbamate acid tert-butyl ester (0.5 mL, 506 mg, 3.2 mmol) was added to 3- (2-bromo-4-trifluoromethyl-thiazol-5-yl) -5 in methanol (1 mL). VI) 7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (100 mg, 0.22 mmol) and triethylamine (0.3 mL, 2.2 mmol) Added to. Methanol was evaporated and heated to 80 ° C. overnight. The reaction was cooled to room temperature and concentrated under vacuum. The residue produced by column chromatography eluting with CH₂ Cl₂ containing 10-50% ethyl acetate was purified to give the title compound (0.11 g, 97%). ES / MS m / z 527.2 (M + 1) ⁺.

The following compounds were prepared essentially as described in Example 31, using the appropriate amine.

[†] Heated at reflux overnight in EtOH. EtOH was evaporated and heated at 110 ° C. for 24 hours.

^{**} Heated at 80 ° C. overnight in EtOH.

^{***} Prepared HCl salt and recrystallized from EtOAc / hexane.

(Example 33)

N1-(3-[2-(1-ethylpropyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl)-Ethane-1,2-diamine, hydrochloride (2-[5-[7-(1-ethylpropyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-3-yl]-4-Trifluoromethyl-thiazol-2-ylamino]-ethyl)-carbamate acid tert-butyl ester (101 mg, 0.192 mmol) was added to 1 M HCl in methanol (1 mL). The reaction was heated at 70 ° C. overnight. The reaction was cooled and concentrated in vacuo from methanol / ethyl acetate. The resulting residue was triturated with ethyl acetate to give the title compound (64 mg, 76%). ES / MS m / z 427.0 (M + 1) ⁺.

The following examples were prepared essentially as described for Example 33.

(Example A)

In Vivo Efficacy Assessment Using In Vitro Binding In order to assess in vivo efficacy, the compounds of the invention were evaluated using in vitro binding. D. R. The compounds were administered to rats by the oral route using the procedure provided in Gehert et al., EJP 539, 145-153 (2005). The binding of ¹²⁵I-sorbazine to the cerebellum was evaluated in vitro as described by Gehert et al. For example, Example 15 resulted in 65% inhibition at 10 mg / kg.

(Example B)

CRF1 filter binding method The limitation of plasmid-based human CRF1 expression in generating recombinant cell lines with sufficient receptor density to develop binding studies is the Phoenix retrovirus expression system licensed from Stanford. Overcome with Membranes were prepared using a stable HEK-hCRF1 cell line and the binding reaction (200 µL) was set up as follows: 50 µL ¹²⁵I-sorbazine (0.2 nM final concentration), 50 µL compound and 100 µL CRF1 membrane (25 µg / reaction). The reaction was incubated at room temperature for 2 hours and stopped by filtering through pretreated FB Millipore glass fiber filter plates (96 wells). The plate was washed twice with ice-cold test buffer (50 mM Tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM MgCl₂, 0.05% BSA, pH 7.2), air dried overnight, and in a MicroBeta counter. Counting was performed using 100 µL Microscint40. Non-specific binding (NSB) was measured in the presence of 0.5 µM unlabeled sorvazine. Usually three measurements were taken and the average data points were plotted by Graph Pad Prism.

Using this test, the exemplified compounds of the invention inhibited the binding of ¹²⁵I-sorbazine (4 nM) with a K_i (inhibition constant) of less than 1 µM in roller / adherent cells. For example, Example 15 showed a K_i of 6.2 nM.

(Example C)

CRF2 filter binding method The limitation of plasmid-based human CRF2 expression in generating recombinant cell lines with sufficient receptor density to develop binding studies is the Phoenix retrovirus expression system licensed from Stanford. Overcome with Membranes were prepared using a stable HEK-hCRF2 cell line and the binding reaction (200 µL) was set up as follows: 50 µL ¹²⁵I-sorbazine (0.2 nM final concentration), 50 µL compound and 100 µL CRF2 membrane (25 µg / reaction). The reaction was incubated at room temperature for 2 hours and stopped by filtering through pretreated FB Millipore glass fiber filter plates (96 wells). The plate was washed twice with ice-cold test buffer (50 mM Tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM MgCl₂, 0.05% BSA, pH 7.2), air dried overnight, and in a MicroBeta counter. Counting was performed using 100 µL Microscint40. Non-specific binding (NSB) was measured in the presence of 0.5 µM unlabeled sorvazine. Alternatively, compounds were evaluated using a scintillation proximity assay. The test procedure was set up as follows: 50 µL of ¹²⁵I-sorbazine (0.2 nM final concentration), 50 µL of compound or unlabeled sorvazine (NSB), and 250 µg of wheat germ agglutinin (WGA) SPA beads and 100 µL containing CRF2 membrane (1.5 µg / reaction). Plates were incubated for 4-5 hours at room temperature and centrifuged at 200 xg for 10 minutes. Bound radioactivity was assessed using a Wallac TriLux scintillation counter. Usually, three measurements were taken to assess binding and the average data points were plotted by Graph Pad Prism. Compounds were screened at the initially fixed concentration and when sufficient activity was observed, a concentration-response curve was subsequently generated.

Particularly exemplified compounds of the invention were tested in the CRF2 binding test and showed weak affinity for the CRF2 receptor. For example, Example 15 showed 11% inhibition at a concentration of 50 µM. This result suggests that the compounds of the invention are selective for the CRF1 receptor (rather than CRF2).

(Example D)

Bioavailability and pharmacokinetic properties The volume of distribution (V_d) relates the amount of drug in the body to the drug concentration in blood or plasma. The volume of distribution refers to the volume of fluid that would be required to contain the entire amount of drug in the body at the same concentration as in blood or plasma. V_d = amount of drug in the body / drug in blood or plasma Concentration (Goodman and Gilman literature). For a 10 mg dose and a plasma concentration of 10 mg / L, the distribution volume would be 1 liter. The volume of distribution reflects the extent to which the drug is present in extravascular tissue. A large volume of distribution reflects that certain compounds tend to bind to tissue components rather than plasma protein binding. In the clinical setting, V_d can be used to determine the loading dose to achieve a steady state concentration.

To test for volume of distribution, male Sprague Dawley rats (N = 3) were dosed with a single intravenous dose of 1 mg / kg. Multiple plasma samples were collected at 0.03-24 hours after administration. The plasma sample was analyzed by LC / MS / MS to determine plasma concentration. Pharmacokinetic calculations in plasma were performed to determine pharmacokinetic parameters including V_d and plasma clearance (Cl_p).

The compounds of the present invention preferably have a suitable bioavailability profile. For example, most commercially available CNS drugs and cardiovascular drugs exhibit a V_d of less than 10 L / Kg for humans. Compared to CRF antagonists, CP154526 (Schulz et al., Proc. Natl. Acad. Sci. (USA), 93: 10477 (1996)) and NB3775 (Chen et al., Drug Development Research, 65: 216 (2005)) when analyzed separately, the rat V_d of 114 L / Kg and 76 L / Kg, respectively, is shown. Example 15 of the present invention, when analyzed separately, shows only 7.2 L / Kg rat V_d after a single intravenous dose of 1 mg / kg.

In addition, plasma clearance (Cl_p) is a measure of the rate of drug removal from the body. According to intravenous administration and first order rate equations, plasma clearance can be determined using the following formula: Cl_p = dose / AUC, where AUC is plasma as a function of time from zero to infinity. The total area under the curve representing the drug concentration in the medium). The referenced CRF antagonists CP154526 and NB37582, when analyzed separately, show rat clearance (Cl_p) of 53 mL / min / kg and 356 mL / min / kg, respectively, after a single intravenous administration, whereas Example 15 shows only 23.6 mL / min / kg of rat Cl_p when analyzed separately.

Patent Citations (10)

Publication number	Priority date	Publication date	Assignee	Title
JP2000050966A *	1999-02-07	2000-03-28	ジヤンセン・ファーマシユ ---アカ・ナームローゼ・フ ニンノートシヤツブ	Pyrazolopyrimidines as CRF receptor antagonists
WO2000059508A2 *	1999-04-05	2000-10-12	Du Pont Pharmaceuticals Company	Pyrazolopyrimidines as crf antagonists
JP2000302993A *	1999-04-02	2000-10-31	Pfizer Prod Inc	Corticotropin releasing factor antagonist for preventing sudden death
JP2002513382A *	1999-07-24	2002-05-08	デュポン ファーマシユ ティカルズ カンパニ---	Azolo triazines and azolopyrimidines
WO2000506375A1 *	2000-12-22	2000-07-14	Sb Pharmco Puerto Rico Inc	Crf receptor antagonists and methods relating thereto
Family To Family Citations				
CZ286882B6	1992-12-17	2000-07-12	Pfizer	Pyrolopyrimidine compounds, intermediates for their preparation and pharmaceutical preparations based thereon
EP0915880B1	1996-07-24	2007-10-10	Bristol-Myers Squibb Pharma Company	Azolo triazines and pyrimidines
JP0621706B2	1995-08-28	2005-02-16	ファイザー・インク	Substituted 6,5-hetero-bicyclic derivatives
DE60032249T2 *	1999-09-30	2007-07-19	Neurogen Corp., Branford	Amino-substituted pyrazolo [1,5-a]-1,5-pyrimidines and pyrazolo [1,5-a]-1,3,5-triazines
SEP20050609B *	2001-03-13	2005-09-12	Squibb Bristol Myers Co	4-(2-Butylamino)-2,7-Dimethyl-8-(Methyl-6-Methoxypyrid-3-yl) Pyrazolo [1,5-A]-1,3,5-Triazine, Its Enantiomers and Pharmaceutically Acceptable Salts, as Corticotropin Releasing Factor Receptor Ligands

* Cited by examiner, † Cited by third party

Non-Patent Citations (1)

Title
JPN6012062715; CHEN, C. et al.: 'Design of 2, 5-dimethyl-3- (6-dimethyl-4-methylpyridin-3-yl) -7-di-propylaminopyrazolo [1, 5-a] pyrimidine'. J. Med. Chem. Vol. 47, No. 19, 2004, p. 4787-4798 *

* Cited by examiner, † Cited by third party

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Publication number	Priority date	Publication date	Assignee	Title
JP2017503030A *	2014-01-21	2017-01-26	ニューロクライン バイオサイ ンシーズ, インコーポレイテッド	CRF1 receptor antagonist for the treatment of congenital adrenocortical hyperplasia
JP2020530831A *	2017-08-14	2020-10-29	スプリウス バイオサイエンス インコーポレイテッド	Corticotropin-releasing factor receptor antagonist
JP2020530832A *	2017-08-14	2020-10-29	スプリウス バイオサイエンス インコーポレイテッド	Corticotropin-releasing factor receptor antagonist
Family To Family Citations				
EP2025874A1	2007-08-15	2009-02-18	sanoft-aventis	Substituted tetra hydro naphthalines, method for their manufacture and their use as drugs
AU2009298757B2 *	2008-10-02	2013-08-01	Eli Lilly And Company	Thiazolyl-pyrazolopyrimidine compounds as synthetic intermediates and related synthetic processes
EP2567959B1	2011-09-12	2014-04-16	Sanoft	6-(4-hydroxy-phenyl)-3-styryl-1h-pyrazolo[3,4-b]pyridine-4-carboxylic acid amide derivatives as kinase inhibitors
PL236355B1 *	2015-04-02	2021-01-11	Celon Pharma Spolka Akcyjna	Derivatives of 7-(morpholin-4-yl)pyrazolo[1,5-d]pyrimidine as inhibitors of kinase P13
CA3059026A1	2016-09-07	2018-03-15	The Regents Of The University Of California	Allosteric corticotropin-releasing factor receptor 1 (crf1) antagonists that decrease p-tau and improve cognition

10/26/22, 11:54 AM

JP2010504344A - Thiazolepyrazolopyrimidines as CRF1 receptor antagonists - Google Patents

JP2021522340A *	2018-04-27	2021-08-30	スプリング・バイオサイエンス・ズ, インク.	Methods for Treating Testicular Adrenal Residual Tumors and Ovarian Adrenal Residual Tumors
CN109134431B *	2018-08-07	2021-05-14	中山大学	Substituted pyrrole chromone compound or pharmaceutically acceptable salt thereof, and preparation method and application thereof
AR120649A1 *	2019-12-04	2022-03-09	Neurocrine Biosciences Inc	CRF RECEPTOR ANTAGONISTS AND METHODS OF USE
GR1018696B *	2020-07-02	2021-10-08	Uni-Pharma K&W Τοτρικ Φαρμακευτικα Εργαστρια Αβεε,	Pyrazolo[1,5-a]pyrimidines as autotaxin inhibitors
WO2022036123A1 *	2020-08-12	2022-02-17	Spruce Biosciences, Inc.	Methods and compositions for treating polycystic ovary syndrome
WO2022046005A1	2020-09-26	2022-03-03	Neurocrine Biosciences, Inc	Cr1 receptor antagonists and methods of use

* Cited by examiner, † Cited by third party, ‡ Family to family citation

Similar Documents

Publication	Publication Date	Title
JP5161226B2	2013-03-13	Thiazolepyrazolopyrimidines as CRF1 receptor antagonists
JP5184336B2	2013-04-17	Thiophenepirazolopyrimidine compounds
US6734185B2	2004-05-11	Pyrrole[3,4-d]pyrimidines as corticotropin releasing factor (CRF) antagonists
KR101432316B1	2014-09-29	4,5-dihydro-[1,2,4]triazolo[4,3-f]pteridines as protein kinase pik1 inhibitors for the treatment of proliferative disorders
MX2012010610A	2012-10-03	Pyrazolopyrazine derivatives and their use as jak and syk inhibitors.
US20190263825A1	2019-08-29	Heteroaromatic compounds as vanin inhibitors
AU2006334364B2	2011-09-29	Imidazo (1,2-a)pyridin-3-yl-acetic acid hydrazides, processes for their preparation and pharmaceutical uses thereof
CA2631634A1	2007-06-21	Tricyclic lactam derivatives, their manufacture and use as pharmaceutical agents

Priority And Related Applications

Priority Applications (3)

Application	Priority date	Filing date	Title
US82626406P	2006-09-20	2006-09-20	US Provisional Application
US60/826,264		2006-09-20	
PCT/US2007/078605	2006-09-20	2007-09-17	Thiazole pyrazolopyrimidines as crf1 receptor antagonists

Legal Events

Date	Code	Title	Description
2010-09-10	A521	Written amendment	Free format text: JAPANESE INTERMEDIATE CODE: A523 Effective date: 20100909
2010-09-10	A621	Written request for application examination	Free format text: JAPANESE INTERMEDIATE CODE: A621 Effective date: 20100909
2012-11-27	TRDD	Decision of grant or rejection written	
2012-12-05	A01	Written decision to grant a patent or to grant a registration (utility model)	Free format text: JAPANESE INTERMEDIATE CODE: A01 Effective date: 20121204
2012-12-20	A61	First payment of annual fees (during grant procedure)	Free format text: JAPANESE INTERMEDIATE CODE: A61 Effective date: 20121213
2012-12-21	R150	Certificate of patent or registration of utility model	Ref document number: 5161226 Country of ref document: JP Free format text: JAPANESE INTERMEDIATE CODE: R150

			Free format text: JAPANESE INTERMEDIATE CODE: R150
2012-12-27	FPAY	Renewal fee payment (event date is renewal date of database)	Free format text: PAYMENT UNTIL: 20151221 Year of fee payment: 3
2015-12-08	R250	Receipt of annual fees	Free format text: JAPANESE INTERMEDIATE CODE: R250
2016-12-06	R250	Receipt of annual fees	Free format text: JAPANESE INTERMEDIATE CODE: R250
2017-12-12	R250	Receipt of annual fees	Free format text: JAPANESE INTERMEDIATE CODE: R250
2018-12-18	R250	Receipt of annual fees	Free format text: JAPANESE INTERMEDIATE CODE: R250
2019-12-17	R250	Receipt of annual fees	Free format text: JAPANESE INTERMEDIATE CODE: R250
2020-12-09	R250	Receipt of annual fees	Free format text: JAPANESE INTERMEDIATE CODE: R250

Concepts

machine-extracted

Download Filter table

Name	Image	Sections	Count	Query match
receptor antagonist		title,abstract,description	4	0.000
CRHR1		title,description	33	0.000
CRHR1		title,description	33	0.000
compounds		claims,abstract,description	147	0.000
Metabolic Syndrome		claims,abstract,description	10	0.000
pharmaceutical composition		claims,abstract,description	8	0.000
2-methyl-2H-[1,2,4] triazol-5-yl		claims,description	46	0.000
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salts		claims,description	42	0.000
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alcohol use disease		claims,description	10	0.000
morpholin-4-yl group		claims,description	10	0.000
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Anxiety		claims,description	7	0.000
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⌘ Irritable Bowel Syndrome	claims,description	7	0.000
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⌘ Pituitary-dependent Cushing's syndrome	claims,description	7	0.000
⌘ Premature Birth	claims,description	7	0.000
⌘ Premenstrual syndrome	claims,description	7	0.000
⌘ Sleep disease	claims,description	7	0.000
⌘ Stomach Ulcer	claims,description	7	0.000
⌘ Visceral Pain	claims,description	7	0.000
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⌘ Fibromyalgia	claims,description	6	0.000
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☞ therapeutic procedure		claims	1	0.000
☞ crf1		abstract,description	25	0.000
☞ disease		abstract,description	9	0.000
☞ Nervous System Disease		abstract,description	6	0.000
☞ neuroendocrine		abstract,description	3	0.000

Show all concepts from the description section

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Bibliographic data

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Pub/Pat no: JP2010504344A
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Applicant(s):
Classification: A61K31/519AI; A61K31/5377AI; A61P1/00AI; A61P1/04AI; A61P3/04AI; A61P9/12AI; A61P13/12AI; A61P15/06AI; A61P15/08AI; A61P15/10AI; A61P21/00AI; A61P25/00AI; A61P25/06AI; A61P25/08AI; A61P25/20AI; A61P25/22AI; A61P25/24AI; A61P25/32AI; A61P25/36AI; A61P29/00AI; A61P35/00AI; A61P37/08AI; C07D487/04AI
Application number: JP20090529316 2007-09-17
Priority number: US20060826264P 20060920; WO2007US78605 20070917;

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A 6 1 K 31/519 (2006.01)

C 07 D 487/04 C S P

4 C 0 8 6

A 6 1 K 31/5377 (2006.01)

A 6 1 K 31/519

A 6 1 P 25/24 (2006.01)

A 6 1 K 31/5377

A 6 1 P 25/22 (2006.01)

A 6 1 P 25/24

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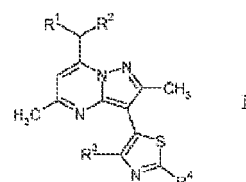
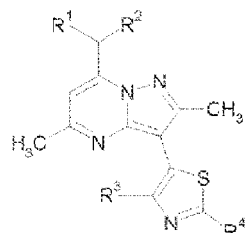
弁理士 坪井 有四郎

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(54) 【発明の名称】 CRF1 受容体アンタゴニストとしてのチアゾールピラゾロピリミジン

(57) 【要約】

本発明は、式 I の化合物、その医薬組成物、ならびに精神障害および神経内分泌系障害、神経系疾患およびメクボリックシンドロームの治療における副腎皮質刺激ホルモン放出因子 1 (CRF1) 受容体アンタゴニストとしてのその使用に関する。



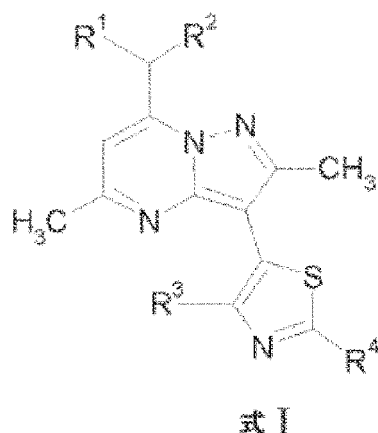
式 I

【特許請求の範囲】

【請求項1】

式Iの化合物

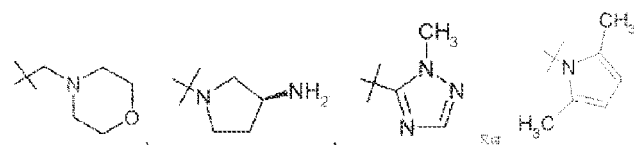
【化1】



(式中、

R¹ および R² は独立に、エチルまたは n-プロピルであり、R³ は水素、Cl、Br、メチル、トリフルオロメチルまたはメトキシであり、R⁴ は水素、Br、R^a R^b N⁻、メトキシメチル、n-ブチル、アセトアミド、ピリジン-4-イル、モルホリン-4-イル、

【化2】



であり、

R^a および R^b は独立に、水素、C₁-C₃ アルキル、H₂NCH₂CH₂-、(CH₃)₃COC(O)NHCH₂CH₂-、または CH₃CH₂CH₂NHCH₂CH₂-である)

またはその薬理的に許容できる塩。

【請求項2】

R³ が Cl、Br、メチルまたはトリフルオロメチルである、請求項1に記載の化合物またはその薬理的に許容できる塩。

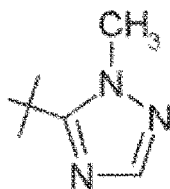
【請求項3】

R³ が Cl または Br である、請求項1または請求項2に記載の化合物またはその薬理的に許容できる塩。

【請求項4】

R⁴ が R^a R^b N⁻、ピリジン-4-イル、モルホリン-4-イル、または

【化3】

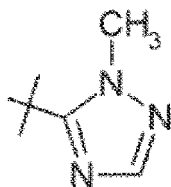


である、請求項1から請求項3のいずれか1項に記載の化合物またはその薬理学的に許容できる塩。

【請求項5】

R⁴ がモルホリン-4-イルまたは

【化4】



である、請求項1から請求項4のいずれか1項に記載の化合物またはその薬理学的に許容できる塩。

【請求項6】

R⁴ がR^a・R^b・N-であり、R^aおよびR^bが独立にC₁ - C₃ アルキルである、請求項1から請求項4のいずれか1項に記載の化合物またはその薬理学的に許容できる塩。

【請求項7】

3-〔4-ブromo-2-(2-メチル-2H-[1,2,4]トリアゾール-3-イル)-チアゾール-5-イル〕-2,5-ジメチル-7-(1-プロピル-ブチル)-ピラゾロ〔1,5-a〕ピリミジンである、請求項1に記載の化合物またはその薬理学的に許容できる塩。

【請求項8】

3-(4-ブromo-2-(2-メチル-2H-[1,2,4]トリアゾール-3-イル)-チアゾール-5-イル)-7-(1-エチル-プロピル)-2,5-ジメチル-ピラゾロ〔1,5-a〕ピリミジンである、請求項1に記載の化合物またはその薬理学的に許容できる塩。

【請求項9】

3-(4-クロロ-2-モルホリン-4-イル-チアゾール-5-イル)-7-(1-エチル-プロピル)-2,5-ジメチル-ピラゾロ〔1,5-a〕ピリミジンである、請求項1に記載の化合物またはその薬理学的に許容できる塩。

【請求項10】

請求項1から請求項9のいずれか1項に記載の化合物またはその薬理学的に許容できる塩と、薬理学的に許容できる賦形剤とを含む、医薬組成物。

【請求項11】

患者のうつ病、不安症、アルコール濫用または薬物濫用、肥満、高血圧、メタボリックシンドローム、過敏性大腸症候群、癲癇、発作、睡眠障害、アレルギー、片頭痛、月経前症候群、不妊性、性的機能不全、先天性副腎過形成、クッシング病、早産、ストレスにより誘発される胃潰瘍、炎症性障害、下垂体または異所性下垂体由来の腫瘍、慢性疲労症候群、線維筋痛、内臓痛または多発性硬化症を治療する方法であって、それを必要とする患者に治療上有効量の請求項1から請求項9のいずれか1項に記載の化合物またはその薬理

学的に許容できる塩を投与することを含む、方法。

【請求項12】

患者の不安症またはうつ病を治療する方法であって、それを必要とする患者に治療上有効量の請求項1から請求項9のいずれか1項に記載の化合物またはその薬理学的に許容できる塩を投与することを含む、方法。

【請求項13】

患者のアルコール濫用または薬物濫用を治療する方法であって、それを必要とする患者に治療上有効量の請求項1から請求項9のいずれか1項に記載の化合物またはその薬理学的に許容できる塩を投与することを含む、方法。

【請求項14】

治療における使用のための、請求項1から請求項9のいずれか1項に記載の化合物またはその薬理学的に許容できる塩。

【請求項15】

うつ病、不安症、アルコール濫用または薬物濫用、肥満、高血圧、メタボリックシンドローム、過敏性大腸症候群、癲癇、発作、睡眠障害、アレルギー、片頭痛、月経前症候群、不妊性、性的機能不全、先天性副腎過形成、クッシング病、早産、ストレスにより誘発される胃潰瘍、炎症性障害、下垂体または異所性下垂体由来の腫瘍、慢性疲労症候群、線維筋痛、内臓痛または多発性硬化症の治療において使用するための、請求項1から請求項9のいずれか1項に記載の化合物またはその薬理学的に許容できる塩。

【請求項16】

不安症またはうつ病の治療において使用するための、請求項1から請求項9のいずれか1項に記載の化合物またはその薬理学的に許容できる塩。

【請求項17】

アルコール濫用または薬物濫用の治療において使用するための、請求項1から請求項9のいずれか1項に記載の化合物またはその薬理学的に許容できる塩。

【発明の詳細な説明】

【技術分野】

【0001】

本発明は、精神障害および神経内分泌系障害、神経系疾患およびメタボリックシンドロームの治療におけるCRF1受容体アンタゴニストとしての新規なチアゾールピラゾロピリミジン化合物、その医薬組成物およびそれらの使用に関する。

【背景技術】

【0002】

副腎皮質刺激ホルモン放出因子(CRF)は、脳下垂体前葉からのプロオピオメラノコルチン(POMC)由来のペプチド分泌の主要な生理的調節因子である41アミノ酸のペプチドである。下垂体でのその内分泌の役割に加えて、CRFの免疫組織化学的局在から、このホルモンが中枢神経系の幅広い視床下部外分布を有し、脳での神経伝達物質または神経修飾物質としての役割と整合した広い範囲の自律神経的効果、電子生理的効果および行為への効果を生じることが証明された。免疫系における生理的、心理的、および免疫学的ストレス因子に対する反応を統合する面でCRFが重要な役割を果たすという証拠もある。

【0003】

CRFは、うつ病および神経不安症を含めた精神障害および神経系疾患、ならびにアルツハイマー病、ハンチントン病、進行性核上麻痺、筋萎縮性側索硬化症、パーキンソン病、癲癇、片頭痛、アルコール濫用および薬物濫用および関連する禁断症状、肥満、メタボリックシンドローム、先天性副腎過形成、クッシング病、高血圧、発作、過敏性大腸症候群、ストレスにより誘発される胃潰瘍、月経前症候群、性的機能不全、早産、炎症性障害、アレルギー、多発性硬化症、内臓痛、睡眠障害、下垂体部腫瘍または異所性下垂体由来の腫瘍、慢性疲労症候群および線維筋痛に関連付けられてきた。

【0004】

CRF受容体亜型(CRF1およびCRF2)が確認されており、これらは脳内に不均一に分布しており、これは機能的多様性の可能性を示唆している。例えば、広く分布した脳CRF1受容体は、環境ストレス因子への曝露に付随する情動性に強く関係する。重要なことは、CRF2受容体ではなくCRF1受容体が、不安惹起様行動の選択を媒介するように見えることである。より離散的な中隔／視床下部分布および別の内在性リガンドの入手可能性は、CRF2受容体の異なる機能的な役割を示唆する。例えば、CRF1受容体よりもCRF2受容体に対して優先的な親和性を有する新規なCRF族の神経ペプチドが、選択的なCRF1アゴニズムで観察される行動活性化の外観を生成することのなく食欲を抑制することが報告されている。他の場合には、CRF2アゴニズムは、CRF1アンタゴニストまたはCRF1遺伝子欠失について報告されている効果に類似の効果をもたらす。例えば、CRF2アゴニストが抗肥満薬として提唱されてきたが、CRF1アンタゴニストも同様に肥満に対する重要な治療法であるかも知れない。

【0005】

CRFアンタゴニストとして有用な特定のピロロ[2, 3-d]ピリミジン、ピロロ[3, 2-d]ピリミジン、ピラゾロ[1, 5-a]ピリミジン、1, 2, 3-トリアゾロ[4, 5-b]ピリジン、およびピラゾロ[1, 5-a]-1, 3, 5-トリアジンが特許文献1、特許文献2、特許文献3、および特許文献4に記載されている。

【国際調査報告】

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International application No. PCT/US2007/078605		
A. CLASSIFICATION OF SUBJECT MATTER INV. C07D487/04 A61K31/505 A61P29/00 A61P25/22 A61P25/24 A61P25/30		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C07D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, BEILSTEIN Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
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☒ Further documents are listed in the continuation of box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "B" document member of the same patent family		
Date of the actual completion of the international search: 22 January 2008		Date of mailing of the international search report: 31/01/2008
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentstein 2 NL - 2280 HV Rijswijk Tel: (+31-70) 340-2040, Tx: 31 651 apo nl, Fax: (+31-70) 340-3016		Authorized officer: Gettins, Marc

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Form PCT/ISB/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT	International application No. PCT/US2007/078605
Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)	
<p>This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:</p> <p>1. ☒ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: <div style="margin-left: 40px;">Although claims 11-13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.</div> </p> <p>2. ☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:</p> <p>3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</p>	
Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)	
<p>This International Searching Authority found multiple inventions in this international application, as follows:</p> <p>1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</p> <p>2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of additional fees.</p> <p>3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:</p> <p>4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:</p> <p style="margin-top: 20px;">Remark on Protest</p> <div style="margin-left: 40px;"> ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee. ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation. ☐ No protest accompanied the payment of additional search fees. </div>	

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(54) Title: INORGANIC SELENIUM FOR TREATMENT OF BENIGN TUMORS

(57) Abstract: The invention relates to the use of selenate or its pharmaceutically acceptable salts, in nutritional or supranutritional amounts, in methods and compositions for inhibiting the growth of benign tumor cells and treating or preventing benign tumors, particularly benign tumors in which the Akt signaling pathway is activated or in which Bcl 2 levels are elevated. Methods of preventing malignant conversion of benign tumors and benign tumor cells to malignant tumors or malignant tumor cells is also described.

“INORGANIC SELENIUM FOR TREATMENT OF BENIGN TUMORS”

FIELD OF THE INVENTION

[0001] This invention relates generally to the use of selenate or its pharmaceutically acceptable salts, especially in supranutritional amounts, in methods and compositions for inhibiting the growth or proliferation of benign tumor cells. The present invention also relates to the use of selenate or a pharmaceutically acceptable salt thereof in combination with at least one other therapy for treating or preventing benign tumors or for inhibiting the growth or proliferation of benign tumor cells. In certain embodiments, the methods of the invention are useful for treating or preventing benign tumors, especially benign tumors in which the Akt signaling pathway is activated or benign tumors in which Bcl-2 levels are elevated. Additionally, the present invention relates to the use of selenate or a pharmaceutically acceptable salt thereof for preventing malignant conversion of benign tumor cells or benign tumors to malignant tumor cells or malignant tumors.

BACKGROUND OF THE INVENTION

[0002] Although many benign tumors are asymptomatic and harmless, some benign tumors occur in positions that cause symptoms such as pain and in the brain or spine symptoms such as loss of mobility, loss of sight etc. Some benign tumors may cause clinical symptoms that are bothersome to a patient, for example, lower urinary tract symptoms (LUTS) resulting from benign prostatic hyperplasia. Some benign tumors may grow to a size that causes damage to tissues, organs or nerves in its vicinity, whilst others may cause serious illness or death if they occur near vital organs or press against vital organs. Some 13,000 deaths per year in the US are attributed to benign tumors and many more benign tumors require treatment. Furthermore, some benign tumors are known to have a high propensity to undergo malignant conversion to produce malignant or cancerous tumors.

[0003] There has been much interest in the use of selenium compounds as cancer-preventive agents. Studies spanning the last 20 years have documented their cancer-preventive action in tumors of the mammary gland (Ip, C. 1981, *Cancer Res.* 41:4386-4390), colon (Reddy *et al.* 1981, *Cancer Res.* 47:5901-5904), lung and prostate (Clark *et al.* 1996, *Jama* 276:1957-1963). Both phase II and III clinical trials for prostate cancer prevention using an organic selenomethionine are currently underway (Nelson *et al.* 1999, *Semin. Urol. Oncol.*, 17(2):91-6).

[0004] Selenium compounds used in chemoprevention studies can broadly be classified into inorganic and organic selenium forms. The typical form of inorganic selenium, sodium selenite, (Na_2SeO_3) is relatively toxic, causing single- and double-strand break DNA

damage, whilst the typical organic selenium entity, selenomethionine (SeMet) is relatively non-toxic and non-DNA-damaging (Lu *et al.* 1995, *Biochem. Pharmacol.* 50(2):213-9; Sinha *et al.* 1996, *Cancer Lett.* 107(2):277-84; Stewart *et al.* 1999, *Free Radic. Biol. Med.* 26(1-2):42-8).

5 **[0005]** Organic selenomethionine, the major constituent of dietary selenium, is incorporated into cellular selenoproteins, such as thioredoxin reductase and glutathione peroxidase *via* a series of complex intermediates (Allan *et al.* 1999, *Annu. Rev. Nutr.* 19:1-16). Since these selenocysteine-containing selenoenzymes, such as the antioxidant glutathione peroxidase and the redox-regulatory thioredoxins, are involved in cellular responses to
10 mutagenic oxidative stress, it has long been proposed that supranutritional levels of selenium intake boosts anticarcinogenic cellular activity by promoting a cellular reducing environment (Allan *et al.* 1999, *supra*).

[0006] It is, however, becoming increasingly clear that the nature of the anti-tumorigenic action of selenium compounds is dependent on the chemical form in which the
15 element is administered (Ip, C. 1998, *J. Nutr.* 128:1845-1854). Organic selenium in the form of methylseleninic acid induces G1 arrest, as well as DNA fragmentation and caspase mediated cleavage of PARP, which are two markers of apoptosis, in DU145 prostate cancer cells (Jiang *et al.* 2001, *Cancer Res.* 61:3062-3070).

[0007] In contrast, selenite induces S-phase arrest and apoptotic DNA
20 fragmentation, which is independent of caspase function (Jiang *et al.* 2002, *Mol. Cancer Ther.* 1:1059-1066). Differences have also been reported for the inorganic selenium compounds, with selenite and selenate inhibiting lymphocyte growth *via* different mechanisms (Spyrou *et al.* 1996, *Cancer Res.* 56:4407-4412). These differences may be explained in part by variations in metabolism and bioavailability between *in vitro* cell culture and *in vivo* studies (Dong *et al.*
25 2003, *Cancer Res.* 63:52-59).

[0008] The role of the PI3K/Akt pathway in uncontrolled cell proliferation is increasingly recognized. Akt is a serine/threonine kinase that is activated in response to membrane receptor stimulated PI3K phosphorylation. Akt, in turn, regulates the activity of several proteins involved in the control of apoptosis, including the Forkhead (FKHR)
30 transcription factors, Bad, Caspase 9, GSK-3 β and mTOR (Vivanco, I. and Sawyers, C.L. 2002, *Nat. Rev. Cancer* 2:289-501). Akt overactivity may occur in benign tumors resulting in lack of apoptosis. Furthermore, higher than normal levels of Bcl-2 proteins may occur in benign tumors, including those that may be susceptible to malignant conversion.

[0009] Radiotherapy resistance in tumor cells has also been linked with
35 upregulation of the PI3K/Akt pathway (Soderlund *et al.* 2005, *Int. J. Oncol.*, 26:25-32; Zhan *et al.* 2004, *Histol. Histopathol.*, 19:915-923; Tanno *et al.* 2004, *Cancer Res.*, 64:3486-3490; Li *et*

al. 2004, *Oncogene*, 23:4594-4602; McKenna *et al.*, 2003, *Genes Chromosomes Cancer*, 28:330-338; Liang *et al.* 2003, *Mol. Cancer Ther.* 2:353-360).

5 **[0010]** Interest in the clinical use of selenium-containing compounds as a chemopreventive agent has become widespread following the publication of Clark *et al.* (1996, *supra*). These results have spurred a number of human clinical trials using supranutritional selenomethionine as a chemopreventive agent for prostate cancer (Meuillet *et al.* 2004, *J. Cell Biochem.* 91:443-458).

10 **[0011]** However, it has been noted that there are marked differences in tumor progression *in vivo*, depending upon the selenium compound administered. For instance, inorganic sodium selenate significantly reduces tumor progression in comparison to organic selenomethionine, methylselenocysteine or selenized yeast (Corcoran *et al.* 2004, *J. Urol.*, 171:907-910). In addition, certain selenium compounds, such as sodium selenite have been found to be toxic to cells when used at certain doses (Jiang *et al.* 2002, *supra*).

SUMMARY OF THE INVENTION

[0012] The present invention is predicated in part on the discovery that a specific type of inorganic selenium compound, namely selenate, significantly inhibits benign tumor cell proliferation, especially when used at high or supranutritional amounts, as compared to other selenium compounds. It has also been found that selenate and its pharmaceutically acceptable salts have an inhibitory effect on benign tumor cells, especially those in which the Akt signaling pathway is activated or those in which Bcl-2 activity is elevated, and have a strong synergistic inhibitory effect on benign tumor cell growth when used in combination with at least one of a cytostatic agent, a cytotoxic agent and a radiotherapy that is optionally administered with a radiosensitizing agent. Further, it has been found that selenate and its pharmaceutically acceptable salts have an inhibitory effect on malignant conversion of benign tumor cells to malignant tumor cells.

[0013] Accordingly, in one aspect, the present invention provides methods for inhibiting the growth or proliferation of benign tumor cells. These methods generally comprise exposing the benign tumor cells to an effective amount of selenate or a pharmaceutically acceptable salt thereof. In some embodiments, the benign tumor cells are tumor cells in which the Akt-signaling pathway is activated. The activation of the Akt signaling pathway involves activation of at least one member selected from Akt, mTOR, GSK-3 β and FKHR. In illustrative examples of this type, the activation of the Akt signaling pathway involves phosphorylation of Akt (e.g., phosphorylation of the Thr 308 and Ser 473 residues of Akt). In other embodiments, the activation of the Akt signaling pathway involves inactivation of PTEN. In some embodiments, the Akt signaling pathway is over-activated. In some embodiments, the benign tumor cells have an abnormally increased Bcl-2 activity. In some embodiments, the amount of selenate or its pharmaceutically acceptable salt, to which the tumor cells are exposed, is a nutritional amount. In other embodiments, the amount is a supranutritional amount. Suitably, the tumor cells are selected from benign adenoma tumor cells, angiofibroma tumor cells, hemangioma tumor cells, leiomyoma tumor cells (fibroid), benign chorangioma tumor cells, cystadenoma tumor cells, dermoid tumor cells, desmoid tumor cells, fibroadenoma tumor cells, fibroma tumor cells, benign ganglioneuroma tumor cells, lipoma tumor cells, meningioma tumor cells, myxoma tumor cells, neurofibroma tumor cells, nevus tumor cells, osteochondroma tumor cells, pheochromocytoma tumor cells, polyposis tumor cells, schwannoma tumor cells, benign teratoma tumor cells, benign thymoma tumor cells and Brenner tumor cells. In specific embodiments, the tumor cells are prostatic intraepithelial neoplasia (PIN) tumor cells, benign prostatic hyperplastic (hypertrophic) tumor cells, ductal carcinoma in situ (DCIS) tumor cells, meningioma tumor cells or benign colonic tumor cells (colon polyps).

[0014] In another aspect, the present invention provides methods for preventing malignant conversion of a benign tumor cell to a malignant tumor cell. These methods generally comprise administering to the subject a therapeutically effective amount of selenate or a pharmaceutically acceptable salt thereof. In some embodiments, the therapeutically effective amount is a nutritional amount. In other embodiments, the therapeutically effective amount is a supranutritional amount. In specific embodiments, the benign tumor cell is a benign colonic or rectal tumor cell, a high grade prostatic intraepithelial neoplasia tumor cell, an atypical small acinar proliferative tumor cell or a ductal carcinoma in situ (DCIS) tumor cell.

[0015] In a further aspect, the present invention provides a use of selenate or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating or preventing a benign tumor.

[0016] In still a further aspect, the present invention provides a use of selenate or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for preventing malignant conversion of benign tumors to malignant tumors.

[0017] In some embodiments of the methods and uses broadly described above, the selenate or its pharmaceutically acceptable salt is administered in combination with at least one of a cytostatic agent, a cytotoxic agent or a radiotherapy that is optionally administered with a radiosensitizing agent. In other embodiments, the selenate is formulated in a composition with at least one cytostatic agent or cytotoxic agent. In still other embodiments, the selenate is formulated in a composition with a radiosensitizing agent for use in combination with radiotherapy.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: graphically represents the growth inhibitory (cytostatic) effect of sodium selenate at 25 μ M, 50 μ M and 100 μ M on prostate stromal cells with time.

Figure 2: graphically represents the growth inhibitory effect of sodium selenate at 25 μ M, 50 μ M and 100 μ M on the proliferation of prostate stromal cells at 4 days (A), 11 days (B) and 18 days (C).

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0018] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0019] The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0020] As used herein, the term "about" refers to a quantity, level, value, dimension, size, or amount that varies by as much as 30%, 20%, or 10% to a reference quantity, level, value, dimension, size, or amount.

[0021] As used herein, the term "Akt signaling pathway activation-inhibiting amount" in the context of treating or preventing a benign tumor or inhibiting the growth of benign tumor cells is meant the administration of an amount or series of doses of selenate, which is effective in antagonizing the Akt signaling pathway, including preventing or reducing activation of Akt by preventing or reducing the expression of Akt or an upstream member of the pathway, or by reducing the level or functional activity of an expression product of the *Akt* gene or of an upstream gene member of the pathway, or by preventing or reducing phosphorylation of Akt. The amount will vary depending on the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall within a relatively broad range that can be determined by routine trials. In specific embodiments, an Akt signaling pathway activation-inhibiting amount is a nutritional or supranutritional amount of selenate.

[0022] As used herein, the term "Bcl-2 activity-reducing amount" in the context of treating or preventing a benign tumor or inhibiting the growth of benign tumor cells is meant the administration of an amount or series of doses of selenate, which is effective in reducing the levels of Bcl-2 activity in benign tumor cells or benign tumors, including downregulating Bcl-2 transcription and modulation of activity of Bcl-2. The amount will vary depending on the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall within a relatively broad range that

can be determined by routine trials. In specific embodiments, a Bcl-2 activity-reducing amount is a nutritional or supranutritional amount of selenate.

[0023] By "benign tumor in which Bcl-2 activity is elevated" is meant a benign tumor which has levels of Bcl-2 protein that are above levels found in normal cells. Normal
5 levels of Bcl-2 protein found in normal cells are very low and generally not detectable. Benign tumors in which Bcl-2 activity is elevated may undergo malignant conversion to malignant tumors in which Bcl-2 activity is elevated. Examples of such benign tumors include colonic polyps and DCIS which may convert to colon cancer or breast cancer respectively.

[0024] As used herein, the term "benign tumor" refers to abnormal growth or
10 proliferation cells in a localised manner. Benign tumors do not invade surrounding tissue or metastasize to other organs. Benign tumors may be asymptomatic, and discovered, for example, by screening, or they may be symptomatic and require treatment. Benign tumors although causing no immediate threat to the subject, may grow and press on other tissues or organs resulting in symptoms of the tumor, for example a meningioma in the brain may grow to a size
15 where it damages the brain or causes malfunction in the brain. In some cases, a benign tumor may undergo malignant conversion to give a malignant tumor or cancer. Illustrative examples of benign tumors include, but are not limited to benign adenomas, angiofibromas, hemangiomas, leiomyomas, benign chorangiomas, cystadenomas, dermoid tumors, desmoid tumors, fibroadenomas, fibromas, benign ganglioneuromas, lipomas, meningiomas, myxomas,
20 neurofibromas, nevus tumors, osteochondromas, pheochromocytomas, polyps such as colonic polyps, schwannomas, benign teratomas, benign thyomas and Brenner tumors. Other types of benign tumors include prostatic intraepithelial neoplasia, benign prostatic hyperplastic (hypertrophic) tumors and ductal carcinoma in situ.

[0025] The phrases "benign tumor in which the Akt pathway is over-activated,"
25 "benign tumor in which Akt is over-activated" and the like refer to a benign tumor in which not only Akt is overexpressed, but the kinase activity of Akt is positively enhanced by phosphorylation of the Thr 308 and Ser 473 residues. Akt is often over-activated in drug-resistant or refractory tumors. Examples of benign tumors in which Akt is over-activated or constitutively activated include, but are not limited to, prostatic intraepithelial neoplasia and
30 breast ductal carcinoma in situ.

[0026] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0027] As used herein the term "cytostatic agent" refers to a substance that can inhibit cell proliferation or cell division without necessarily killing the cell. Suitably, the cytostatic agent inhibits the proliferation of tumor cells.

5 [0028] The term "cytotoxic agent" or "cytotoxic therapy" as used herein refers to a substance or therapy that is harmful to cells and ultimately causes cell death. In some embodiments, the cytotoxic agent harms rapidly dividing cells such as tumor cells and causes tumor cell death, especially tumor cell death while not causing damage to or causing less damage to non-tumor cells. An example of a cytotoxic therapy is radiotherapy.

10 [0029] As used herein the terms "drug resistant" and "refractory" refer to a tumor cell which is unresponsive or partially unresponsive to treatments normally used to treat the cancer or kill the tumor cell.

[0030] As used herein the term "in combination with" refers to the treatment of cancer or exposure of a tumor cell to at least two agents such that their effects on the tumor cell occur, at least in part, over the same time period. Administration of at least two agents may occur simultaneously in a single composition, or each agent may be simultaneously or sequentially administered in separate compositions.

[0031] The phrase "inhibiting growth of tumor cells" is taken to mean that tumor cell growth is ceased or reduced and cell proliferation or cell division is ceased or reduced. This is also known as cytostasis. The growth of tumor cells can be measured in terms of weight or volume or cell number or cellular metabolic activity, i.e. MTT assay.

20 [0032] The term "malignant conversion" as used herein refers to the conversion of benign tumors or benign tumor cells into malignant tumors or malignant tumor cells. Once malignant conversion has occurred, the tumor or tumor cells may invade other tissues or metastasize to other organs or tissues, and the subject has a cancerous tumor. Examples of benign tumors that undergo malignant conversion include colonic polyps and DCIS which may be converted to colon cancer or breast cancer respectively.

[0033] As used herein, the term "nutritional amount" includes an amount of selenium that provides an average daily intake. In the US, the average daily intake is 80-120 µg/day.

30 [0034] By "pharmaceutically acceptable carrier" it is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in topical, local or systemic administration.

[0035] The term "pharmaceutically acceptable salt" as used herein in relation to selenate refers to metal ion salts which are toxicologically safe for human and animal administration. For example, suitable metal ion salts of selenate include, but are not limited to,

sodium, potassium, lithium, magnesium, calcium, iron, nickel, zinc, ammonium and alkylammonium salts. A preferred salt of selenate is the sodium salt, Na_2SeO_4 .

[0036] The term "radiotherapy" as used herein refers to the treatment or exposure of a tumor or tumor cells to high energy radiation. The effectiveness of radiotherapy may be enhanced by selenate or its pharmaceutically acceptable salt. Furthermore, radiotherapy may be further enhanced by administration of radiosensitizing agent. Illustrative examples of radiosensitizing agents include but are not limited to efaproxiral, etanidazole, fluosol, misonidazole, nimorazole, temoporfin and tirapazamine.

[0037] The terms "subject" or "individual" or "patient", used interchangeably herein, refer to any subject, particularly a vertebrate subject and more particularly a mammalian subject, for whom prophylaxis or treatment is desired. Suitable vertebrate animals that fall within the scope of the invention include, but are not limited to, primates, avians, livestock animals (e.g., pigs, sheep, cows, horses, donkeys), laboratory test animals (e.g., rabbits, mice, rats, guinea pigs, hamsters), companion animals (e.g., cats and dogs) and captive wild animals (e.g., foxes, deer, dingoes). A preferred subject is a human in need of treatment or prophylaxis of a benign tumor or may be at risk of a benign tumor undergoing malignant conversion to a malignant tumor. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

[0038] The term "supranutritional" as used herein, refers to an amount which is greater than the amount considered as a nutritional requirement. The average daily intake of selenium in the US is 80-120 $\mu\text{g/day}$. A supranutritional amount of selenium provides selenium to a subject at above the recommended daily allowance. For example, a supranutritional amount of selenium may be 3 $\mu\text{g/kg}$ to 20 mg/kg per day, 0.015 mg/kg to 20 mg/kg , 0.15 mg/kg to 20.0 mg/kg , 0.1 mg/kg to 14 mg/kg , 0.1 mg/kg to 13 mg/kg , 0.1 mg/kg to 12 mg/kg , 0.1 mg/kg to 10 mg/kg , 0.1 mg/kg to 9 mg/kg , 0.1 mg/kg to 8 mg/kg , 0.1 mg/kg to 7 mg/kg , 0.1 mg/kg to 6 mg/kg , 0.15 mg/kg to 5 mg/kg , 0.15 mg/kg to 4 mg/kg , 0.15 mg/kg to 3 mg/kg , 0.15 mg/kg to 2 mg/kg , 0.15 mg/kg to 1 mg/kg , especially 0.07 mg/kg to 6.5 mg/kg or 0.1 mg/kg to 14 mg/kg or 0.15 mg/kg to 5 mg/kg per day, and more especially 0.07 mg/kg to 2 mg/kg per day.

[0039] As used herein, the term "effective amount" in the context of treating or preventing a benign tumor or inhibiting the growth of benign tumor cells is meant the administration of an amount of selenate or a pharmaceutically acceptable salt thereof, either in a single dose or as part of a series of doses, that is effective for inhibiting the growth and/or proliferation of benign tumor cells or for causing benign tumor cell death or for preventing metastatic conversion of a benign tumor to a malignant tumor. The effective amount will vary depending on the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated, and the formulation of the composition, the assessment of

the medical situations and other relevant factors. It is expected that the amount will fall within a relatively broad range that can be determined through routine trials. In specific embodiments, an effective amount is a nutritional or supranutritional amount. In some embodiments, an effective amount is an Akt-signaling pathway activation inhibiting amount or a Bcl-2 activity-reducing amount.

2: *Methods for inhibiting tumor cell growth and proliferation and for preventing malignant conversion*

[0040] The present invention is predicated in part on the determination that selenate, as opposed to other forms of selenium such as selenite, is effective in inhibiting the growth or proliferation of benign tumor cells, especially those in which the Akt signaling pathway is activated or in which Bcl-2 activity is elevated. Accordingly, in one aspect, the present invention provides methods for inhibiting the growth or proliferation of benign tumor cells, wherein the methods generally comprise exposing the tumor cells to an effective amount, such as an Akt signaling pathway activation-inhibiting amount or a Bcl-2 activity-reducing amount of selenate or a pharmaceutically acceptable salt thereof. In some embodiments of the invention, the amount of selenate or its pharmaceutically acceptable salt thereof is a nutritional amount. In other embodiments, the amount of selenate or its pharmaceutically acceptable salt is a supranutritional amount, which is generally from about 0.015 mg/kg to 20.0 mg/kg, usually from about 0.07 mg/kg to 6.5 mg/kg or 0.1 mg/kg to 14 mg/kg or from about 0.15 mg/kg to 5 mg/kg, especially 0.07 mg/kg to 2 mg/kg per day.

[0041] The present invention can be used effectively against various types of tumor cells. Illustrative examples of benign tumors that may be treated or prevented include, but are not limited to benign adenomas, angiofibromas, hemangiomas, leiomyomas, benign chorangiomas, cystadenomas, dermoid tumors, desmoid tumors, fibroadenomas, fibromas, benign ganglioneuromas, lipomas, meningiomas, myxomas, neurofibromas, nevus tumors, osteochondromas, pheochromocytomas, polyps, schwannomas, benign teratomas, benign thyomas and Brenner tumors. In some embodiments, the tumor is a prostatic intraepithelial neoplasia, colonic polyps, atypical small acinar proliferative tumor cells, benign prostatic hyperplastic (hypertrophic) tumor cells (benign prostatic hyperplasia) or a ductal carcinoma in situ. In a related aspect, the invention provides methods for treating or preventing a benign tumor in which Akt is activated, wherein the methods generally comprise administering to a subject in need thereof an Akt signaling pathway activation-inhibiting amount of selenate or a pharmaceutically acceptable salt thereof. In a further related aspect, the invention provides methods for treating or preventing a benign tumor in which Bcl-2 activity is elevated, wherein the methods generally comprise administering to a subject in need thereof a Bcl-2 activity-reducing amount of selenate or a pharmaceutically acceptable salt thereof. In some

embodiments, the amount of selenate or pharmaceutically acceptable salt thereof is a nutritional amount. In other embodiments, the amount is a supranutritional amount as broadly defined above.

5 [0042] In some embodiments, the tumor cell is drug resistant. In some embodiments, the tumor cell has an overactive Akt pathway.

10 [0043] In certain embodiments, the selenate or its pharmaceutically acceptable salt is administered in combination with at least one cytostatic agent or cytotoxic agent. Non-limiting examples of cytostatic agents are selected from: (1) kinase inhibitors, illustrative examples of which include Iressa®, Gleevec, Tarceva™, (Erlotinib HCl), BAY-43-9006, inhibitors of the split kinase domain receptor tyrosine kinase subgroup (e.g., PTK787/ZK 222584 and SU11248); (2) receptor kinase targeted antibodies, which include, but are not limited to, Trastuzumab (Herceptin®), Cetuximab (Erbix™), Bevacizumab (Avastin™), Rituximab (ritusan®), Pertuzumab (Omnitarg™); (3) mTOR pathway inhibitors, illustrative examples of which include rapamycin and CCI-778; (4) Apo2L/Trail, anti-angiogenic agents 15 such as but not limited to endostatin, combrestatin, angiostatin, thrombospondin and vascular endothelial growth inhibitor (VEGI); (5) antineoplastic immunotherapy vaccines, representative examples of which include activated T-cells, non-specific immune boosting agents (i.e., interferons, interleukins); (6) hormonal antineoplastic agents, non-limiting examples of which include Nilutamide, Cyproterone acetate, Anastrozole, Exemestane, Tamoxifen, Raloxifene, 20 Bicalutamide, Aminoglutethimide, Leuporelin acetate, Toremifene citrate, Letrozole, Flutamide, Megestrol acetate and Goserelin acetate; (7) gonadal hormones such as but not limited to Cyproterone acetate and Medoxyprogesterone acetate; (8) antimetabolites, illustrative examples of which include Cytarabine, Fluorouracil, Gemcitabine, Topotecan, Hydroxyurea, Thioguanine, Methotrexate, Colaspase, Raltitrexed and Capicitabine; (9) anabolic agents, such as but not limited to, Nandrolone; (10) adrenal steroid hormones, illustrative examples of which 25 include Methylprednisolone acetate, Dexamethasone, Hydrocortisone, Prednisolone and Prednisone.

[0044] The methods of the present invention may be employed in combination with other known treatments for benign tumors, for instance but not limited to, surgery, 30 chemotherapy and radiotherapy. In some embodiments, the selenate or its pharmaceutically acceptable salt is used in combination with radiotherapies, such as but not limited to, conformal external beam radiotherapy (50-100 Grey given as fractions over 4-8 weeks), either single shot or fractionated, high dose rate brachytherapy, permanent interstitial brachytherapy, systemic radio-isotopes (e.g., Strontium 89). In some embodiments the radiotherapy may be administered 35 in combination with a radiosensitizing agent. Illustrative examples of radiosensitizing agents include but are not limited to efaproxiral, etanidazole, fluosol, misonidazole, nimorazole,

temoporfin and tirapazamine. In other embodiments, selenate or its pharmaceutically acceptable salt is used in combination with a tumorectomy.

[0045] In some embodiments, the cytostatic agent is a nucleic acid molecule, suitably an antisense or siRNA recombinant nucleic acid molecule. In other embodiments, the
5 cytostatic agent is a peptide or polypeptide. In still other embodiments, the cytostatic agent is small molecule. The cytostatic agent may be a cytotoxic agent that is suitably modified to enhance uptake or delivery of the agent. Non-limiting examples of such modified cytotoxic agents include, but are not limited to, pegylated or albumin-labelled cytotoxic drugs.

[0046] In embodiments where the benign tumor is benign prostatic hypertrophy
10 (benign prostatic hyperplasia) or prostatic intraepithelial neoplasia, the selenate or pharmaceutically acceptable salt thereof may be administered in combination with 5-alpha reductase inhibitors such as finasteride or dutasteride; alpha adrenergic receptor blockers such as terazosin (Hytrin), doxazosin (cardura), alfuzosin, tamsulosin (flomax) and Prazosin (Minipress); selective estrogen receptor modulators (SERMs) such as toremifene citrate
15 (Acapodene), tamoxifen and raloxifen; selective androgen receptor modulators (SARMs) such as prostarine; or phytotherapeutics such as those derived from the American dwarf palm tree (saw palmetto or *Serenoa repens*), African plum tree (*Pygeum africanum*), pumpkin seeds, rye pollen extracts, South African star grass roots, stinging nettle roots and the purple cone flower.

[0047] In embodiments where the benign tumor is colonic polyps, the selenate or
20 pharmaceutically acceptable salt thereof may be administered in combination with non steroidal anti inflammatory drugs such as aspirin, ibuprofen, naproxene; or Cox-2 inhibitors such as sulindac, celocoxib (celebrex), refocoxib (vioxx) and lumiracoxib.

[0048] In embodiments where the benign tumor is ductal carcinoma in situ the
25 selenate or pharmaceutically acceptable salt thereof may be administered in combination with selective estrogen receptor modulators such as tamoxifen, raloxifen and toremifene citrate.

[0049] In embodiments where the benign tumor is meningioma, the selenate or
pharmaceutically acceptable salt thereof may be administered in combination with progesterone receptor antagonists such as mifepristone; immune modulating agents such as interferon; chemotherapy such as hydroxyurea and external beam radiation.

[0050] Certain embodiments of the present invention are directed to methods for
30 treating or preventing a benign tumor in a subject, which methods generally comprise administering to the subject a therapeutically effective amount of selenate or a pharmaceutically acceptable salt thereof. To practice these methods, the person managing the subject can determine the effective dosage form of selenate or its pharmaceutically acceptable salts for the
35 particular condition and circumstances of the subject. A therapeutically effective amount of

selenate is one that is effective for the treatment or prevention of the benign tumor, including the prevention of incurring a symptom (e.g., proliferation of tumor cells), holding in check such symptoms, and/or treating existing symptoms associated with the tumor (e.g., pain, tenderness, headaches, menstrual irregularity, urinary retention, cosmetic effect, shortness of breath, altered bowel habit, malena, haematemesis, focal neurological deficit). In some embodiments, the therapeutically effective amount is a nutritional amount. In other embodiments, the amount is a supranutritional amount of selenate or its pharmaceutically acceptable salt. In specific embodiments, the selenate is suitably in the form of the salt, sodium selenate (Na_2SeO_4).

[0051] Modes of administration, amounts of selenate administered, and selenate formulations, for use in the methods of the present invention, are discussed below. The tumor to be treated may be determined by measuring one or more diagnostic parameters indicative of the course of the disease, compared to a suitable control. In the case of a human subject, a "suitable control" may be the individual before treatment, or may be a human (e.g., an age-matched or similar control) treated with a placebo. In accordance with the present invention, the treatment or prevention of a benign tumor includes and encompasses without limitation: (i) preventing a benign tumor in a subject who may be predisposed to the benign tumor but has not yet been diagnosed with the benign tumor and, accordingly, the treatment constitutes prophylactic treatment for the benign tumor; (ii) inhibiting tumorigenesis, i.e., arresting the development of the benign tumor; (iii) relieving symptoms resulting from the benign tumor; or (iv) preventing malignant conversion of a benign tumor.

[0052] The methods of the present invention are suitable for treating an individual who has been diagnosed with a benign tumor, who is suspected of having a benign tumor, who is known to be susceptible and who is considered likely to develop a benign tumor, who is considered likely to develop a recurrence of a previously treated benign tumor, or who has a benign tumor which may undergo malignant conversion to a malignant tumor.

[0053] In specific embodiments of the above methods, the benign tumor is a high grade prostatic intraepithelial neoplasia (HGPIN), colonic polyp or ductal carcinoma in situ (DCIS) that may undergo malignant conversion to prostate cancer, colon cancer or breast cancer respectively. In other embodiments, the tumor to be treated may be a meningioma.

[0054] Exemplary subjects for treatment with the methods of the invention are vertebrates, especially mammals. In certain embodiments, the subject is selected from the group consisting of humans, sheep, cattle, horses, bovine, pigs, dogs and cats. A preferred subject is a human.

[0055] The selenate or pharmaceutically acceptable salt may be formulated by following any number of techniques known in the art of drug delivery. Selenate or its pharmaceutically acceptable salts may of course be administered by a number of means keeping

in mind that all formulations are not suitable for every route of administration. Selenate or its pharmaceutically acceptable salts can be administered in solid or liquid form. The application may be oral, rectal, nasal, topical (including buccal and sublingual), or by inhalation. Selenate or its pharmaceutically acceptable salts may be administered together with conventional pharmaceutical acceptable adjuvant, carriers and/or diluents.

[0056] The solid forms of application comprise tablets, capsules, powders, pills, pastilles, suppositories and granular forms of administration. They may also include carriers or additives, such as flavors, dyes, diluents, softeners, binders, preservatives, lasting agents and/or enclosing materials. Liquid forms of administration include solutions, suspensions and emulsions. These may also be offered together with the above-mentioned additives.

[0057] Solutions and suspensions of selenate or pharmaceutically acceptable salt thereof, assuming a suitable viscosity for ease of use, may be injected. Suspensions too viscous for injection may be implanted using devices designed for such purposes, if necessary. Sustained release forms are generally administered *via* parenteral or enteric means. Parenteral administration is another route of administration of the selenate or a pharmaceutically acceptable salt thereof used to practice the invention. "Parenteral" includes formulations suitable for injection and for nasal, vaginal, rectal, and buccal administration.

[0058] The administration of selenate or its pharmaceutically acceptable salts may involve an oral prolonged dose formulation. Oral dose formulations are preferably administered once daily to three times daily in the form of a sustained release capsule or tablet, or alternatively as an aqueous based solution. Selenate or its pharmaceutically acceptable salt may be administered intravenously either daily, continuously, once a week or three times a week.

[0059] The administration of selenate or its pharmaceutically acceptable salts may include daily administration, preferably once daily in the form of a sustained release capsule or tablet, or once daily as an aqueous solution.

[0060] Combinations of selenate or its pharmaceutically acceptable salt and at least one cytostatic agent or a cytotoxic agent or a radiosensitizing agent may be administered in solid or liquid form in a single formulation or composition or in separate formulations or compositions. In some embodiments, the selenate or its pharmaceutically acceptable salt and the cytostatic agent(s) or cytotoxic agent(s) or radiosensitizing agent(s) are administered orally as a single tablet or capsule or separate tablets or capsules. In other embodiments, the selenate or its pharmaceutically acceptable salt and the cytostatic agent(s) or cytotoxic agent(s) or radiosensitizing agent(s), are administered intravenously in a single composition or separate compositions.

[0061] The present invention also provides pharmaceutical compositions for treating or preventing a benign tumor or preventing a benign tumor undergoing malignant conversion, generally comprising a supranutritional amount, suitably from about 0.5 mg to about 1.0 g of selenate or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. In some embodiments, the selenate or its pharmaceutically acceptable salt is in an amount of about 5.0 mg to about 700 mg or 5 mg to 450 mg. In illustrative examples, the selenate or its pharmaceutically acceptable salt is an amount of about 0.07 mg to 6.5 mg, 5 mg to 450 mg or 7.5 mg to 250 mg, especially 50 mg to 200 mg, for example, 50 mg to 100 mg or 100 mg to 150 mg for a single or divided daily dose. The pharmaceutical composition may be for treating a benign tumor in which Akt is activated or over-activated or a benign tumor in which Bcl-2 activity is elevated.

[0062] In some embodiments, the pharmaceutical compositions further comprise at least one cytostatic agent or cytotoxic agent. In other embodiments, the pharmaceutical compositions may further comprise a radiosensitizing agent for use with radiotherapy. In other embodiments, the pharmaceutical composition may further comprise a second agent that is suitable for treating a benign tumor, such as 5- α -reductase inhibitors, α -adrenergic receptor blockers, selective estrogen receptor modulators, selective androgen receptor modulators, phytotherapeutics, anti-inflammatory drugs, Cox-2 inhibitors, progesterone receptor antagonists, immune modulating agents or chemotherapy.

[0063] The pharmaceutical composition of the present invention may include any additional components that are non-immunogenic and biocompatible with selenate, as well as capable of bioabsorption, biodegradation, elimination as an intact molecule. The formulation may be supplied in a ready-to-use form or may be supplied as a sterile powder or liquid requiring vehicle addition prior to administration. If sterility is desired, the formulation may be made under sterile conditions, the individual components of the mixture may be sterile, or the formulation may be sterile filtered prior to use. Such a solution can also contain appropriate pharmaceutically acceptable carriers, such as but not limited to buffers, salts, excipients, preservatives, etc.

[0064] In some embodiments, sustained release oral formulations are used for administering selenate or its pharmaceutically acceptable salt in the methods of the invention. These formulations generally comprise selenate or its pharmaceutically acceptable salt having decreased solubility in order to delay absorption into the bloodstream. In addition, these formulations may include other components, agents, carriers, etc., which may also serve to delay absorption of the selenate or its pharmaceutically acceptable salt. Microencapsulation, polymeric entrapment systems, and osmotic pumps, which may or may not be bioerodible, may

also be used to allow delayed or controlled diffusion of the selenate or a pharmaceutically acceptable salt thereof from a capsule or matrix.

5 [0065] The selenate or its pharmaceutically acceptable salts can be used solus or as part of another agent. Accordingly, the present invention also contemplates an agent that comprises selenate or a pharmaceutically acceptable salt thereof for the treatment of a benign tumor, especially one in which Akt is activated or in which Bcl-2 activity is elevated, or for the prevention of malignant conversion of a benign tumor to a malignant tumor.

10 [0066] In order that the nature of the present invention may be more clearly understood and put into practical effect, particular preferred embodiments thereof will now be described with reference to the following non-limited examples.

EXAMPLES

EXAMPLE 1

Isolation of primary benign tumor cell lines

- 5 [0067] Primary meningioma tissue samples will be obtained from the Royal Melbourne Hospital under a protocol approved by the Institutional Review Board. Primary cultures of meningiomas will be established according to established protocols (Friend KE, Radinsky R, McCutcheon IE. Growth hormone receptor expression and function in meningiomas: effect of a specific receptor antagonist. *J Neurosurg* 1999; 91:93-9).
- 10 [0068] Briefly, tumor tissue will be collected at the time of resection from patients with benign, or atypical, meningioma as determined by neuropathologic review using WHO 2000 criteria. Tumor fragments will be immediately transferred to the laboratory for further processing, carefully minced into small pieces, and treated with 0.4% trypsin/EDTA to dissociate the cells. The cells will be washed with PBS supplemented with 200 units/mL
- 15 penicillin and 200 µg/mL streptomycin and transferred to 100-mm² tissue culture dishes containing DMEM supplemented with 10% FCS, 100 units/mL penicillin, and 100 µg/mL streptomycin. The cells will be grown in humidified incubators at 37°C with 5% CO₂ for 5 days, with periodic medium changes and then allowed to reach confluence. Low-passage cells (<7 passages) will be used for proliferation experiments.

20

EXAMPLE 2

Cell proliferation assays

Benign tumor growth curve

- 25 [0069] Benign meningioma tumor growth curves will be analysed by allowing 2 x 10⁵ tumor cells. The cells will be allowed to attach overnight in culture dishes. After 16 hours the medium will be changed to include sodium selenate in the presence of serum at the indicated concentrations and allowed to grow until specified time points. Supernatants and cells will then be harvested, combined and viable cells assessed by Trypan Blue exclusion assay. Experiments will be performed in triplicate.

30

MTT Growth Assay

- [0070] MTT Growth Assays will be performed by plating 1 x 10³ meningioma tumor cells per well in a 96 well plate and allowing the cells to attach overnight. At 16 hours the medium will be replaced with fresh media containing sodium selenate (5-250 µM). For
- 35 experiments determining the additional effect of PI3K inhibition on sodium selenate growth

inhibition, LY294002 (Promega, Madison, WI, USA) will be added at a concentration of 10-50 μ M with the sodium selenate. The controls for this experiment will then receive an equal volume of LY294002 diluent (DMSO). MTT growth assays will then be performed according to the manufacturer's protocol (Sigma). Experiments will be performed in triplicate.

5

EXAMPLE 3

The effect of sodium selenate on the growth/proliferation or survival of benign tumor cells.
Primary human prostate stromal cell lines as a model for human benign prostatic hyperplasia
(BPH)

10 MATERIALS AND METHODS

[0071] **Cell Culture.** The prostate stromal (PrS) cell line was obtained from Cambrex Bio Science (Walkersville, MC, USA, www.cambrex.com) and was routinely cultured in Stromal Cell Medium BulletKit (Clonetics SCGM BulletKit, CC-3205) purchased from Cambrex. Complete growth medium was prepared from the BulletKit containing stromal cell
15 basal medium supplemented with 0.1% human fibroblast growth factor B, 0.1% insulin, 5% fetal bovine serum and 0.1% gentamicin sulphate amphotericin-B according to supplier specification. Cells were cultured at 37°C in 5% CO₂.

[0072] ***In Vitro* – growth proliferation study.** 4 x 10³ PrS cells were plated per well in a Falcon 6-well plate and were allowed to attach overnight at 5%CO₂/ 37°C in complete
20 growth medium. The medium was replaced with fresh complete growth medium containing various concentration of sodium selenate (25, 50 and 100 μ M) and were allowed to grow until specified time points (day 4, 11 and 18). Cells were trypsinised according to supplier specification and viable cells were assessed by trypan blue exclusion assay at the specified time points indicated above. Experiments were performed in triplicate per selenate treatment dose.

25 [0073] **Statistical analysis.** Data are presented as mean $\pm$ SEM, unless otherwise indicated. Differences between treatment groups were analyzed using Fisher's least significant difference test with significance assumed at p<0.01, ANOVA. All statistical analysis was performed using STATGRAPHICS statistical software (Virginia, USA).

[0074] The results are shown in Table 1 and Figures 1 and 2.

Table 1. Growth inhibitory (cytostatic) effect of sodium selenate on prostate stromal cell growth over time.

Treatment Groups	No. of days in culture	No. of viable cells (mean)
Complete growth medium (control)	1	40000.0
25 μ M Selenate	1	40000.0
50 μ M Selenate	1	40000.0
100 μ M Selenate	1	40000.0
Complete growth medium (control)	4	44666.7
25 μ M Selenate	4	48000.0
50 μ M Selenate	4	15666.7
100 μ M Selenate	4	11666.7
Complete growth medium (control)	11	163333.0
25 μ M Selenate	11	61333.3
50 μ M Selenate	11	19333.3
100 μ M Selenate	11	4000.0
Complete growth medium (control)	18	480000.0
25 μ M Selenate	18	81333.3
50 μ M Selenate	18	7666.7
100 μ M Selenate	18	2962.7

5 [0075] The results show that 25 μ M selenate effectively impedes prostate stromal cell growth after 11 days in culture. Higher concentrations of selenate, 50 μ M and 100 μ M are very effective at controlling prostate stromal cell growth.

 [0076] , The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

10 [0077] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

 [0078] Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Those of skill in the art will therefore appreciate that, in light of the
15 instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appended claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of inhibiting the growth or proliferation of benign tumor cells comprising exposing the benign tumor cells to an effective amount of selenate or a pharmaceutically acceptable salt thereof.
- 5 2. A method according to claim 1, wherein the benign tumor cells are tumor cells in which the Akt-signaling pathway is activated or over-activated.
3. A method according to claim 1, wherein the benign tumor cells have abnormally increased Bcl-2 activity.
4. A method according to claim 1, wherein the benign tumor cells are selected
10 from benign adenoma tumor cells, angiofibroma tumor cells, hemangioma tumor cells, leiomyoma tumor cells (fibroid), benign chorangioma tumor cells, cystadenoma tumor cells, dermoid tumor cells, desmoid tumor cells, fibroadenoma tumor cells, fibroma tumor cells, benign ganglioneuroma tumor cells, lipoma tumor cells, meningioma tumor cells, myxoma tumor cells, neurofibroma tumor cells, nevus tumor cells, osteochondroma tumor cells,
15 pheochromocytoma tumor cells, polyposis tumor cells, schwannoma tumor cells, benign teratoma tumor cells, benign thymoma tumor cells and Brenner tumor cells.
5. A method according to claim 1, wherein the benign tumor cells are selected from prostatic intraepithelial neoplasia (PIN) tumor cells, benign prostatic hyperplastic (hypertrophic) tumor cells, ductal carcinoma in situ (DCIS) tumor cells, meningioma tumor
20 cells or benign colonic tumor cells (colon polyps).
6. A method of treating or preventing a benign tumor in a subject comprising administering to the subject an effective amount of selenate or a pharmaceutically acceptable salt thereof.
7. A method according to claim 6, wherein the effective amount of selenate or a
25 pharmaceutically acceptable salt thereof is a nutritional amount.
8. A method according to claim 6, wherein the effective amount of selenate or a pharmaceutically acceptable salt thereof is a supranutritional amount.
9. A method according to claim 6, wherein the benign tumor is selected from benign adenomas, angiofibromas, hemangiomas, leiomyomas, benign chorangiomas,
30 cystadenomas, dermoid tumors, desmoid tumors, fibroadenomas, fibromas, benign ganglioneuromas, lipomas, meningiomas, myxomas, neurofibromas, nevus tumors, osteochondromas, pheochromocytomas, polyps, schwannomas, benign teratomas, benign thymomas and Brenner tumors.
10. A method according to claim 6, wherein the benign tumor is selected from
35 prostatic intraepithelial neoplasia, colonic polyps, atypical small acinar proliferative tumor cells, benign prostatic hyperplastic (hypertrophic) tumor cells or a ductal carcinoma in situ.

11. A method according to claim 6, further comprising administration in combination with another known treatment for benign tumors.

12. A method according to claim 11, wherein the other known treatments for benign tumors are selected from surgery, radiotherapy and chemotherapy.

5 13. A method according to claim 6, further comprising administering at least one cytotoxic or cytostatic agent or a radiosensitizing agent or a second agent used to treat benign tumors.

10 14. A method according to claim 13, wherein the cytostatic agent is selected from kinase inhibitors, receptor kinase targeted antibodies, mTOR pathway inhibitors, Apo2L/Trail antiangiogenic agents, antineoplastic immunotherapy vaccines, hormonal antineoplastic agents, gonadal hormones, antimetabolites, anabolic agents, adrenal steroid hormones and microtubule-stabilizing agents.

15 15. A method according to claim 13, wherein the radiosensitizing agent is selected from efaproxiral, etanidazole, fluosol, misonidazole, nimorazole, temoporfin and tirapazamine.

16. A method according to claim 13, wherein the second agent used to treat benign tumors is selected from 5- α -reductase inhibitors, α -adrenergic receptor blockers, selective estrogen receptor modulators, selective androgen receptor modulators, phytotherapeutics, non-steroidal anti-inflammatory drugs, Cox-2 inhibitors, progesterone receptor antagonists, immune modulating agents and chemotherapy.

20 17. A method of preventing benign tumor cells or a benign tumor undergoing malignant conversion to malignant tumor cells or a malignant tumor in a subject, comprising administering to the subject an effective amount of selenate or a pharmaceutically acceptable salt thereof.

25 18. A method according to claim 17, wherein the effective amount of selenate or a pharmaceutically acceptable salt thereof is a nutritional amount.

19. A method according to claim 17, wherein the effective amount of selenate or a pharmaceutically acceptable salt thereof is a supranutritional amount.

30 20. A method according to claim 17, wherein the benign tumor cells are benign colonic or rectal tumor cells, high grade prostatic intraepithelial neoplasia tumor cells, atypical small acinar proliferative tumor cells or ductal carcinoma in situ tumor cells.

21. A method according to claim 17, wherein the benign tumor is a high grade prostatic intraepithelial neoplasia, a colonic polyp or a ductal carcinoma in situ.

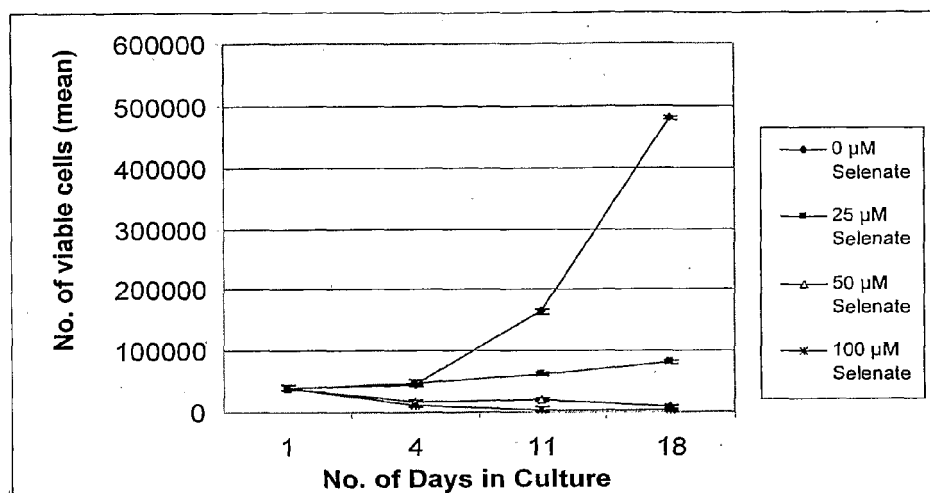
35 22. A pharmaceutical composition comprising selenate or a pharmaceutically acceptable salt thereof and at least one of a cytostatic agent, a cytotoxic agent, a radiosensitizing agent or a second agent used to treat benign tumors and a pharmaceutically acceptable carrier.

23. Use of selenate or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating or preventing a benign tumor.

24. Use of selenate or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for preventing benign tumor cells or a benign tumor undergoing malignant conversion to malignant tumor cells or a malignant tumor.

- 1/2 -

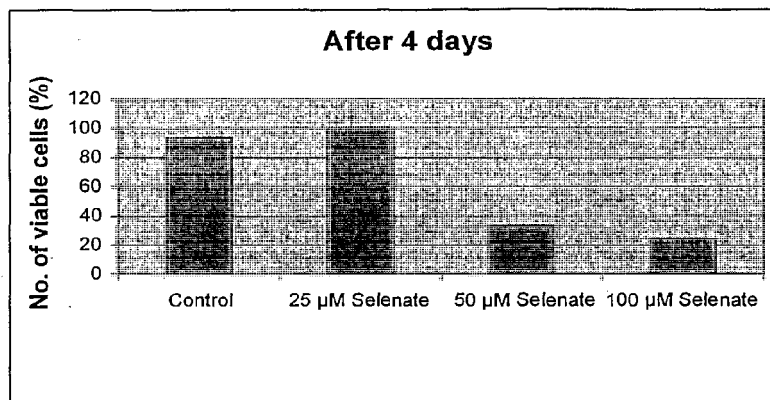
FIGURE 1



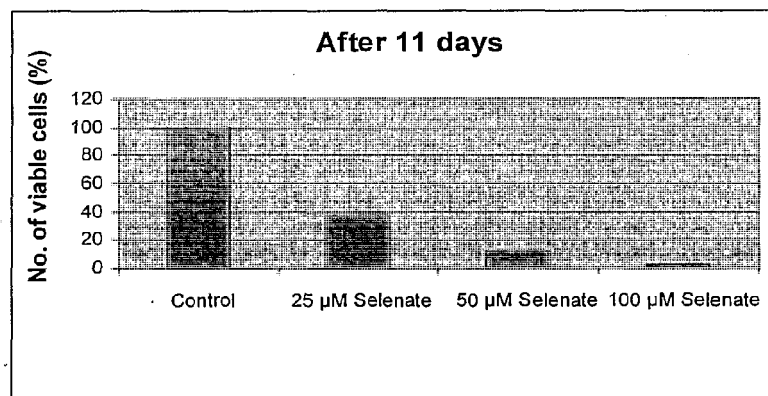
- 2/2 -

FIGURE 2

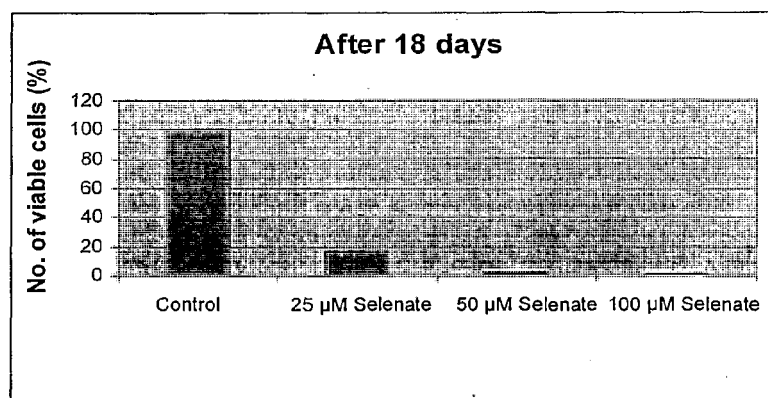
A



5 B



C



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000393

A. CLASSIFICATION OF SUBJECT MATTER		
Int. Cl.		
A61K 33/04 (2006.01) A61P 35/00 (2006.01)		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPIDS and Medline. Keywords: selenate, cancer, tumour, tumor, neoplasm, benign and related terms.		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2005/115472 A2 (Abbott Laboratories), 8 December 2005 see page 9 line 31, page 13 line 5 to page 14 line 25, page 27 line 16 to page 30 line 25, page 31 line 4 to page 34 line 30.	1-4, 6-9, 11-14, 17-19, 22-24
Y	see page 9 line 31, page 13 line 5 to page 14 line 25, page 27 line 16 to page 30 line 25, page 31 line 4 to page 34 line 30.	1-24
X	DAVIS, C.D. ET AL The Chemical Form of Selenium Influences 3,2'-Dimethyl-4-aminobiphenyl-DNA Adduct Formation in Rat Colon. Journal of Nutrition, 1999, volume 129, pages 63-69 see abstract and page 63, right hand column, 2 nd paragraph.	6-10, 17-21, 23-24
Y	see abstract and page 63, right hand column, 2 nd paragraph.	1-24
☒ Further documents are listed in the continuation of Box C ☒ See patent family annex		
* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 08 May 2007		Date of mailing of the international search report 11 MAY 2007
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929		Authorized officer Steven Chew AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : (02) 6283 2248

INTERNATIONAL SEARCH REPORT

International application No.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2005/048925 A2 (The Board of Trustees of the Leland Stanford Junior University), 2 June 2005 see claim 1 and paragraphs 0025, 0044, 0049, 0051, 0070 and 0080.	11-16, 22
Y	CLARK, L.C. ET AL Plasma Selenium Concentration Predicts the Prevalence of Colorectal Adenomatous Polyps. Cancer Epidemiology, Biomarkers and Prevention, January/February 1993, volume 2, pages 41-46 (see abstract)	1-24
P,X	WO2006/032074 A1 (University of Melbourne), 30 March 2006 (see whole document)	1-24
A	WO2001/054688 A1 (Merck and Co Inc.), 2 August 2001	
A	CORCORAN, N.M. ET AL Inorganic Selenium Retards Progression of Experimental Hormone Refractory Prostate Cancer. Journal of Urology, February 2004, volume 171, pages 907-910	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2007/000393

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	2005115472	CA	2564592	US	2006034944		
WO	2005048925						
WO	2006032074	AU	2005287854				
WO	0154688	AU	36533/01	CA	2396593	EP	1253921
		US	6486204	US	2001047022		
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
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BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
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HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
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SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
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Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii))

— as to the applicant's entitlement to claim the priority of the
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Published:

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(54) Title: CRF1 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

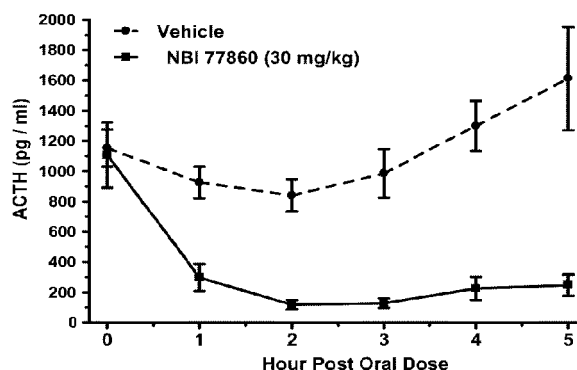


FIG. 2

(57) Abstract: CRF₁ receptor antagonists have the potential to directly inhibit ACTH release in patients with CAH and thereby allow normalization of androgen production while using lower, more physiologic doses of hydrocortisone, and thus reducing treatment-associated side effects.

TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

BACKGROUND

Technical Field

Compositions and methods for treating congenital adrenal hyperplasia
5 are described herein.

Description of the Related Art

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive genetic disorders that result in little or no cortisol biosynthesis. The most frequent form of the disease is 21-hydroxylase deficiency caused by mutations in the *CYP21A2* gene
10 located on chromosome 6p21, which accounts for approximately 95% of CAH cases (see, e.g., Speiser et al., *Int. J. Pediatr. Endocrinol.* 2010:494173 (2010) for a review). These mutations can range from complete loss of enzyme activity required for synthesis of cortisol in the adrenal cortex to a spectrum of partial loss, which results in disease severity that is a direct consequence of a specific mutation. This continuum of 21-
15 hydroxylase deficiency has been broadly classified into salt-wasting and simple-virilizing forms, grouped as classical CAH, and the milder form known as non-classic CAH (NCCAH) or “late-onset” CAH, which is usually diagnosed in late childhood or early adulthood. Non-classic CAH patients are either homozygous or compound heterozygotes, often with a classical CAH allele. These patients have sufficient enzyme
20 activity (> 20-50% of normal) such that they do not have salt-wasting or cortisol deficiency and have normal genitalia at birth, and many remain asymptomatic throughout life (Trapp et al., *Steroids* 77(4):342-46 (2012)). In the less frequent form of the disease, which accounts for 5% of cases, mutation of the 11 β -hydroxylase gene *CYP11B1* results in CAH (11 β -OH CAH).

25 Both genetic mutations result in congenital adrenal hyperplasia, cortisol deficiency and excessive adrenocorticotrophic hormone (ACTH) production with overproduction of androgens. These patients require lifelong management with

glucocorticoids and the attendant problems associated with such treatment. Accordingly, a significant need exists for treatment regimens to improve the health, well-being, quality of life, and to manage related disorders in patient with CAH.

BRIEF SUMMARY

5 Corticotropin-releasing factor (CRF) activates the CRF₁ receptor, a class B G protein-coupled receptor (GPCR). CRF₁ antagonists have the potential to directly inhibit ACTH release in patients with CAH, thereby allowing normalization of androgen production while using lower, more physiologic doses of hydrocortisone, and reducing treatment-associated side effects.

10 In one embodiment, a method is provided for treating CAH by administering to a subject in need thereof an effective amount of a CRF₁ antagonist, including (but not limited to) bedtime administration.

In a more specific embodiment, the CRF₁ antagonist has a dissociation half-life ($t_{1/2}$) in excess of 30 minutes, and in another embodiment in excess of 40
15 minutes, and in another embodiment in excess of 50 minutes.

Embodiment 1. A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF₁ receptor antagonist having a dissociation half-life in excess of 30 minutes.

Embodiment 2. The method of Embodiment 1 wherein the CRF₁
20 receptor antagonist has a dissociation half-life in excess of 40 minutes.

Embodiment 3. The method of Embodiment 1 wherein the CRF₁ receptor antagonist has a dissociation half-life in excess of 50 minutes.

Embodiment 4. The method of any one of Embodiments 1-3 wherein the CRF₁ receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-
25 (methoxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

Embodiment 5. The method of any one of Embodiments 1-3 wherein the CRF₁ receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543,

antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

Embodiment 6. The method of any one of Embodiments 1-5 wherein the CRF₁ receptor antagonist is administered at bedtime.

5 Embodiment 7. The method of any one of Embodiments 1-6 wherein the CRF₁ receptor antagonist is administered at or before the expected circadian release of ACTH.

Embodiment 8. The method of Embodiment 7 wherein the CRF₁ receptor antagonist is administered 3-4 hours before the expected circadian release of
10 ACTH.

Embodiment 9. A method for reducing 17-OHP and ACTH levels in a subject who has Congenital Adrenal Hyperplasia (CAH), said method comprising administering to the subject a CRF₁ receptor antagonist at bedtime.

Embodiment 10. The method of Embodiment 9, wherein the CRF₁
15 receptor antagonist is administered at or before the expected circadian release of ACTH.

Embodiment 11. The method of Embodiment 9 or Embodiment 10, wherein the CRF₁ receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

20 Embodiment 12. The method of any one of Embodiments 9-11, wherein the CRF₁ receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methoxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

Embodiment 13. The method of any one of Embodiments 9-11, wherein
25 the CRF₁ receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

Embodiment 14. A CRF₁ receptor antagonist for use in treating Congenital Adrenal Hyperplasia (CAH), wherein the CRF₁ receptor antagonist has a
30 dissociation half-life in excess of 30 minutes.

Embodiment 15. The CRF₁ receptor antagonist of 14, wherein the CRF₁ receptor antagonist has a dissociation half-life in excess of 40 minutes.

Embodiment 16. The CRF₁ receptor antagonist of Embodiment 14, wherein the CRF₁ receptor antagonist has a dissociation half-life in excess of 50 minutes.

Embodiment 17. The CRF₁ receptor antagonist of any one of Embodiments 14-16, wherein the CRF₁ receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

Embodiment 18. The CRF₁ receptor antagonist of any one of Embodiments 14-16, wherein the CRF₁ receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

Embodiment 19. The CRF₁ receptor antagonist of any one of Embodiments 14-18, wherein the CRF₁ receptor antagonist is suitable for administration at bedtime.

Embodiment 20. The CRF₁ receptor antagonist of any one of Embodiments 14-19, wherein the CRF₁ receptor antagonist is suitable for administration at or before the expected circadian release of ACTH.

Embodiment 21. The CRF₁ receptor antagonist of any one of Embodiments 14-20, wherein the CRF₁ receptor antagonist is suitable for administration 3-4 hours before the expected circadian release of ACTH.

In other embodiments, the methods and uses described above and herein further comprise reducing the amount of a glucocorticoid or mineralocorticoid by at least 10%, 15%, 20%, 30%, 40%, 50%, 60% from the recommended daily dose of GC (such as hydrocortisone (HC), prednisone, prednisolone, dexamethasone, or fludrocortisone) administered to a full grown subject (*e.g.*, a human subject) who has CAH. In other embodiments, the methods and uses described above and herein further comprise reducing the amount of a glucocorticoid or mineralocorticoid by at least 10%, 15%, 20%, 30%, 40%, 50%, 60% from the recommended daily dose of GC (*e.g.*,

hydrocortisone) or mineralocorticoid (e.g., fludrocortisone) administered to a growing subject (e.g., a human subject) who has CAH.

These and other embodiments will be apparent upon reference to the following detailed description. To this end, various references are set forth herein that
5 describe in more detail certain background information, procedures, compounds and compositions, and are each hereby incorporated by reference in their entirety.

Terms not specifically defined herein should be given the meanings that would be given to them by one of skill in the art in light of the disclosure and the context. As used in the specification, however, unless specified to the contrary, the
10 terms have the meaning indicated. Reference throughout this specification to “one embodiment” or “an embodiment” means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the phrases “in one embodiment” or “in an
15 referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

Also, as used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a non-human animal” may refer to one or
20 more non-human animals, or a plurality of such animals, and reference to “a cell” or “the cell” includes reference to one or more cells and equivalents thereof (e.g., plurality of cells) known to those skilled in the art, and so forth. When steps of a method are described or claimed, and the steps are described as occurring in a particular order, the description of a first step occurring (or being performed) “prior to” (*i.e.*, before) a
25 second step has the same meaning if rewritten to state that the second step occurs (or is performed) “subsequent” to the first step. The term “about” when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary between 1% and 15% of the stated
30 number or numerical range. It should also be noted that the term “or” is generally

employed in its sense including “and/or” unless the content clearly dictates otherwise. The term, “at least one,” for example, when referring to at least one compound or to at least one composition, has the same meaning and understanding as the term, “one or more.”

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic of steroid synthetic pathways in the adrenal gland. The most common form of CAH is caused by 21-hydroxylase (also called 21- α hydroxylase) deficiency resulting in a decrease of cortisol and increase in androgens such as testosterone and estrogen. A more rare type of classical CAH is 11 β -hydroxylase deficiency.

Figure 2 presents a graph illustrating the effect of Compound I (NBI-77860) on ACTH concentration in adrenalectomized rats. Rats received 30 mg/kg of Compound I (NBI-77860) orally. Data are presented as mean plasma concentration of ACTH ($\pm$ SEM).

Figures 3A-C present graphs illustrating the effect of CRF₁ receptor antagonists, differentiated by their dissociation half-life, on ACTH concentration in adrenalectomized rats.

Figure 4 presents the study design schematic for the clinical trial described in Example 6 that was designed to assess the safety, tolerability and plasma exposure of NBI-77860, as well as the effect of this compound on endogenous levels of HPA (hypothalamic-pituitary-adrenal) axis hormones.

Figure 5 presents the data for mean 17-OHP (upper panel) and ACTH levels (lower panel) throughout the 24-hour postdose period for the clinical trial described in Example 6.

Figure 6 presents the response of a specific individual subject for 17-OHP (upper panel) and ACTH levels (lower panel) following administration of 300 mg and 600 mg NBI-77860 and placebo over time.

DETAILED DESCRIPTION

As described herein, CRF₁ receptor antagonists have been found to directly inhibit ACTH release in patients with CAH and thereby allow normalization of androgen production. Administration of a CRF₁ receptor antagonist permits use of
5 lower, more physiologic doses of hydrocortisone in subjects with CAH and thus reduces treatment-associated side effects.

Newborn screening for CAH is performed by immunoassay to measure 17-OHP levels in heel-stick capillary blood specimens obtained within the first 72 hours of life. The blood sample is analyzed for 17-OHP by commercially available
10 dissociation-enhanced lanthanide fluoroimmunoassay (DELFI; PerkinElmer, Waltham Massachusetts) (White et al., J. Pediatr. 163:10-12 (2013)).

Second-tier screening tests utilizing biochemical and molecular genetic testing methods, performed between 8 and 14 days of life, are employed by nine states in the United States and strongly recommended by an additional 5 states. The
15 biochemical method includes immunoassay with organic solvent extraction or liquid chromatography followed by tandem mass spectrometry to measure steroid ratios of 17-OHP, androstenedione, and 21-deoxycortisol to cortisol (*see, e.g.,* Speiser et al., *Int. J. Pediatr. Endocrinol.* 2010:494173, 2010). The genetic screen looks for *CYP21A2* mutations that are associated with CAH. While not widely employed in the U.S., the
20 addition of a second screening could potentially improve the sensitivity of the overall screening process, where sensitivity of the first screen alone is approximately 72%.

In absence of results from the newborn screening, female infants with classical CAH are typically identified due to the presence of ambiguous genitalia. Males have normal genitalia at birth and therefore are not diagnosed unless newborn
25 screening is conducted or other medical complications come to attention. Infants who are not initially diagnosed with CAH and suffer from the salt-wasting form of the disease are later diagnosed in the setting of poor weight gain, vomiting, hyperkalemia and hyponatremia within the first few weeks of life.

Treatment of CAH is based on normalization of hormone and steroid
30 levels using a variety of medications from diagnosis in infancy through adulthood.

Glucocorticoids are the current standard treatment in CAH and are used both to correct the endogenous cortisol deficiency and for reducing the elevated ACTH levels from the pituitary, which drives increased androgen production. Unlike the treatment of Addison's disease (adrenal insufficiency), in which cortisol replacement is sufficient, the treatment of CAH must also reduce ACTH production, to control the subsequent androgen excess as well. Thus, the goals of glucocorticoid treatment include cortisol replacement and suppression of ACTH to prevent virilization and menstrual disturbances in women and to inhibit testicular adrenal rest tumors in men. Mineralocorticoid replacement is needed to achieve normal plasma renin activity for maintenance of regular blood pressure, electrolyte balance, and volume status in those patients with the salt-wasting form of CAH.

The regimen of glucocorticoid treatment must support normal physiology and also ensure that sufficient cortisol is available during events that may elicit a strong stress response (*e.g.*, intercurrent illness, exercise, hypotension). Careful monitoring is also necessary to avoid the development of iatrogenic Cushing's syndrome due to glucocorticoid overtreatment in an effort to adequately suppress androgen production, or Addisonian syndrome due to under-treatment. Overtreatment with mineralocorticoids may cause hypertension while under-treatment may lead to low blood pressure, salt loss, fatigue and increased requirements for glucocorticoids. Typical laboratory tests for monitoring treatment efficacy include measurement of plasma concentrations of 17-OHP, androstenedione, testosterone, renin activity, and electrolytes.

Adult patients with CAH have an increased prevalence of risk factors for cardiovascular disease including obesity, hypertension, and insulin resistance (*see, e.g.*, Kim et al., *Semin. Reprod. Med.* 27(4):316-21 (2009)). A study of a large cohort of pediatric and adult CAH patients (n=244) demonstrated that patients are prescribed a variety of glucocorticoid treatment regimens yet frequently suffer from poor hormonal control and the aforementioned adverse outcomes (*see, e.g.*, Finkelstein et al., *J. Clin. Endocrinol Metab.* 97(12):4429-38 (2012)).

Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids (usually hydrocortisone in children but often more potent agents with narrow therapeutic indices, such as dexamethasone, in adults) and, if necessary for salt-wasting, mineralocorticoids (usually fludrocortisone). The glucocorticoid doses
5 required to achieve sufficient suppression of excess androgens, however, are usually well above the normal physiologic dose used for cortisol replacement alone as in patients with Addison's disease. This increased exposure to glucocorticoids can lead to iatrogenic Cushing's syndrome, increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density in CAH patients (*see, e.g., Elnecave et al., J. Pediatr. Endocrinol. Metab.* 21:1155-62 (2008); King et al., *J. Clin. Endocrinol. Metab.* 91(3):8656-59 (2006); Migeon et al., *Endocrinol. Metab. Clin. North Am.* 30:193-206 (2001)).

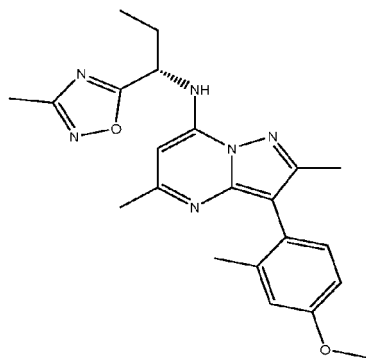
Corticotropin-releasing factor (CRF) was isolated from ovine hypothalami and identified as a 41-amino acid peptide. CRF has been found to produce
15 profound alterations in endocrine, nervous, and immune system function. CRF is believed to be the major physiological regulator of the basal and stress-induced release of adrenocorticotrophic hormone ("ACTH"), β -endorphin, and other pro-opiomelanocortin ("POMC")-derived peptides from the anterior pituitary (*see, e.g., Vale et al., Science* 213:1394-1397, 1981). Secretion of CRF causes release of ACTH
20 from corticotrophs in the anterior pituitary via binding to the CRF₁ receptor, a member of the class B family of G-protein coupled receptors.

Due to the physiological significance of CRF, the development of biologically-active small molecules having significant CRF₁ receptor binding activity and which are capable of antagonizing the CRF₁ receptor remains a desirable goal and
25 has been the subject of ongoing research and development for the treatment of anxiety, depression, irritable bowel syndrome, post-traumatic stress disorder, and substance abuse.

The pituitary hormone ACTH, under the control of hypothalamic corticotropin-releasing factor (CRF), stimulates uptake of cholesterol and drives the
30 synthesis of pregnenolone initiating steroidogenesis in the adrenal gland (*see* Figure 1).

The adrenal cortex is comprised of three zones, which produce distinct classes of hormones many of which are driven by ACTH mobilizing cholesterol through this pathway. Deficiencies in these enzymes as a result of mutation or deletion cause the substrate concentrations to increase. In the most common form of CAH resulting from mutations or deletions in the 21-hydroxylase gene (CYP21A2), potent androgens are produced by the adrenal because of the accumulation of the steroid precursors, progesterone and 17-hydroxyprogesterone (17-OHP). Plasma levels of 17-OHP can reach 10-1000 times the normal concentration in these cases. These increases result in the overproduction of androgens, specifically androstenedione, testosterone, and dihydroxytestosterone causing virilization in females. In addition, 21-hydroxylase deficiency in CAH causes insufficient biosynthesis of glucocorticoids and mineralocorticoids, specifically cortisol and aldosterone. Cortisol is a critical negative feedback regulator of hypothalamic CRF secretion and pituitary ACTH release. The lack of glucocorticoid synthesis and release eliminates the restraint on the hypothalamus and pituitary, which causes ACTH levels to increase. The excessive ACTH stimulation causes hypertrophy of the zona fasciculata and zona reticularis resulting in adrenal hyperplasia.

In an embodiment, a CRF₁ receptor antagonist useful for the treatment of CAH is NBI-77860, 2,5-dimethyl-3-[2-methyl-4-(methoxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine (also referred to as “Compound I” herein), and which has the following structure.



Compound I

Compound I is a potent CRF₁ antagonist possessing a binding pK_i = 8.2 (see, e.g., Tellet et al., *Bioorg. Med. Chem. Lett.* 20:7259, 2010 and Int'l. Patent Appl. Publ. No. WO 2006/044958, both of which references are incorporated by reference herein in their entirety). As described herein, Compound I has potent ACTH lowering effects as shown in adrenalectomized rats.

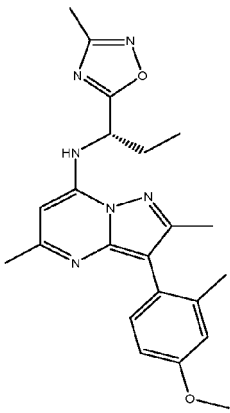
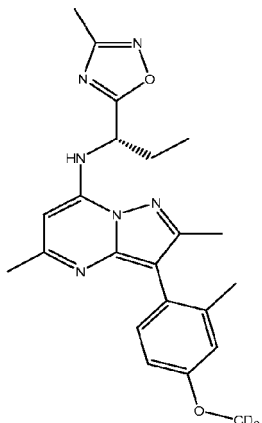
In another embodiment, the CRF₁ receptor antagonist useful for the treatment of CAH is a small molecule antagonist as described in U.S. 6,586,456, US 6,806,282, US 6,531,475, US 6,664,261, US 6,610,678, WO 98/08846, WO 98/11075, WO 99/10350, WO 2000/059888, WO 2006/044821, WO 2006/102194, WO 2006/107784, WO 2006/116412, WO 2006/126718, WO 2007/069565, WO 2007/069671, WO 2008/036541, WO 2008/036579, WO 2008/051533, WO 2008/082003, WO 2008/083070, WO 2008/136377, WO 2009/008552, WO 2009/144632, WO 2010/014280, WO 2010/014687, WO 2010/015628, WO 2010/015655, WO 2010/062718, WO 2010/096426, WO 2011/043387, WO 2011/092293, WO 2011/095450, WO 2011/092290, and WO 2011/043381.

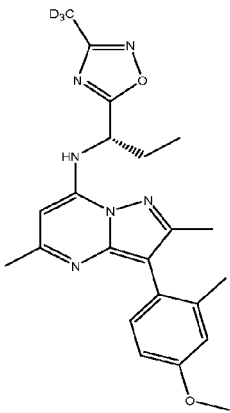
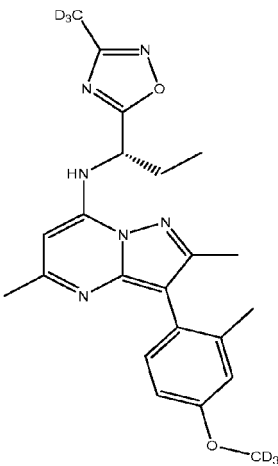
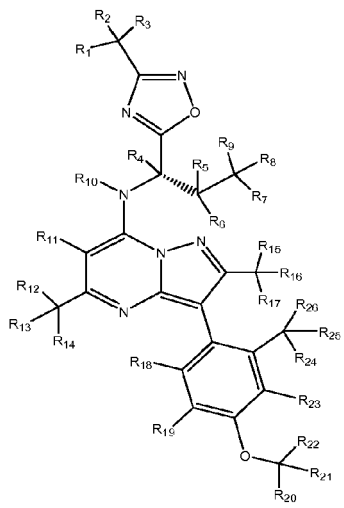
In still another embodiment, the CRF₁ receptor antagonist is NBI-30775, CP-316,311, pexacerfont, emicerfont, SSR-125543 [4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-(2-propyn-1-yl)-2-thiazolamine], SSR-126374, ONO-2333, NBI-34041, JNJ-19567470, GSK586529, PF-00572778, CP-376395, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), and DMP904.

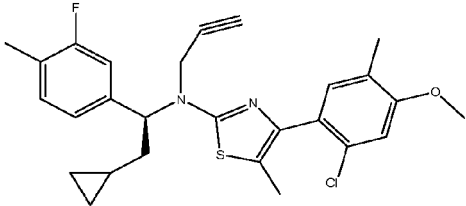
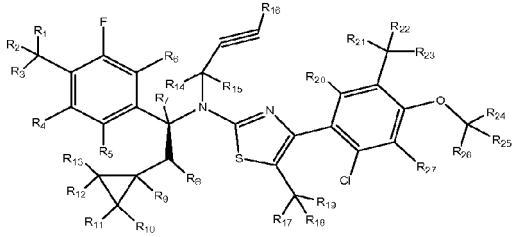
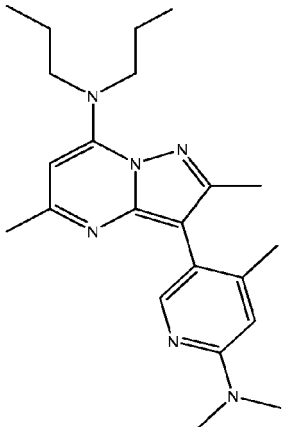
In yet another embodiment, the CRF₁ receptor antagonist has a dissociation half-life ($t_{1/2}$) in excess of 30 minutes, and in another embodiment in excess of 40 minutes, and in another embodiment in excess of 50 minutes. The dissociation half-life of a particular CRF₁ receptor antagonist is determined by the technique disclosed in Example 3. Representative CRF₁ receptor antagonists of these embodiments include Compound I, NBI-30775, NBI-34041, SSR-125543A, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), and DMP904.

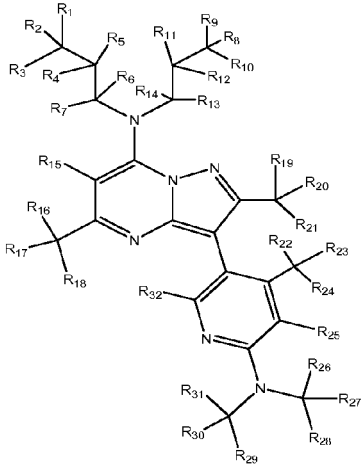
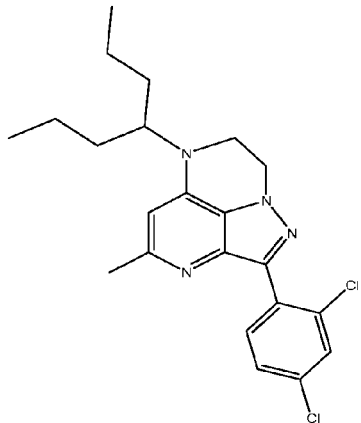
With respect to the compounds described herein, it should also be understood that when a particular position is designated as hydrogen, that such hydrogen (H) may be replaced with deuterium (D). Incorporation of deuterium in place of hydrogen is known to produce significant effects on the physiological and pharmacological activities of the substituted compound. To this end, it should be understood that deuterium replacement of hydrogen means that the abundance of deuterium at that position is substantially greater than the natural abundance of deuterium. Accordingly, in one embodiment representative compounds include the following.

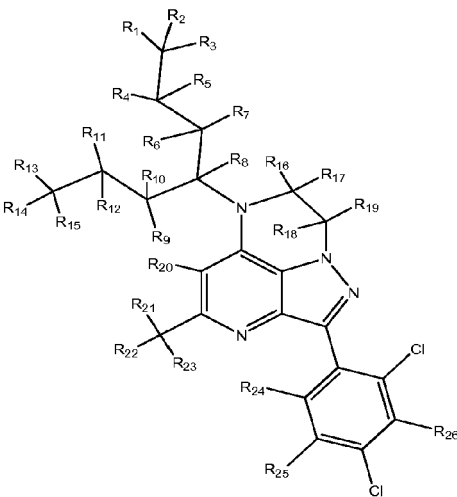
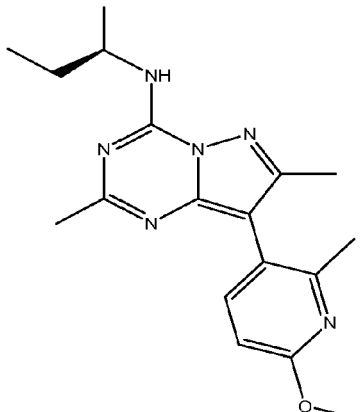
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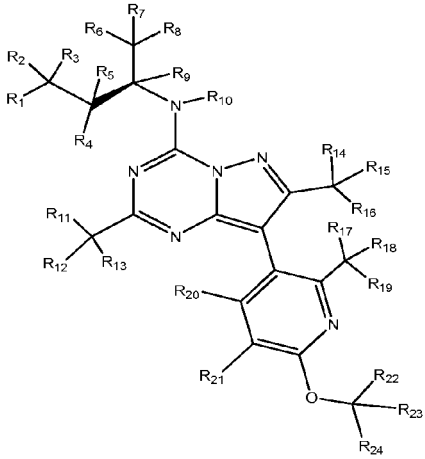
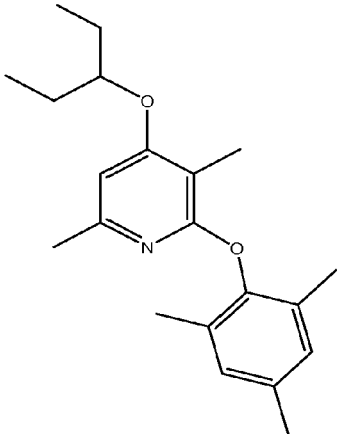
Cpd.	Structure	Chemical Name
NBI-77860		2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine
Deuterated NBI-77860		2,5-dimethyl-3-[2-methyl-4-(d ₃ -methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine

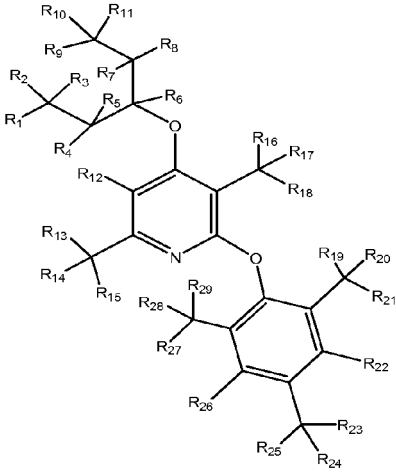
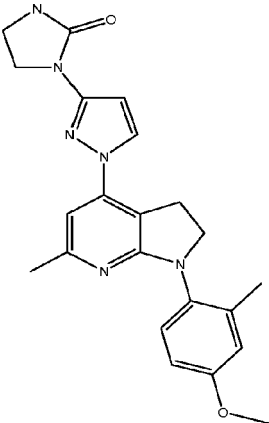
Cpd.	Structure	Chemical Name
Deuterated NBI-77860		2,5-dimethyl-3-[2-methyl-4-(methoxy)phenyl]-N-[(1S)-1-(3-d ₃ -methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine
Deuterated NBI-77860		2,5-dimethyl-3-[2-methyl-4-(d ₃ -methoxy)phenyl]-N-[(1S)-1-(3-d ₃ -methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine
Deuterated NBI-77860		

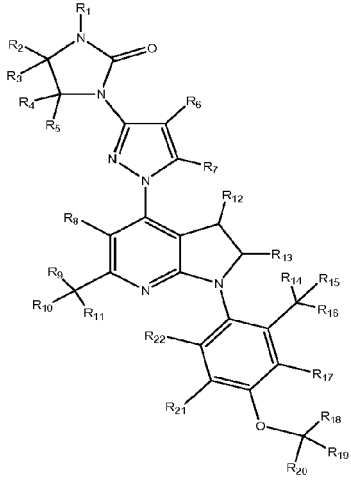
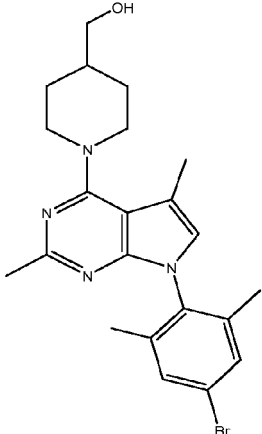
Cpd.	Structure	Chemical Name
	R ₁ -R ₂₆ independently H or D	
SSR-125543		4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-(2-propyn-1-yl)-2-thiazolamine
Deuterated SSR-125543	 <p>R₁-R₂₇ independently H or D</p>	
NBI-30775		5-[7-(dipropylamino)-2,5-dimethylpyrazolo[1,5-a]pyrimidin-3-yl]-N,N,4-trimethylpyridin-2-amine

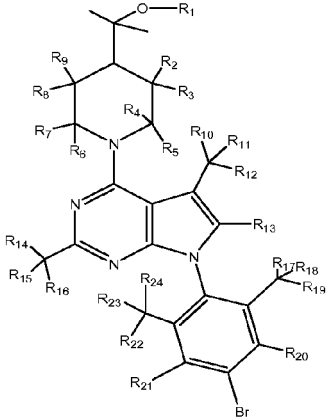
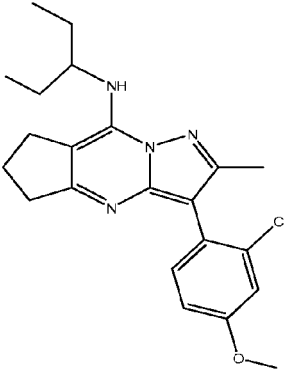
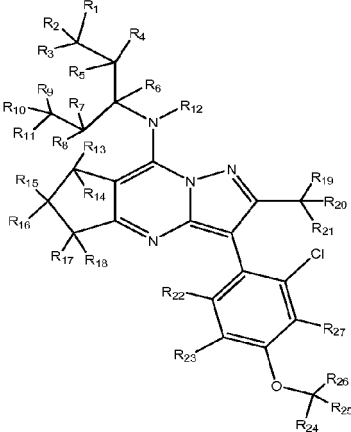
Cpd.	Structure	Chemical Name
Deuterated NBI-30775	 <p>R₁-R₃₂ independently H or D</p>	
NBI-34041		3-(2,4-dichlorophenyl)- 9-(heptan-4-yl)-6- methyl- 1,2,5,9- tetraazatricyclo[6.3.1.0 ⁴ , 12]dodeca- 2,4(12),5,7- tetraene

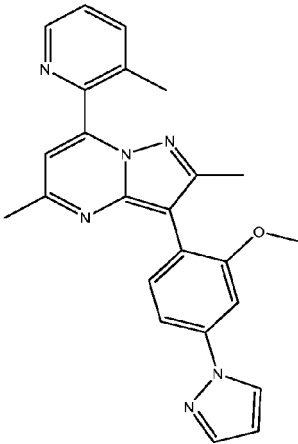
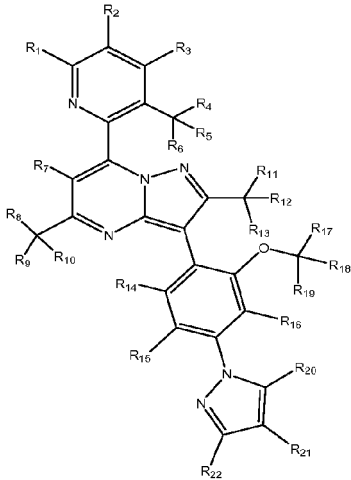
Cpd.	Structure	Chemical Name
Deuterated NBI-34041	 <p>R₁-R₂₆ independently H or D</p>	
Pexacerfont		N-[(2R)-butan-2-yl]-8-(6-methoxy-2-methylpyridin-3-yl)-2,7-dimethylpyrazolo[1,5-a][1,3,5]triazin-4-amine

Cpd.	Structure	Chemical Name
Deuterated Pexacerfont	 <p>R₁-R₂₄ independently H or D</p>	
CP-316,311		3,6-dimethyl-4-(pentan-3-yloxy)-2-(2,4,6-trimethylphenoxy)pyridine

Cpd.	Structure	Chemical Name
Deuterated CP-316,311	 <p>R₁-R₂₉ independently H or D</p>	
GSK876008		

Cpd.	Structure	Chemical Name
Deuterated GSK876008	 <p>The structure shows a complex polycyclic molecule with multiple R-substituents labeled R₁ through R₂₂. It includes a pyrazole ring, a pyridine ring, and a benzene ring with an ether linkage. The substituents are distributed across these rings and their fused systems.</p> <p>R₁-R₂₂ independently H or D</p>	
CRA5626/R3 17573	 <p>The structure shows a polycyclic molecule with a pyrazole ring, a pyridine ring, and a benzene ring. It features a hydroxymethyl group (-CH₂OH) attached to the pyrazole ring and a bromine atom (Br) on the benzene ring.</p>	

Cpd.	Structure	Chemical Name
Deuterated CRA5626/R3 17573	 <p>R₁-R₂₄ independently H or D</p>	
ONO2333Ms		10-(2-chloro-4-methoxyphenyl)-11-methyl-N-(pentan-3-yl)-1,8,12-triazatricyclo[7.3.0.0 ^{3,7}]dodeca-2,7,9,11-tetraen-2-amine
Deuterated ONO2333	 <p>R₁-R₂₇ independently H or D</p>	

Cpd.	Structure	Chemical Name
NBI76169		
Deuterated NBI76169	 <p data-bbox="597 1346 945 1377">R₁-R₂₂ independently H or D</p>	

In another embodiment, any of the aforementioned compounds may incorporate stable or radioactive isotopes. Accordingly, also contemplated is use of isotopically-labeled compounds identical to those described herein, wherein one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into these compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as, but not limited to

deuterium as discussed above (2H), as well as 3H, 13C, 14C, 15N, 18O, 17O, 31P, 32P, 35S, 18F, and 36Cl, respectively. Certain isotopically-labeled compounds, for example those into which radioactive isotopes such as 3H and 14C are incorporated, are also useful in drug or substrate tissue distribution assays. Tritiated hydrogen (3H) and carbon-14 (14C) isotopes are particularly preferred for their ease of preparation and detectability. Substitution with heavier isotopes such as deuterium (2H) can provide certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dose requirements and, therefore, may be preferred in some circumstances. Isotopically-labeled compounds can generally be prepared by performing procedures routinely practiced in the art.

Effect on Plasma ACTH in Adrenalectomized Rats

Adrenalectomy eliminates circulating corticosterone (the primary glucocorticoid) in rats and removes the negative feedback control of the HPA axis at both the hypothalamic and pituitary (corticotroph cells) levels and thus chronically elevates plasma ACTH (*see, e.g., Mims et al., J. Natl. Med. Assoc.* 69:145-47 (1977)). Intravenous injection of peptide CRF₁ receptor antagonists have been demonstrated to reduce the high plasma ACTH levels in adrenalectomized (ADX) rats (*see, e.g., Rivier et al., J. Med. Chem.* 12:42:3175-82 (1999)). These findings were recapitulated with the small molecule NBI-77860 (Compound I). In adrenalectomized rats, Compound I has potent capability for lowering ACTH. The maximum reduction in ACTH correlated with peak plasma concentrations of NBI-77860; however, the duration of the ACTH-lowering effect exceeded drug plasma exposure. In adrenalectomized rats, a predictable relationship therefore exists between integrated plasma exposure of NBI-77860 and *in vivo* efficacy following oral administration.

The effectiveness of a compound as a CRF receptor antagonist may be determined by various assay methods. CRF antagonists described herein may be capable of inhibiting the specific binding of CRF to its receptor and consequently antagonizing activities associated with CRF. A compound may be assessed for activity as a CRF antagonist by one or more generally accepted assays including the assay

described in the Examples. CRF antagonists useful for the methods described herein include compounds that demonstrate affinity for CRF receptor.

Without wishing to be bound by theory, in the treatment of CAH, CRF receptor antagonists would potentially block the release of ACTH from pituitary corticotrophs, thereby decreasing the production of androgens, and allow a more refined treatment paradigm for replacement of cortisol. Animal and human studies have shown the pharmacologic effect of Compound I (NBI-77860) on ACTH release. Standard biomarker assessments used by endocrinologists when monitoring treatment efficacy may be used for monitoring the effects of this CRF₁ receptor antagonist. Plasma levels of 17-OHP, androstenedione, testosterone, cortisol and ACTH, as well as urinary metabolites of these steroids, are easily measured in both children and adults giving rapid and meaningful data regarding treatment impact.

Pharmaceutical Compositions and Methods of Treatment

The present disclosure further provides for pharmaceutical compositions comprising any one of the CRF antagonist compounds described herein and a pharmaceutically acceptable excipient for use in the methods for treating CAH. A pharmaceutically acceptable excipient is a physiologically and pharmaceutically suitable non-toxic and inactive material or ingredient that does not interfere with the activity of the active ingredient; an excipient also may be called a carrier. The CRF antagonist compounds may be formulated in a pharmaceutical composition for use in treatment or preventive (or prophylactic) treatment (*e.g.*, reducing exacerbation of CAH disease, or occurrence or recurrence of one or more symptoms of the disease). The methods and excipients described herein are exemplary and are in no way limiting. Pharmaceutically acceptable excipients are well known in the pharmaceutical art and described, for example, in Rowe *et al.*, Handbook of Pharmaceutical Excipients: A Comprehensive Guide to Uses, Properties, and Safety, 5th Ed., 2006, and in *Remington: The Science and Practice of Pharmacy* (Gennaro, 21st Ed. Mack Pub. Co., Easton, PA (2005)). Examples of pharmaceutically acceptable excipients include sterile saline and phosphate buffered saline at physiological pH. Preservatives, stabilizers, dyes, buffers,

and the like may be provided in the pharmaceutical composition. In addition, antioxidants and suspending agents may also be used.

Therapeutic and/or prophylactic benefit includes, for example, an improved clinical outcome, both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow or retard (lessen) an undesired physiological change or disorder, or to prevent or slow or retard (lessen) the expansion or severity of such disorder. As discussed herein, beneficial or desired clinical results from treating a subject include, but are not limited to, abatement, lessening, or alleviation of symptoms that result from or are associated the disease, condition, or disorder to be treated; decreased occurrence of symptoms; improved quality of life; longer disease-free status (*i.e.*, decreasing the likelihood or the propensity that a subject will present symptoms on the basis of which a diagnosis of a disease is made); diminishment of extent of disease; stabilized (*i.e.*, not worsening) state of disease; delay or slowing of disease progression; amelioration or palliation of the disease state; and remission (whether partial or total), whether detectable or undetectable; and/or overall survival. "Treatment" can also mean prolonging survival when compared to expected survival if a subject were not receiving treatment. Subjects in need of treatment include those who already have the condition or disorder as well as subjects prone to have or at risk of developing the disease, condition, or disorder, and those in which the disease, condition, or disorder is to be prevented (*i.e.*, decreasing the likelihood of occurrence of the disease, disorder, or condition). A subject may be a human or non-human mammal (e.g., rat, mouse, dog, cat, livestock, zoo animal).

Optimal doses may generally be determined using experimental models and/or clinical trials. The optimal dose may depend upon the body mass, weight, or blood volume of the subject. In general, the amount of a compound described herein, that is present in a dose ranges from about 0.1 mg to about 30 mg per kg weight of the subject. In certain embodiments, a single dose is about 50-1000 mg. The use of the minimum dose that is sufficient to provide effective therapy is usually preferred. Subjects may generally be monitored for therapeutic effectiveness by clinical evaluation and using assays suitable for the condition being treated or

prevented, which assays will be familiar to those having ordinary skill in the art and are described herein. The level of a compound that is administered to a subject may be monitored by determining the level of the compound in a biological fluid, for example, in the blood, blood fraction (*e.g.*, serum), and/or in the urine, and/or other
5 biological sample from the subject. Any method practiced in the art to detect the compound may be used to measure the level of compound during the course of a therapeutic regimen.

The dose of a composition comprising at least one of the compounds described herein for treating CAH or a related disease or disorder may depend upon the
10 subject's condition, that is, stage of the disease, severity of symptoms caused by the disease, general health status, as well as age, gender, and weight, and other factors apparent to a person skilled in the medical art. Similarly, the dose of the compound may be determined according to parameters understood by a person skilled in the medical art.

15 The pharmaceutical compositions described herein that comprise at least one of the CRF₁ antagonist compounds described herein may be administered to a subject in need by any one of several routes that effectively deliver an effective amount of the compound. Such administrative routes include, for example, oral, parenteral, enteral, rectal, intranasal, buccal, sublingual, intramuscular, and transdermal.
20 Compositions administered by these routes of administration and others are described in greater detail herein.

Administration of the compounds or compositions disclosed includes nighttime administration or administration prior to sleep (*i.e.*, bedtime administration). As used herein, bedtime administration refers to dosing intended to deliver clinically
25 relevant concentrations of the CRF₁ antagonist at or before (such as 2-5 hours before) the expected circadian release of ACTH. Since this ACTH release is typically at 1-2 A.M., and since most orally administered drugs have a T_{max} of several hours, dosing at 10 P.M., for example, which is 3-4 hours in advance of the expected circadian release of ACTH is desirable. This same pre-pulse bedtime dosing may be adapted for shift
30 workers (*e.g.*, those who work at night and sleep during the day), in which case

administration will not necessarily occur at nighttime. Administration is therefore dependent upon the expected circadian release of ACTH, and can vary depending upon the individual's (*i.e.*, subject, patient) particular work and sleep patterns. In certain embodiments, a CRF₁ receptor antagonist described herein (for example, NBI-77860 or
5 any one of NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904) is administered between about 2-5 hours prior to (*i.e.*, before, in advance of) the expected circadian release of ACTH. In other embodiments, the CRF₁ receptor antagonist is administered to the subject between about 2-4 hours or 3-5 hours prior to
10 the expected circadian release of ACTH. In a more specific embodiment, CRF₁ receptor antagonist is administered to the subject between about 3-4 hours prior to the expected circadian release of ACTH.

Also provided herein is a method for reducing 17-OHP and/or ACTH levels in a subject (*i.e.*, patient, individual) who has CAH by administering a CRF₁
15 receptor antagonist. In certain embodiments, the antagonist is administered at or before the expected circadian release of ACTH. In other certain embodiments, the CRF₁ receptor antagonist is administered between about 2-4 hours or between about 3-5 hours prior to the expected circadian release of ACTH. In a more specific embodiment, CRF₁ receptor antagonist is administered to the subject between about 3-4 hours prior
20 to the expected circadian release of ACTH.

The methods described herein that comprise administering a CRF₁ receptor antagonist to a female subject in need thereof in the manner described herein that causes a decrease in level of ACTH and 17-OHP in the subject may result in decreased release of androgens such as testosterone and androstenedione. The dose of
25 glucocorticoids may concomitantly be decreased by a clinically significant amount, which in turn results in decreased side effects.

The amount of glucocorticoids (GC) and mineralocorticoids (MC) for maintenance therapy in patients who have CAH and who are young and growing and for maintenance therapy in patients who are full grown patients is known to a person
30 skilled in the art. For example, guidelines are described in Speiser et al. (*J. Clin.*

Endocrinol. Metab. 95:4133-60 (2010), incorporated by reference in its entirety) and shown in the Tables 1 and 2 therein. Particularly, for young, growing patients who have CAH, persons skilled in the art appreciate that too high dosing with GCs can impede statural growth in the patient. Accordingly, the methods described herein for
5 treating a patient with a CRF₁ receptor antagonist may comprise reducing the dose of a GC in a clinically significant manner.

In certain embodiments, methods for treating CAH by administering a CRF₁ receptor antagonist may further comprise administering a GC at a dose lower than the currently recommended dose of a GC for treating a subject who has CAH. When
10 the subject is a fully grown patient, the dose of a GC, such as the dose of hydrocortisone (HC), prednisone, prednisolone, dexamethasone, or fludrocortisone recommended for maintenance therapy in a fully grown subject may be decreased by about 10%, 15%, 20%, 30%, 40%, 50%, 60% or more from the recommended doses of 15-25 mg/day HC; 5-7.5 mg/day prednisone, 4-6 mg/day prednisolone; 0.25-0.5 mg/day
15 dexamethasone, or 0.05-0.2 mg/day of fludrocortisone. In a subject who is a growing patient with CAH, the recommended dose of GCs, such as HC may be decreased from the total recommended dose of 10-15 mg/m² per day and/or the total dose of fludrocortisone of 0.05-0.2 mg/day may each be decreased by about 10%, 15%, 20%, 30%, 40%, 50%, 60% or more in a subject who receives a CRF₁ receptor antagonist as
20 described herein. In an embodiment, the methods described herein comprising administering to a subject in need thereof an effective amount of a CRF₁ antagonist results in a clinically significant reduction of ACTH levels relative to placebo. In a specific embodiment, administering to a subject in need thereof an effective amount of a CRF₁ antagonist results in a clinically significant reduction of ACTH levels relative to
25 placebo, wherein that reduction is at least 25%. In another embodiment, administering to a subject in need thereof an effective amount of a CRF₁ antagonist results in a clinically significant reduction of ACTH levels relative to placebo, wherein that reduction is at least 50%. See Speiser et al., *supra*, for guidelines regarding administering glucocorticoids and mineralocorticoids.

In another embodiment, the methods described herein comprising administering to a subject in need thereof an effective amount of a CRF₁ antagonist results in a clinically significant reduction of 17-OHP levels relative to placebo. In another specific embodiment, administering to a subject in need thereof an effective
5 amount of a CRF₁ antagonist results in a clinically significant reduction of 17-OHP levels relative to placebo wherein that reduction is at least 25%. In still another embodiment, administering to a subject in need thereof an effective amount of a CRF₁ antagonist results in a clinically significant reduction of 17-OHP levels relative to placebo wherein that reduction is at least 50%.

10 In an embodiment, the methods described herein comprising administering to a subject in need thereof an effective amount of a CRF₁ antagonist results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo. In a certain embodiment, administering to a subject in need thereof an effective amount of a CRF₁ antagonist results in a clinically significant reduction of
15 both ACTH and 17-OHP levels relative to placebo wherein the reduction is at least 25%. In another specific embodiment, administering to a subject in need thereof an effective amount of a CRF₁ antagonist results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo wherein the reduction is at least 50%.

20 The pharmaceutical compositions may be in the form of a solution. Alternatively, they may be in the form of a solid, such as powder, tablets, or the like. A composition comprising any one of the compounds described herein may be formulated for sustained or slow release. Such compositions may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous
25 implantation, or by implantation at the desired target site. Sustained-release formulations may contain the compound dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Excipients for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The
30 amount of active compound contained within a sustained release formulation depends

upon the site of implantation, the rate and expected duration of release, and the nature of the condition to be treated or prevented.

For oral formulations, at least one of the compounds described herein can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders; with disintegrators; with lubricants; and if desired, with diluents, buffering agents, moistening agents, preservatives, coloring agents, and flavoring agents. The compounds may be formulated with a buffering agent to provide for protection of the compound from low pH of the gastric environment and/or an enteric coating. A compound included in the compositions may be formulated for oral delivery with a flavoring agent, *e.g.*, in a liquid, solid or semi-solid formulation and/or with an enteric coating. Oral formulations may be provided as gelatin capsules, which may contain the active compound along with powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar carriers and diluents may be used to make compressed tablets.

EXAMPLES

EXAMPLE 1

CRF RECEPTOR BINDING ACTIVITY

CRF antagonists as used in the methods described herein may be evaluated for binding activity to the CRF receptor by a standard radioligand binding assay as generally described by Grigoriadis et al. (*see, e.g., Mol. Pharmacol* vol 50, pp 679-686, 1996) and Hoare et al. (*see, e.g., Mol. Pharmacol* 63: 751-765, 2003.) By utilizing radiolabeled CRF ligands, the assay may be used to evaluate the binding activity of the compounds described herein with any CRF receptor subtype.

Briefly, the binding assay involves the displacement of a radiolabeled CRF ligand from the CRF receptor. More specifically, the binding assay is performed in 96-well assay plates using 1-10 μ g cell membranes from cells stably transfected with

human CRF receptors. Each well receives about 0.05 mL assay buffer (*e.g.*, Dulbecco's phosphate buffered saline, 10 mM magnesium chloride, 2 mM EGTA) containing compound of interest or a reference ligand (for example, sauvagine, urocortin I, or CRF), 0.05 mL of [¹²⁵I] tyrosine - sauvagine (final concentration ~150 pM or approximately the K_D as determined by Scatchard analysis) and 0.1 mL of a cell membrane suspension containing the CRF receptor. The mixture is incubated for 2 hours at 22°C followed by separation of the bound and free radioligand by rapid filtration over glass fiber filters. Following three washes, the filters are dried and radioactivity (Auger electrons from ¹²⁵I) is counted using a scintillation counter. All radioligand binding data may be analyzed using the non-linear least-squares curve-fitting programs Prism (GraphPad Software Inc) or XLfit (ID Business Solutions Ltd).

EXAMPLE 2

CRF₁ RECEPTOR AGONIST ACTIVITY

As reported in Fleck *et al.* (*J. Pharmacology and Experimental Therapeutics*, 341(2):518-531, 2012) (hereinafter "Fleck *et al.*" and incorporated by reference in it's entirety) the activity of previously identified CRF₁ receptor antagonists are presented. Such activity is reported as the kinetically derived affinity (K_i) calculated from the association (*k*₁) and dissociation (*k*₋₁) rate constants by the following equation:

$$K_i = k_{-1}/k_1$$

Also as reported in Fleck *et al.*, the kinetic K_i of the CRF₁ receptor antagonists listed in Table 1 below have been reported:

Table 1
Representative CRF₁ Receptor Antagonists

Ligand	<i>k</i> ₁ (10 ⁶ M ⁻¹ min ⁻¹)	Kinetic K _i (nM)
NBI-27914	9.4 ± 3	25

Ligand	k_1 ($10^6 \text{M}^{-1} \text{min}^{-1}$)	Kinetic K_i (nM)
CP-316,311	13 ± 2	12
NBI-46200	6.2 ± 2	22
DMP696	7.7 ± 2	9.5
pexacerfont	2.6 ± 0.1	19
NBI-35965	20 ± 2	2.3
ONO-2333Ms	4.4 ± 2.2	15
antalarmin	3.4 ± 0.6	3.9
NBI-34041	8.3 ± 2.0	1.7
DMP904	18 ± 1	0.38
NBI-30775	14 ± 2.0	0.36
SSR125543A	33 ± 5	0.049

By this same technique, the kinetic K_i of Compound I (NBI-77860) was found to be as follows:

Table 1 (cont.)

Ligand	k_1 ($10^6 \text{M}^{-1} \text{min}^{-1}$)	Kinetic K_i (nM)
NBI-77860	0.24 ± 0.05	48 ± 9

5

EXAMPLE 3

DISSOCIATION HALF-LIFE ($t_{1/2}$) OF CRF₁ RECEPTOR ANTAGONISTS

The dissociation half-life ($t_{1/2}$) of a CRF₁ receptor antagonist as used in the methods described herein is evaluated by the technique described in Fleck *et al.* As described therein, the dissociation rate constant for labeled and unlabeled ligands is

denoted as k_{-1} , while the half-life of drug dissociation from the receptor ($t_{1/2}$), which is equal to the median residence time, is calculated from the dissociation rate constant (k_{-1}) by the following equation:

$$t_{1/2} = 0.693/k_{-1}$$

- 5 As reported in Fleck *et al.*, the dissociation half-life ($t_{1/2}$) of the CRF₁ receptor antagonists listed in Table 2 below have been reported.

Table 2
Dissociation Half-Life of Representative Compounds

Ligand	k_{-1} (min⁻¹)	Dissociation $t_{1/2}$ (min)
NBI 27914	0.27 ± 0.07	2.6
CP-316,311	0.17 ± 0.04	4.1
NBI-46200	0.13 ± 0.002	5.3
DMP696	0.095 ± 0.02	7.3
pexacerfont	0.049 ± 0.001	14
NBI-35965	0.048 ± 0.005	16
ONO-2333Ms	0.063 ± 0.029	17
antalarmin	0.013 ± 0.002	53
NBI-34041	0.013 ± 0.002	53
DMP904	0.0072 ± 0.002	96
NBI-30775	0.0054 ± 0.0006	130
SSR125543A	0.0016 ± 0.0003	430

- 10 By this same technique, the dissociation half-life of Compound I (NBI-77860) was found to be as follows.

Table 2 (cont.)

Ligand	k_{-1} , min^{-1}	Dissociation $t_{1/2}$, min
NBI-77860	0.012 ± 0.0003	58

Accordingly, CRF₁ receptor antagonists that have a dissociation half-life ($t_{1/2}$) in excess of 30 minutes include (but are not limited to) antalarim, NBI-34041, DMP904, NBI-30775, SSR125543A, and NBI-77860 (Compound I). These same compounds are also representative of CRF₁ receptor antagonists having a dissociation half-life ($t_{1/2}$) in excess of 40 minutes, and a dissociation half-life ($t_{1/2}$) in excess of 50 minutes.

EXAMPLE 4

10 LOWERING OF ACTH IN ADRENALECTOMIZED RATS

Compound I (NBI-77860) (*see, e.g.,* Tellew et al., *Bioorg. Med. Chem. Lett.* 2010, 20:7259; WO2006044958) is a potent CRF₁ antagonist possessing a binding pK_i of 8.2, a kinetic K_i of 49 nM (Table 1 above), and a Dissociation $t_{1/2}$ of 58 minutes (Table 2 above).

15 Intravenous injection of peptide CRF₁ receptor antagonists have been demonstrated to reduce the high plasma ACTH levels in adrenalectomized (ADX) rats (*see, e.g.,* Rivier et al., *J. Med. Chem.* 12:42:3175-82 (1999)). These findings were recapitulated with the small molecule NBI-77860. When administered orally to ADX rats (n=6/group), 30 mg/kg single doses of NBI-77860 significantly lowered the plasma
20 ACTH levels for up to 5 hours (*see* Figure 2). The duration of efficacy correlated with peak plasma concentrations, with a time course exceeding drug plasma exposure. In adrenalectomized rats, a predictable relationship exists between integrated plasma exposure of NBI-77860 and *in vivo* efficacy following oral administration.

25 Fleck *et al.* also report the effects of CRF₁ receptor antagonists differentiated by their dissociation half-life on plasma ACTH levels in adrenalectomized rats; namely, NBI 30775, NBI 34041 and NBI 35965. At the highest

dose (10 mg/kg), all three ligands reduced ACTH acutely (1 hr post-injection, Figure 3A-C). After a longer duration, a clear difference emerged between NBI 35965 and the other two ligands. The ACTH level returned to the vehicle level by 2 hr for NBI 35965 (Fig. 3C), whereas the response was sustained for 4-6 hr for NBI 30775 and NBI 34041 (Fig. 3A and 3B).

EXAMPLE 5

PHARMACODYNAMIC EFFECTS OF COMPOUND I IN HUMANS

In this study, pharmacodynamic effects of NBI-77860 (Compound I) on human subjects were evaluated by observing the effect of single oral doses on HPA axis response produced by metyrapone (0.04 g/kg) with respect to placebo and to a single dose of alprazolam (0.75 mg). Metyrapone blocks cortisol synthesis in the adrenal, thereby mimicking the cortisol deficiency of CAH, and is associated with an acute reflex rise ACTH levels.

The primary analysis was performed by determining the ACTH values between 30 min and 4 h post dose (AUC (30 min to 4 h)). In this analysis, when compared to placebo, the following observations were made. A significant reduction of ACTH AUC (30 min to 4 h) was observed with the alprazolam and the NBI-77860, 400 mg treatments. A non-significant reduction in ACTH AUC (30 min to 4 h) was seen with the NBI-77860, 50 mg treatment. An increase in AUC (30 min to 4 h) was seen with the NBI-77860, 10 mg treatment.

In the analysis performed on the ACTH values between 2 h to 4 h post-dose (AUC (2 h to 4 h)) (the period when exposure reached the C_{max} value), there was a significant reduction of ACTH levels in the NBI-77860, 400 mg and 50 mg treatment periods compared with the placebo treatment period; however, the reduction was not seen with the NBI-77860 at the 10 mg dose.

EXAMPLE 6
CLINICAL STUDY

In this study, NBI-77860 (Compound I) was evaluated in the clinical study entitled “A Phase 1, Single-Blind, Placebo-Controlled, Fixed-Sequence, Single-Dose Study to Evaluate the Safety and Tolerability of NBI-77860 in Adult Females with Congenital Adrenal Hyperplasia” (IND 117,388). The study was a single-blind, placebo-controlled, single center, fixed-sequence, single-dose clinical trial in adult female classical CAH patients. It was designed to assess the safety, tolerability, and plasma exposure of NBI-77860, as well as the effect of this compound on endogenous levels of HPA axis hormones.

A total of 8 female subjects, ages 19 to 58, with a medical diagnosis of classical 21-hydroxylase deficiency CAH were administered single bedtime doses (hs) of NBI-77860 300 mg, 600 mg, and placebo during three separate treatment periods (see study design schematic in Figure 4). The subjects’ usual morning dose of concurrent steroidal treatment was delayed until after the 16-hour postdose blood samples were collected (*i.e.*, until approximately 1400h).

The PD endpoints for this study included the HPA axis biomarkers of interest in this patient population; namely, 17-hydroxyprogesterone (17-OHP; as the primary PD endpoint), adrenocorticotrophic hormone (ACTH), androstenedione, testosterone, and serum cortisol levels. The initial analysis of PD variables was an examination of the grouped subject data for each biomarker, expressed as a mean percent change from predose levels for the two active dosing conditions relative to the placebo condition. The data for mean 17-OHP and ACTH levels throughout the 24-hour postdose period are provided in Figure 5.

Consistent and clinically meaningful reductions from predose levels of both 17-OHP and ACTH were observed throughout the postdose period following administration of NBI-77860 relative to placebo in these CAH patients. In addition to the group mean data, individual responses were evaluated and treatment “responders” were conservatively defined as those subjects with at least a 50% decrease in 17-OHP and ACTH under active NBI-77860 relative to placebo during the peak morning period.

This responder analysis yielded a sizeable responder rate of 50% in the study (none of these subjects were responders during the initial placebo treatment period). Furthermore, the 300 mg dose yielded nearly identical effects on 17-OHP and ACTH as the 600 mg dose. An example of an individual subject response for these biomarkers is
5 provided in Figure 6.

The various embodiments described above can be combined to provide further embodiments. All U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications
10 referred to in this specification and/or listed in the Application Data Sheet, including U.S. Provisional Application Serial Nos. 61,929,941, filed January 21, 2014; 61,981,033, filed April 17, 2014; and 62/069,155, filed October 27, 2014, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and
15 publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible
20 embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

I claim the following:

1. A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF₁ receptor antagonist having a dissociation half-life in excess of 30 minutes.
2. The method of claim 1 wherein the CRF₁ receptor antagonist has a dissociation half-life in excess of 40 minutes.
3. The method of claim 1 wherein the CRF₁ receptor antagonist has a dissociation half-life in excess of 50 minutes.
4. The method of claim 1 wherein the CRF₁ receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methoxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).
5. The method of claim 1 wherein the CRF₁ receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.
6. The method of any one of claims 1-5 wherein the CRF₁ receptor antagonist is administered at bedtime.
7. The method of any one of claims 1-5 wherein the CRF₁ receptor antagonist is administered at or before the expected circadian release of ACTH.

8. The method of claim 7 wherein the CRF₁ receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

9. A method for reducing 17-OHP and ACTH levels in a subject who has Congenital Adrenal Hyperplasia (CAH), said method comprising administering to the subject a CRF₁ receptor antagonist at bedtime.

10. The method of claim 9, wherein the CRF₁ receptor antagonist is administered at or before the expected circadian release of ACTH.

11. The method of claim 9, wherein the CRF₁ receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

12. The method of any one of claims 9-11, wherein the CRF₁ receptor antagonist is Compound 1 (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

13. The method of any one of claims 9-11, wherein the CRF₁ receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

14. A CRF₁ receptor antagonist for use in treating Congenital Adrenal Hyperplasia (CAH), wherein the CRF₁ receptor antagonist has a dissociation half-life in excess of 30 minutes.

15. The CRF₁ receptor antagonist of claim 14, wherein the CRF₁ receptor antagonist has a dissociation half-life in excess of 40 minutes.

16. The CRF₁ receptor antagonist of claim 14, wherein the CRF₁ receptor antagonist has a dissociation half-life in excess of 50 minutes.

17. The CRF₁ receptor antagonist of claim 14, wherein the CRF₁ receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methoxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

18. The CRF₁ receptor antagonist of claim 14, wherein the CRF₁ receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

19. The CRF₁ receptor antagonist of any one of claims 14-18, wherein the CRF₁ receptor antagonist is suitable for administration at bedtime.

20. The CRF₁ receptor antagonist of any one of claims 14-18, wherein the CRF₁ receptor antagonist is suitable for administration at or before the expected circadian release of ACTH.

21. The CRF₁ receptor antagonist of any one of claims 14-18, wherein the CRF₁ receptor antagonist is suitable for administration 3-4 hours before the expected circadian release of ACTH.

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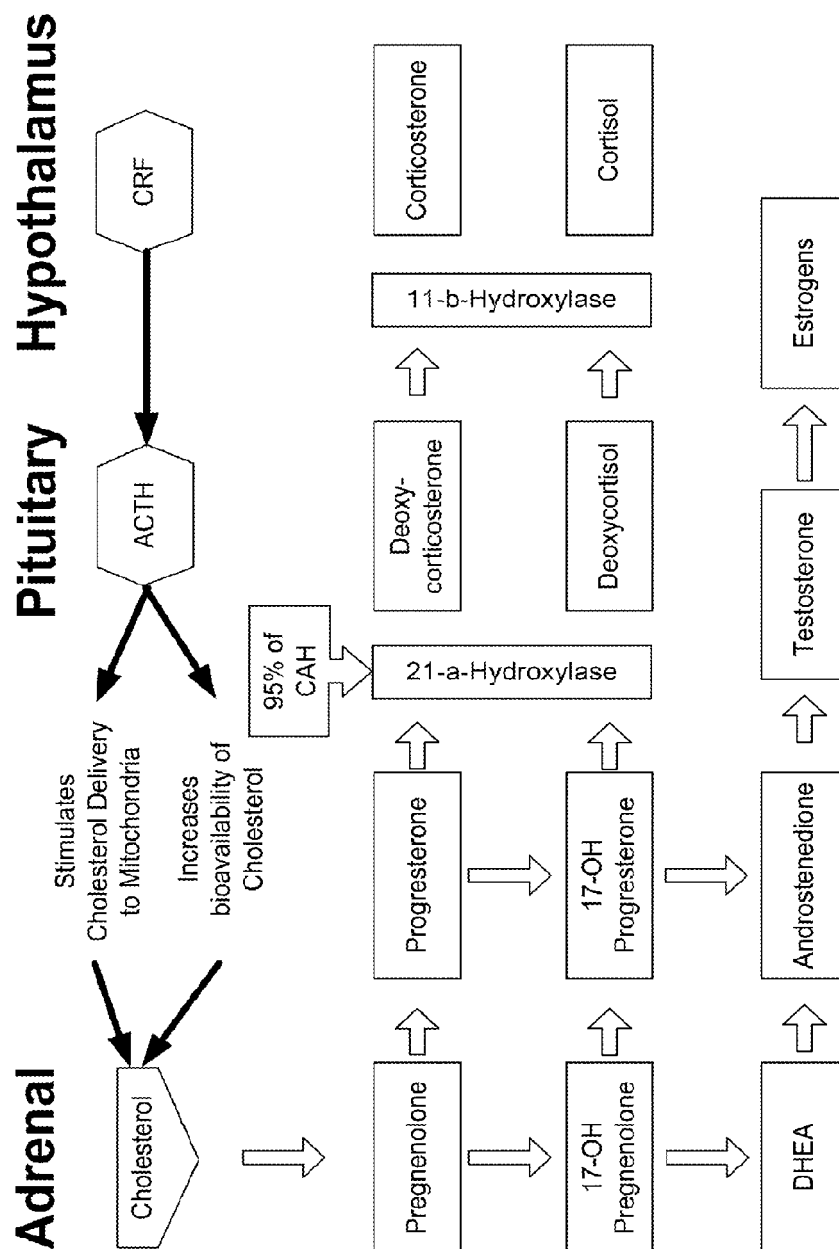


FIG. 1

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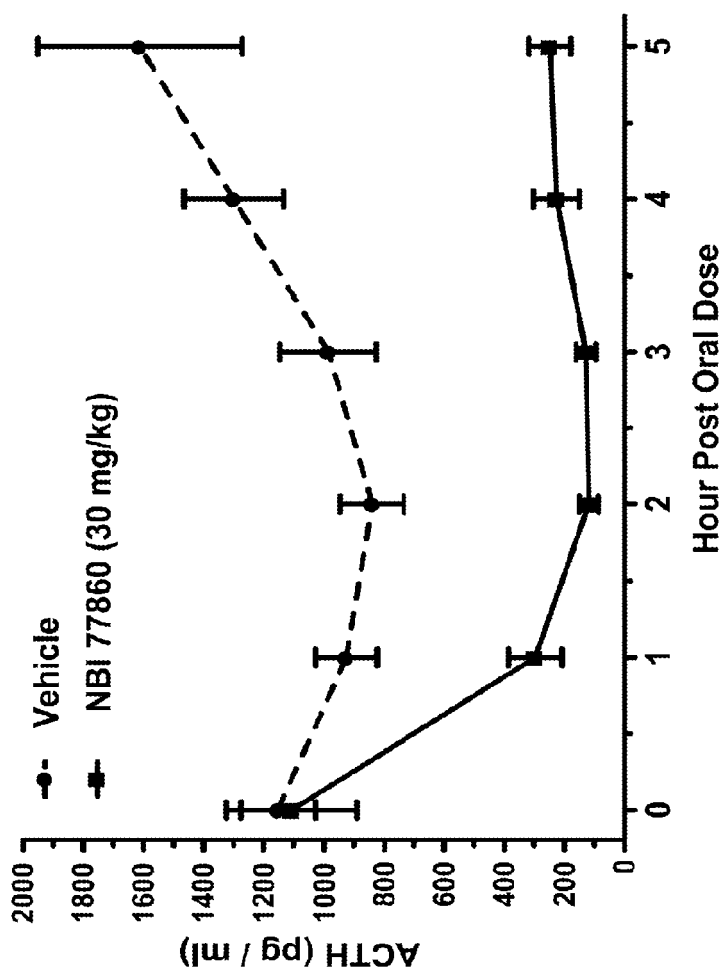
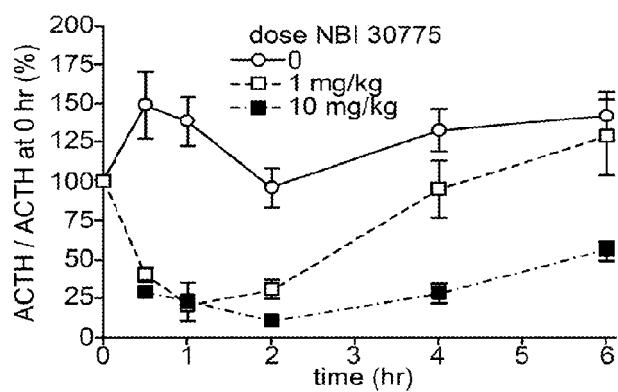
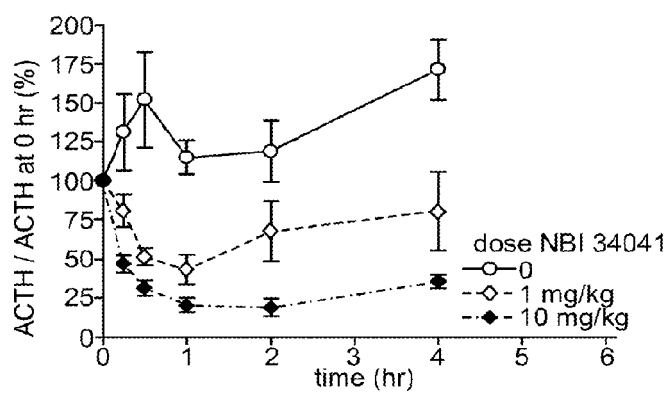
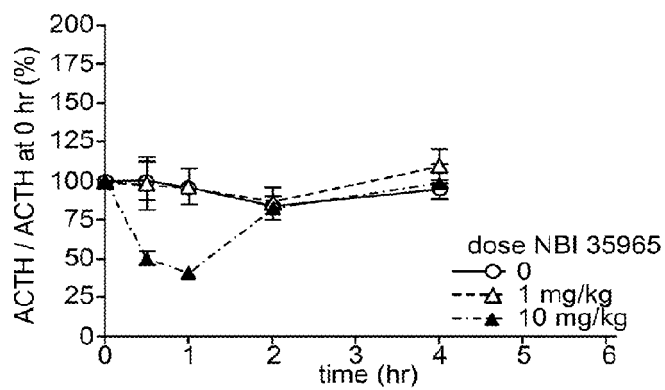
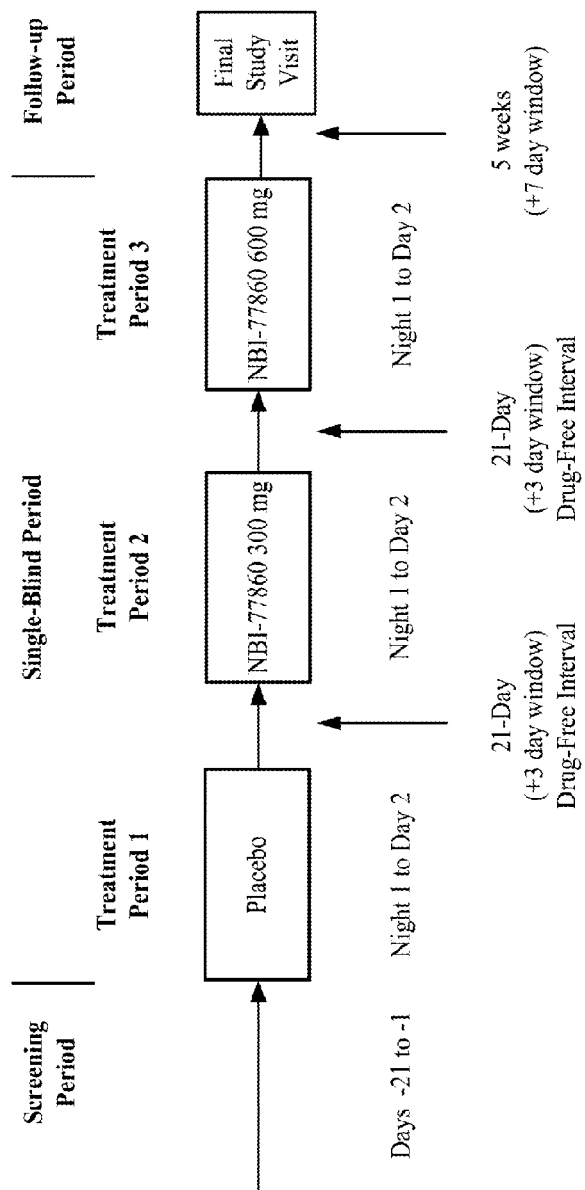


FIG. 2

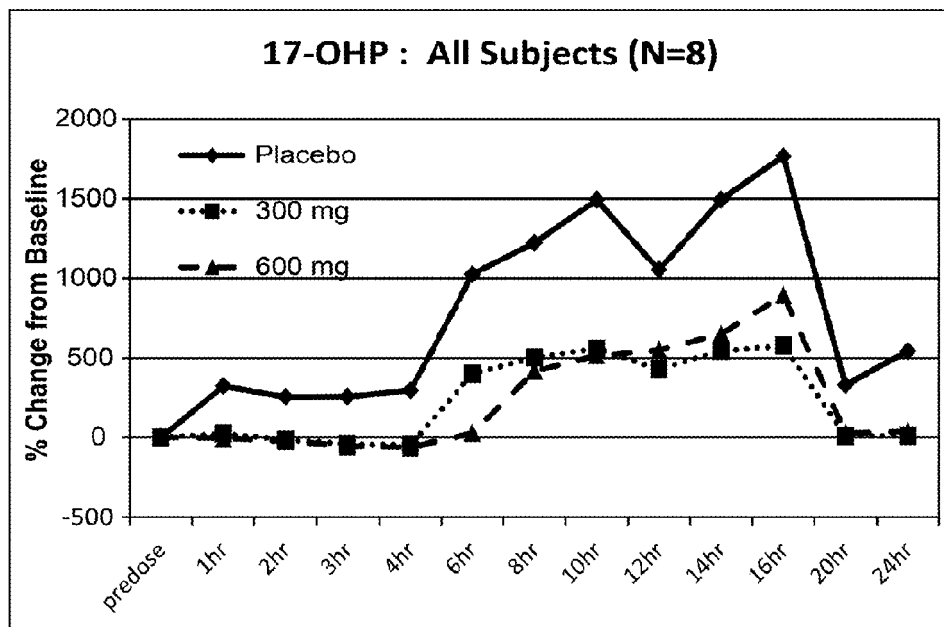
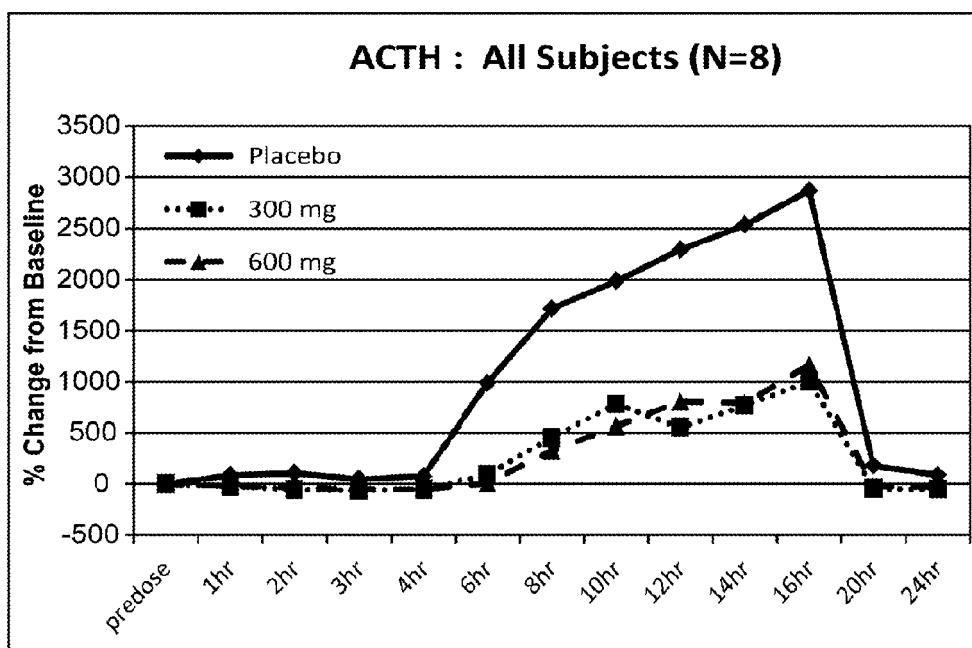
3/6**FIG. 3A****FIG. 3B****FIG. 3C**

SUBSTITUTE SHEET (RULE 26)

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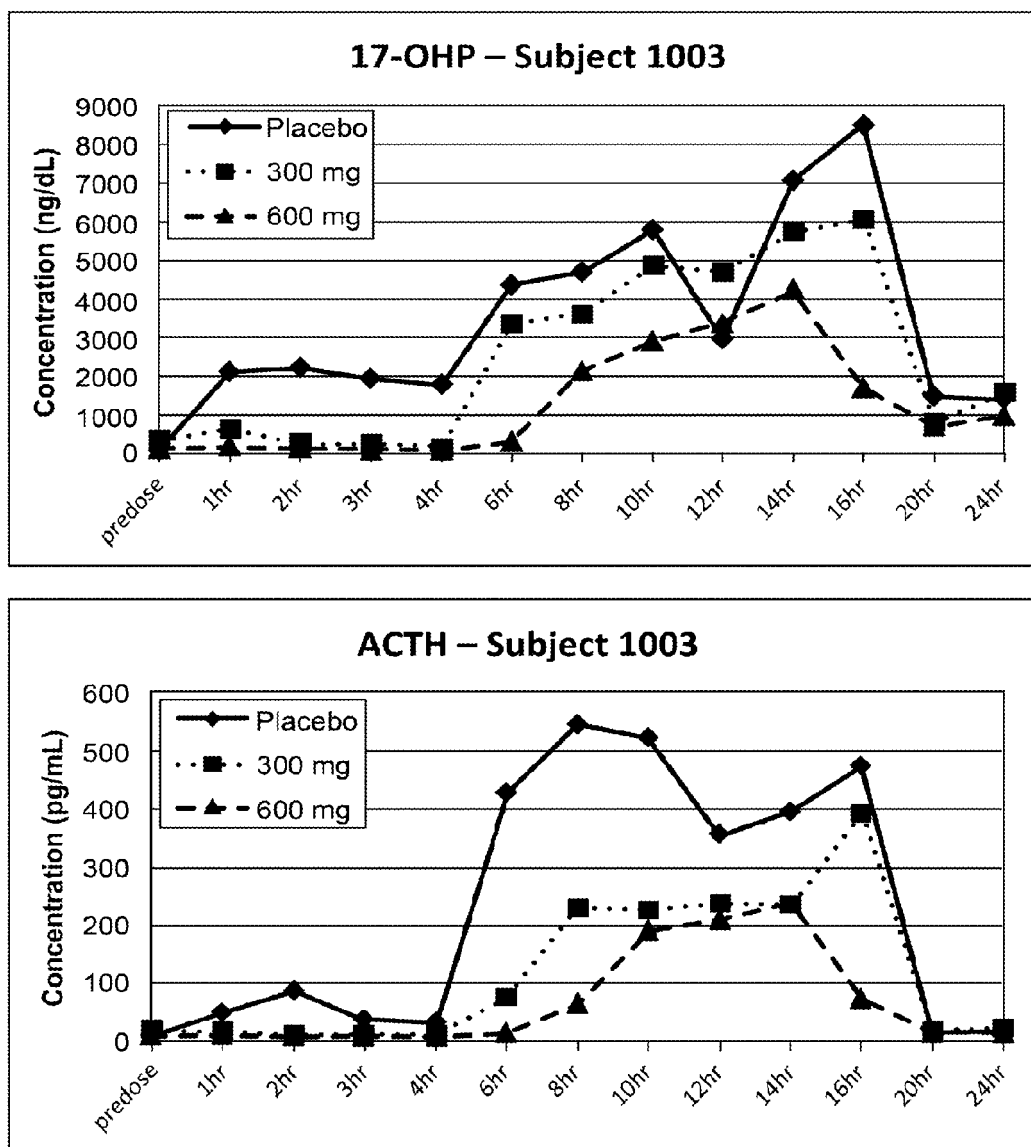
**FIG. 4**

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**FIG. 5**

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**FIG. 6**

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/012315

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K31/427 A61K31/4985 A61K31/519 ADD.				
According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EP0-Internal, EMBASE, BIOSIS				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	US 8 030 304 B2 (CHEN ZHAOGEN [US] ET AL) 4 October 2011 (2011-10-04) cited in the application	1-3, 14-16		
Y	column 2, lines 13-46 column 2, line 56 -----	1-21		
X	ZOUMAKIS E ET AL: "Corticotropin-releasing hormone receptor antagonists", EUROPEAN JOURNAL OF ENDOCRINOLOGY, vol. 155, no. Suppl. 1, November 2006 (2006-11), pages S85-S91, XP002738177, ISSN: 0804-4643	1-3,5, 14-16,18		
Y	page S86, left-hand column, paragraph 3; table 1 ----- -/-	1-21		
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.				
* Special categories of cited documents : <table border="0"> <tr> <td style="vertical-align: top;"> "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </td> <td style="vertical-align: top;"> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family </td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family			
Date of the actual completion of the international search		Date of mailing of the international search report		
8 April 2015		21/04/2015		
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Engl, Brigitte		

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/012315

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Y	NEWFIELD ET AL: "ACTH receptor blockade: A novel approach to treat congenital adrenal hyperplasia, or Cushing's disease", MEDICAL HYPOTHESES, EDEN PRESS, PENRITH, US, vol. 74, no. 4, 1 April 2010 (2010-04-01), pages 705-706, XP026975604, ISSN: 0306-9877 [retrieved on 2010-03-18] the whole document -----	1-21
Y	JOHN E TELLEW ET AL: "Discovery of NBI-77860/GSK561679, a potent corticotropin-releasing factor (CRF) receptor antagonist with improved pharmacokinetic properties", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 20, no. 24, 19 October 2010 (2010-10-19), pages 7259-7264, XP028129093, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2010.10.095 [retrieved on 2010-10-24] cited in the application page 7264, left-hand column, lines 17-21 -----	1-21
Y	CHEN C ET AL: "NBI 30775 (R121919), an orally active antagonist of the corticotropin-releasing factor (CRF) type-1 receptor for the treatment of anxiety and depression", DRUG DEVELOPMENT RESEARCH 200508 US, vol. 65, no. 4, August 2005 (2005-08), pages 216-226, XP002738174, ISSN: 0272-4391 page 219, left-hand column, line 28 - page 220, left-hand column, line 4 ----- -/--	1-21

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/012315

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>WEBSTER E L ET AL: "In vivo and in vitro characterization of Antalarmin, a nonpeptide corticotropin releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation", ENDOCRINOLOGY, THE ENDOCRINE SOCIETY, US, vol. 137, no. 12, 1 January 1996 (1996-01-01), pages 5747-5750, XP000651739, ISSN: 0013-7227, DOI: 10.1210/EN.137.12.5747 the whole document -----</p>	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2015/012315

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2010222339 A1	02-09-2010
		WO 2008036579 A1	27-03-2008

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27 March 2008 (27.03.2008)

PCT

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A61K 31/505 (2006.01) *A61P 25/24* (2006.01)
A61P 29/00 (2006.01) *A61P 25/30* (2006.01)

(74) **Agents:** HOSTETTLER, Danica et al.; Eli Lilly and Company, Patent Division, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

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(72) Inventors; and

(75) **Inventors/Applicants** (for US only): **CHEN, Zhaogen** [US/US]; 735 Canal Court #B, Indianapolis, Indiana 46202 (US). **HAMDOUCHI, Chafiq, Hamdouchi** [ES/US]; 14469 Twin Oaks Drive, Carmel, Indiana 46032 (US). **HEMBRE, Erik, James** [US/US]; 1332 North New Jersey Street, Indianapolis, Indiana 46202 (US). **HIP-SKIND, Philip, Arthur** [US/US]; 4255 Cabin Court, New Palestine, Indiana 46163 (US). **MYERS, Jason, Kenneth** [US/US]; 8021 Springwater Circle, Indianapolis, Indiana 46256 (US). **TAKAKUWA, Takako** [JP/US]; 7653 Germander Lane, Indianapolis, Indiana 46237 (US). **TOTH, James, Lee** [US/US]; 4642 South 625 West, Knightstown, Indiana 46148 (US).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BI, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

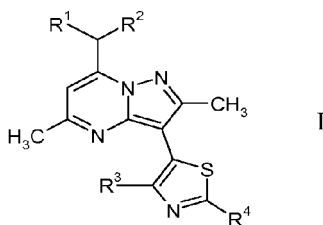
(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(54) **Title:** THIAZOLE PYRAZOLOPYRIMIDINES AS CRF1 RECEPTOR ANTAGONISTS

(57) **Abstract:** The present invention relates to compounds of Formula (I), pharmaceutical compositions thereof, and use thereof as corticotropin releasing factor 1 (CRF1) receptor antagonists in the treatment of psychiatric and neuroendocrine disorders, neurological diseases, and metabolic syndrome.

THIAZOLE PYRAZOLOPYRIMIDINES AS CRF1 RECEPTOR ANTAGONISTS

FIELD OF THE INVENTION

This invention relates to novel thiazole pyrazolopyrimidine compounds,
5 pharmaceutical compositions thereof, and use thereof as CRF1 receptor antagonists in the treatment of psychiatric and neuroendocrine disorders, neurological diseases, and metabolic syndrome.

BACKGROUND OF THE INVENTION

10 Corticotropin releasing factor (CRF) is a 41 amino acid peptide that is the primary physiological regulator of proopiomelanocortin (POMC) derived peptide secretion from the anterior pituitary gland. In addition to its endocrine role at the pituitary gland, immunohistochemical localization of CRF has demonstrated that the hormone has a broad
15 extrahypothalamic distribution in the central nervous system and produces a wide spectrum of autonomic, electrophysiological and behavioral effects consistent with a neurotransmitter or neuromodulator role in the brain. There is also evidence that CRF plays a significant role in integrating the response in the immune system to physiological, psychological, and immunological stressors.

CRF has been implicated in psychiatric disorders and neurological diseases
20 including depression and anxiety, as well as the following: Alzheimer's disease, Huntington's disease, progressive supranuclear palsy, amyotrophic lateral sclerosis, Parkinson's disease, epilepsy, migraine, alcohol and substance abuse and associated withdrawal symptoms, obesity, metabolic syndrome, congenital adrenal hyperplasia, Cushing's disease, hypertension, stroke, irritable bowel syndrome, stress-induced gastric
25 ulceration, premenstrual syndrome, sexual dysfunction, premature labor, inflammatory disorders, allergies, multiple sclerosis, visceral pain, sleep disorders, pituitary tumors or ectopic pituitary-derived tumors, chronic fatigue syndrome and fibromyalgia.

CRF receptor subtypes, CRF1 and CRF2, have been identified and are distributed heterogeneously within the brain thereby suggesting potential functional diversity. For
30 example, widely distributed brain CRF1 receptors are strongly implicated in emotionality accompanying exposure to environmental stressors. Significantly, CRF1, not CRF2, receptors appear to mediate select anxiogenic like behaviors. A more discrete

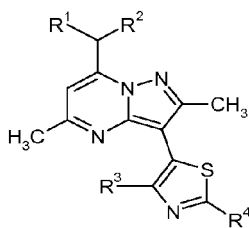
septal/hypothalamic distribution and the availability of alternative endogenous ligands suggest a different functional role for the CRF2 receptor. For example, a novel CRF-family neuropeptide with preferential affinity for CRF2 relative to CRF1 receptors is reported to suppress appetite without producing the profile of behavioral activation
 5 observed with selective CRF1 agonism. In other cases, CRF2 agonism produces similar effects to those reported for CRF1 antagonists or CRF1 gene deletion. For example, while CRF2 agonists have been proposed as antiobesity agents, CRF1 antagonists may be an important treatment for obesity as well.

Certain pyrrolo[2,3-*d*]pyrimidines, pyrrolo[3,2-*d*]pyrimidines, pyrazolo[1,5-*a*]pyrimidines, 1,2,3-triazolo[4,5-*b*]pyridines, and pyrazolo[1,5-*a*]-1,3,5-triazines, useful
 10 as CRF antagonists, are described in WO 94/13676, WO 97/29109, WO 98/08847, and WO 98/03510.

The present invention provides novel thiazole pyrazolopyrimidines useful as CRF1 receptor antagonists. In view of the above, it is desirable to discover new
 15 efficacious and selective antagonists of CRF1 as potentially valuable therapeutic agents for the treatment of psychiatric and neuroendocrine disorders, neurological diseases, and metabolic syndrome. Further, since a majority of commercial CNS and cardiovascular drugs exhibit unfavorable bioavailability and pharmacokinetic profiles, it is also desirable to discover new compounds with favorable bioavailability and pharmacokinetic profiles
 20 relative to known CRF antagonists such as CP154526 and NBI30775.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a compound of Formula I



Formula I

25

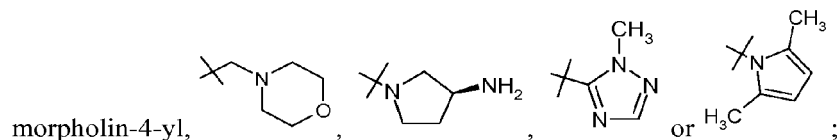
wherein:

R¹ and R² are independently ethyl or *n*-propyl;

3

R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl or methoxy;

R^4 is hydrogen, Br, $R^a R^b N$ -, methoxymethyl, n-butyl, acetamido, pyridin-4-yl,



- R^a and R^b are independently hydrogen, C_1 - C_3 alkyl, $H_2NCH_2CH_2$ -,
 5 $(CH_3)_3COC(O)NHCH_2CH_2$ -, or $CH_3CH_2CH_2NHCH_2CH_2$ -,
 or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention provides a pharmaceutical composition comprising: a compound of Formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

In another embodiment, the present invention relates to a method of treating depression, anxiety, alcohol or substance abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep disorders, allergy, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric ulceration, inflammatory disorders, pituitary or ectopic pituitary-derived tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis in a patient, comprising: administering to a patient in need thereof a therapeutically effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the treatment of depression, anxiety, alcohol or substance abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep disorders, allergy, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric ulceration, inflammatory disorders, pituitary or ectopic pituitary-derived tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis.

In another embodiment, the present invention relates to a compound of Formula I, or a pharmaceutically acceptable salt thereof, for use in the treatment of depression,

anxiety, alcohol or substance abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep disorders, allergy, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric ulceration, inflammatory disorders, 5 pituitary or ectopic pituitary-derived tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis.

In another embodiment, the present invention relates to a compound of Formula I, or a pharmaceutically acceptable salt thereof, for use in therapy.

10

DETAILED DESCRIPTION OF THE INVENTION

As used above, and throughout the description of the invention, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

"Alkyl" means a saturated aliphatic hydrocarbon group, which may be straight or branched, having 1 to 5 carbon atoms in the chain.

15

"Pharmaceutically acceptable excipient" refers to a pharmaceutically acceptable formulation carrier, solution, or additive to enhance the formulation characteristics. Such excipients must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof and are well known to the skilled artisan (see e.g. *Remingtons Pharmaceutical Sciences*, 19th Edition, Mack Publishing Company, 1995).

20

"Pharmaceutically acceptable salts" refers to the relatively non-toxic, inorganic and organic acid addition salts, and base addition salts, of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds. In particular, acid addition salts can be prepared by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and 25 isolating the salt thus formed (see e.g. *Remingtons Pharmaceutical Sciences*, 19th Edition, Mack Publishing Company, 1995).

"Therapeutically effective amount" or "effective amount" means the amount of the compound of formula I of the present invention or pharmaceutical composition containing a compound of formula I of the present invention that will elicit the biological 30 or medical response of or desired therapeutic effect on a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

The terms "treatment," "treat," "treating," and the like, are meant to include both slowing and reversing the progression of a disorder. These terms also include alleviating, ameliorating, attenuating, eliminating, or reducing one or more symptoms of a disorder or condition, even if the disorder or condition is not actually eliminated and even if progression of the disorder or condition is not itself slowed or reversed. The term "treatment" and like terms also include preventive (e.g., prophylactic) and palliative treatment. Prevention of the disease is manifested by a prolonging or delaying of the onset of the symptoms of the disease.

The symbol " " in a molecular structure indicates the position of attachment for that particular substituent.

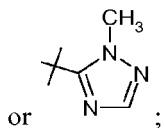
When any variable occurs more than one time in any constituent or in formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds. In choosing compounds of the present invention, one of ordinary skill in the art will recognize that the various substituents, i.e. R^1 , R^2 , etc., are to be chosen in conformity with well-known principles of chemical structure connectivity.

Under standard nomenclature used throughout this disclosure, the terminal portion of the designated side chain is described first, followed by the adjacent functionality toward the point of attachment. For example, an arylcarbonylaminoalkyl substituent is equivalent to aryl-C(O)-NH-alkyl-.

The present invention contemplates the following embodiments, which can alternatively be further combined:

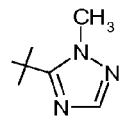
- (a) A compound of Formula I wherein R^1 and R^2 are ethyl;
- (b) A compound of Formula I wherein R^1 and R^2 are *n*-propyl;
- (c) A compound of Formula I wherein R^3 is Cl, Br, methyl or trifluoromethyl;
- (d) A compound of Formula I wherein R^3 is Cl;
- (e) A compound of Formula I wherein R^3 is Br;
- (f) A compound of Formula I wherein R^4 is R^aR^bN -, pyridin-4-yl, morpholin-4-yl,

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(g) A compound of Formula I wherein R⁴ is morpholin-4-yl;



(h) A compound of Formula I wherein R⁴ is

(i) A compound of Formula I wherein R^a and R^b are independently C₁-C₃alkyl;

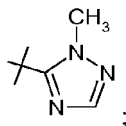
(j) A compound of Formula I wherein R¹ and R² are ethyl, R³ is Cl, and R⁴ is morpholin-4-yl;

(k) A compound of Formula I wherein R¹ and R² are *n*-propyl, R³ is Cl, and R⁴ is morpholin-4-yl;

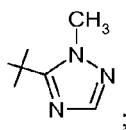
(l) A compound of Formula I wherein R¹ and R² are ethyl, R³ is Br, and R⁴ is morpholin-4-yl;

(m) A compound of Formula I wherein R¹ and R² are *n*-propyl, R³ is Br, and R⁴ is morpholin-4-yl;

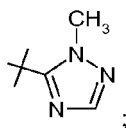
(n) A compound of Formula I wherein R¹ and R² are ethyl, R³ is Cl, and R⁴ is



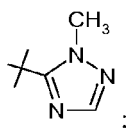
(o) A compound of Formula I wherein R¹ and R² are *n*-propyl, R³ is Cl, and R⁴ is



(p) A compound of Formula I wherein R¹ and R² are ethyl, R³ is Br, and R⁴ is



(q) A compound of Formula I wherein R¹ and R² are *n*-propyl, R³ is Br, and R⁴ is



(r) Use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, for treating depression or anxiety;

- (s) Use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, for treating alcohol or substance abuse;
- (t) Use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, for treating alcohol or substance abuse and associated withdrawal symptoms;
- 5 (u) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a K_i value for CRF1 binding of ≤ 500 nM;
- (v) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a K_i value for CRF1 binding of ≤ 50 nM;
- 10 (w) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a K_i value for CRF1 binding of ≤ 5 nM;
- (x) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a K_i value for CRF1 binding of ≤ 500 nM and selectively binding to CRF1 (i.e., lower K_i) relative to CRF2;
- (y) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a K_i value for CRF1 binding of ≤ 50 nM and selectively binding to CRF1 (i.e., lower K_i) relative to CRF2;
- 15 (z) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a K_i value for CRF1 binding of ≤ 5 nM and selectively binding to CRF1 (i.e., lower K_i) relative to CRF2; and/or
- 20 (aa) Particular exemplified compounds with superior bioavailability and pharmacokinetic profiles relative to some known CRF antagonists (e.g., CP154526 and NBI30775), such as Example 15.

The compounds of the present invention are preferably formulated as
 25 pharmaceutical compositions administered by a variety of routes. Preferably, such compositions are for oral administration. Such pharmaceutical compositions and processes for preparing same are well known in the art (see, e.g., *Remington: The Science and Practice of Pharmacy*, A. Gennaro, et al., eds., 19th ed., Mack Publishing Co., 1995).

30 The compounds of Formula I are generally effective over a wide dosage range. For example, dosages per day normally fall within the range of about 0.0001 to about 30

mg/kg of body weight. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, and therefore the above dosage range is not intended to limit the scope of the invention in any way. It will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound or compounds administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms.

The compounds of Formula I are CRF-1 antagonists and, as such, are useful for treating a condition which is treatable by reducing CRF1 receptor tone or stimulation. Corticotropin releasing factor (CRF), a 41 amino acid peptide that is the primary physiological regulator of proopiomelanocortin (POMC) derived peptide secretion from the anterior pituitary gland [J. Rivier et al., *Proc. Natl. Acad. Sci (USA)* 80:4851 (1983); W. Vale et al., *Science* 213:1394 (1981)], has been linked to a number of medical conditions. For example, in addition to its endocrine role at the pituitary gland, immunohistochemical localization of CRF has demonstrated that the hormone has a broad extrahypothalamic distribution in the central nervous system and produces a wide spectrum of autonomic, electrophysiological and behavioral effects consistent with a neurotransmitter or neuromodulator role in the brain [W. Vale et al., *Rec. Prog. Horm. Res.* 39:245 (1983); G. F. Koob, *Persp. Behav. Med.* 2:39 (1985); E. B. De Souza et al., *J. Neurosci.* 5:3189 (1985)]. There is also evidence that CRF plays a significant role in integrating the response in the immune system to physiological, psychological, and immunological stressors [see, e.g., J. E. Blalock, *Physiological Reviews* 69:1 (1989); J. E. Morley, *Life Sci.* 41:527 (1987)].

CRF is involved in psychiatric disorders and neurological diseases including depression and anxiety [D. M. Nielsen, *Life Sci.* 78:909-919; H. E. Kunzel et al., *J. Psychiatr. Res.* 37:525-533; D. R. Gehlert et al., *Eur. J. Pharmacol.* 509:145-153]. A role for CRF has also been postulated in the etiology and pathophysiology of Alzheimer's disease, Huntington's disease, progressive supranuclear palsy and amyotrophic lateral sclerosis, as they relate to the dysfunction of CRF neurons in the central nervous system [for a review, see: E. B. De Souza, *Hosp. Practice* 23:59 (1988)]. Chronic administration of CRF has been shown to produce impairment of the dopamine system suggesting a role

in Parkinson's disease [E. Izzo et al., *Pharmacol. Biochem. Behav.* 81:701-708 (2005)]. Other neurological disorders in which CRF is involved include epilepsy [T. Z. Baram et al., *Brain Res.* 770:89-95 (1997)] and migraine [T. C. Theoharides et al., *Endocrinology* 136:5745-5750 (1995)]. CRF has been implicated in alcohol and substance abuse and associated withdrawal symptoms [D. H. Overstreet et al., *Pharmacol. Biochem. Behav.* 77:405-413; Y. Shaham et al., *Psychopharmacology (Berl)* 137:184-190]. Moreover, there is evidence that CRF has a role in various endocrine disorders and cardiovascular diseases such as obesity [E. Timofeeva and D. Richard, *Neuroendocrinology* 66:327-340 (1997)], metabolic syndrome [A. M. Ward et al., *Metabolism* 53:720-726(2004)], congenital adrenal hyperplasia [D. P. Merke and G. B. Cutler Jr., *Endocrinol. Metab. Clin. North Am.* 30:121-135 (2001)], Cushing's disease [M. Labeur et al., *Curr. Drug Targets Immune Endocr. Metabol. Disord.* 4:335-342 (2004)], hypertension [R. J. Briscoe, et al., *Brain Res.* 881:204-207 (2000)], and stroke [S. L. Stevens et al., *J. Cereb. Blood Flow Metab.* 23:1151-1159 (2003)]. Gastric disturbances such as irritable bowel syndrome [Y. Tache et al., *Eur J. Surg. Suppl.* 16-22 (2002)] and stress-induced gastric ulceration [K. E. Gabry et al., *Mol. Psychiatry* 7:474-483, 433 (2002)] have been shown to be related to CRF. In addition, there is indication that CRF has a role in various areas of human female health, for example, premenstrual syndrome [F. Facchinetti et al., *Psychosom. Med.* 56:418-422 (1994)], infertility [L. Ghizzoni et al., *Endocrinology* 138:4806-4811 (1997)], sexual dysfunction [J. E. Jones et al., *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283:R591-597 (2002)], and premature labor [P. D. Wadhwa et al., *Am. J. Obstet. Gynecol.* 191:1063-1069 (2004)]. There is also evidence that CRF has a significant role in the immune system indicating therapeutic potential for treating inflammatory disorders [A. Gravanis and A. N. Margioris, *Curr. Med. Chem.* 12:1503-1512 (2005)], allergies [L. K. Singh et al., *Brain Behav. Immun.* 13:225-239 (1999)], multiple sclerosis and other autoimmune disorders [C. Benou et al., *J. Immunol.* 174:5407-5413 (2005)]. In addition to the preceding, CRF has been implicated in visceral pain [M. Nijssen et al., *Neurogastroenterol. Motil.* 17:423-432 (2005)], sleep disorders [T. M. Buckley and A. F. Schatzberg, *J. Clin. Endocrinol. Metab.* 90:3106-3114(2005)], pituitary tumors or ectopic pituitary-derived tumors [K. D. Dieterich et al., *J. Clin. Endocrinol. Metab.* 83:3327-3331 (1998)], chronic fatigue syndrome and

fibromyalgia [G. Neeck and L. J. Crofford, *Rheum. Dis. Clin. North Am.* 26:989-1002 (2000)].

CRF receptor subtypes, CRF1 and CRF2, have been identified and are distributed heterogeneously within the brain [D. T. Chalmers et al., *TIPS* 17:166-72 (1996)] thereby suggesting potential functional diversity [S. C. Heinrichs et al., *Regul. Peptides* 71:15 (1997)]. For example, widely distributed brain CRF1 receptors are strongly implicated in emotionality accompanying exposure to environmental stressors [G. Liebsch et al., *Regul. Peptides* 59: 229-39 (1995); D. W. Schulz, *PNAS* 93: 10477-82 (1996)]. Significantly, CRF1, not CRF2, receptors appear to mediate select anxiogenic like behaviors [Heinrichs et al., 1997]. A more discrete septal/hypothalamic distribution [D. T. Chalmers et al., *J. Neurosci.* 15(10): 6340-50 (1995)] and the availability of alternative endogenous ligands [J. Vaughan et al., *Nature* 378: 287-92 (1995)] suggest a different functional role for the CRF2 receptor [Heinrichs et al., 1997]. For example, a novel CRF-family neuropeptide with preferential affinity for CRF2 relative to CRF1 receptors is reported to suppress appetite without producing the profile of behavioral activation observed with selective CRF1 agonism (H. Tezval et al., *PNAS* 101(25): 9468-9473 (2004)]. In some cases, CRF2 agonism produces similar effects to those reported for CRF1 antagonists or CRF1 gene deletion [S. C. Heinrichs, *Trends in Pharmacological Sciences* 20(8):311-5 (1999)]. For example, while CRF2 agonists have been proposed as antiobesity agents, CRF1 antagonists may be an important treatment for obesity as well [C. Contoreggi et al., *Neuroendocrinology* 80(2):111-23 (2004)].

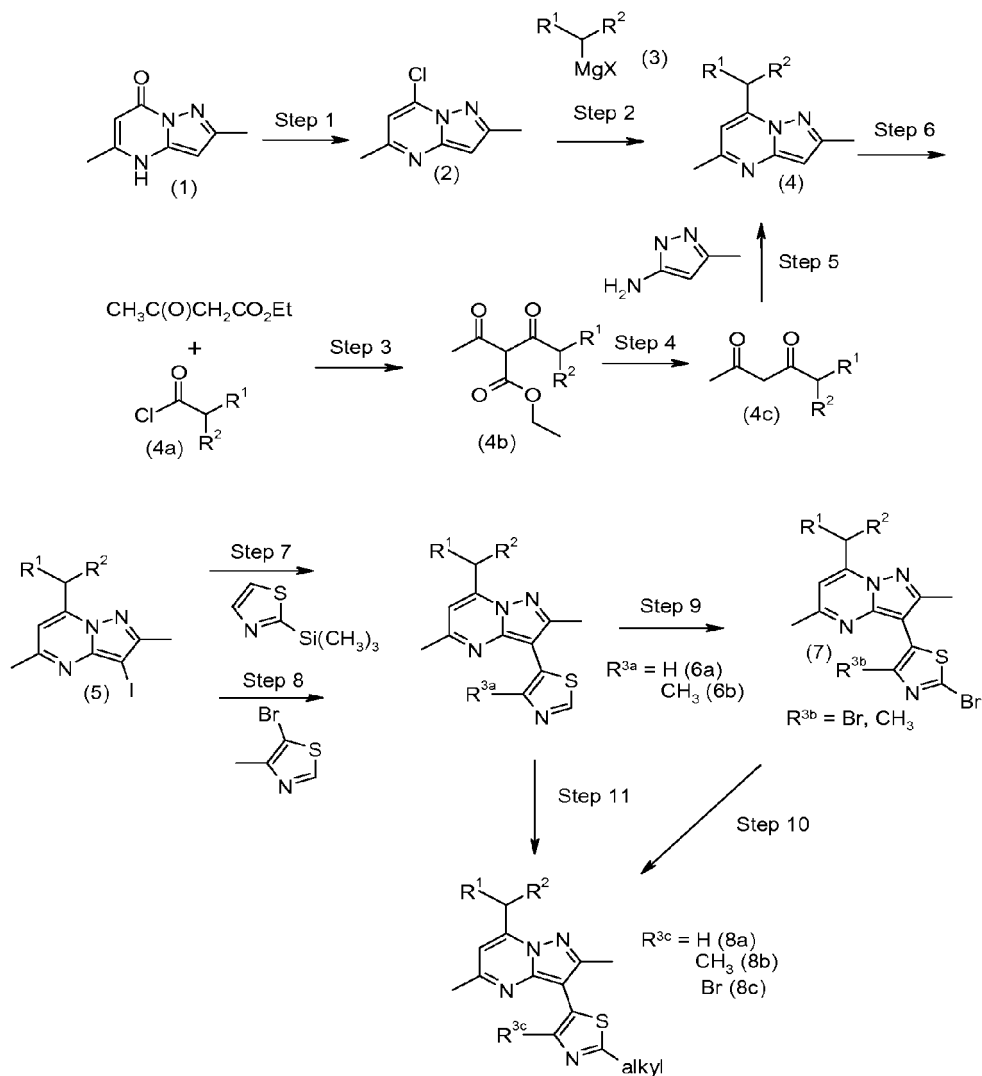
PREPARING COMPOUNDS OF THE INVENTION

All of the compounds of the present invention can be chemically prepared, for example, by following the synthetic routes set forth below. However, the following discussion is not intended to be limiting to the scope of the present invention in any way. For example, the specific synthetic steps for each of the routes described may be combined in different ways, or in conjunction with steps from different schemes, to prepare additional compounds of Formula I. The products of each step can be recovered by conventional methods including extraction, evaporation, precipitation, chromatography, filtration, trituration, crystallization, and the like. In the Schemes below

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all substituents, unless otherwise indicated, are as previously defined and suitable reagents are well known and appreciated in the art.

Scheme 1



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Formation of a compound of formula (6a,b), (7), or 8(a-c) can be carried out in accordance with reactions as depicted in Scheme 1. An appropriate compound of formula

(6a,b), (7), or (8a-c) is one in which R^1 and R^2 are as defined for formula I and $R^{3a} = H$ or CH_3 , $R^{3b} = Br$ or CH_3 and $R^{3c} = H$, CH_3 , or Br .

In Scheme 1, Step 1, the pyrazolo[1,5-a]pyrimidine-7-one of formula (1) is converted to 7-chloro-2,5-dimethyl- pyrazolo[1,5-a]pyrimidine using phosphorous
5 oxychloride and dimethylaniline in an inert solvent, such as toluene, at the reflux temperature of the solvent.

In Step 2, a Grignard reagent of formula (3) ($X = Cl$ or Br) is reacted with the chloride of formula (2), in an inert solvent such as toluene, at reflux temperature to provide the 7-alkyl pyrazolopyrimidine of formula (4).

10 Alternatively, a 7-alkyl pyrazolopyrimidine of formula (4) can be obtained as shown in Steps 3, 4, and 5. In Step 3 ethyl acetoacetate is acylated with an acid halide of formula (4a) in the presence of magnesium chloride to provide a diketo-ester of formula (4b). The diketo-ester of formula (4b) is decarboxylated under Krapcho conditions to provide the diketone of formula (4c). For example, (4b) is heated in dimethyl sulfoxide at
15 a temperature of about 130 to 170 °C to perform the decarboxylation. In Step 5, the diketone of formula (4c) is cyclized with 3-amino-5-methylpyrazole to give the 7-alkyl pyrazolopyrimidine of formula (4) in a protic solvent such as methanol, ethanol, or acetic acid. Preferred conditions use acetic acid at a temperature of about 0 to 60 °C.

The pyrazolopyrimidine of formula (4) is functionalized to an iodo
20 pyrazolopyrimidine of formula (5) in Step 6 using an excess of N-iodosuccinimide in acetonitrile.

In Scheme 1, Step 7 or 8, the iodo pyrazolopyrimidine of formula (5) is reacted with a thiazole zinc halide in a Negishi cross-coupling reaction to provide a thiazolyl
pyrazolopyrimidine of formula (6a) or (6b) (Jensen, J.; Skjaerback, N.; Vedso, P.
25 *Synthesis* 2001, 128). The thiazole zinc halide is generated using methods well known to those skilled in the art. For example, in Step 7, 2-trimethylsilylthiazole is treated with *n*-, *sec*-, or *tert*-butyl lithium, followed by lithium-zinc exchange with $ZnCl_2$. The organozinc reagent is coupled with the iodo pyrazolopyrimidine of formula (5) in the presence of a palladium catalyst, for example, dichloro[1,1'-bis(diphenyl-
30 phosphino)ferrocene]palladium (II) dichloromethane, in an inert solvent, such as THF, at reflux temperature for about 12 to 36 hours, to provide a thiazole pyrazolopyrimidine of formula (6a).

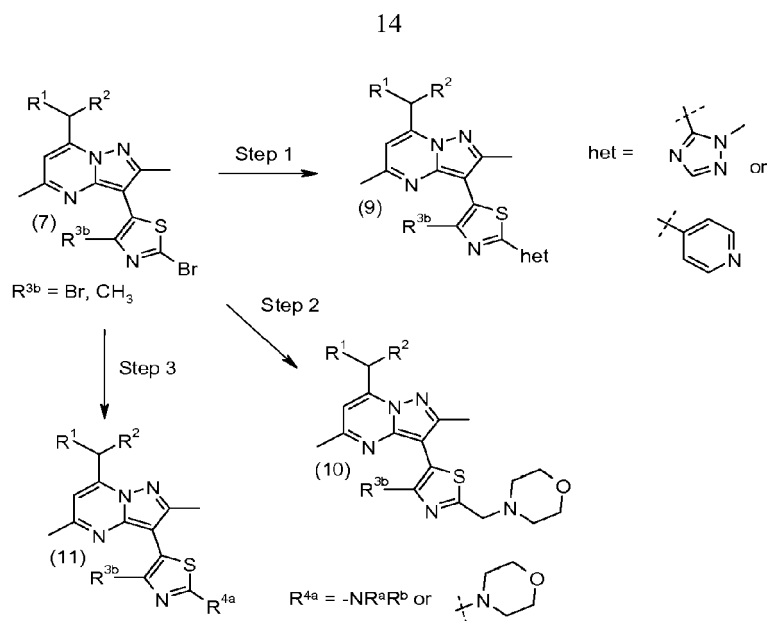
Alternatively, in Step 8, a thiazole zinc bromide is generated using 5-bromo-4-methylthiazole and zinc metal, and used in the Negishi cross-coupling essentially as described for Step 7, to provide a thiazole pyrazolopyrimidine of formula (6b).

In Scheme 1, Step 9, a thiazole of formula (6a,b) is brominated to give a bromo or
5 dibromo thiazole of formula (7), wherein $R^{3b} = \text{Br}$ or CH_3 . The thiazole is brominated with either 1 or 2 eq of N-bromosuccinimide, depending on whether R^{3a} is CH_3 or H, respectively.

An alkyl thiazole of formula (8a-c) is obtained from either a thiazole of formula (6a,b) in Step 11, or from a bromothiazole of formula (7) in Step 10. In Step 10, halogen-
10 lithium exchange with *n*-, *sec*-, or *t*-butyl lithium provides a thiazole lithium reagent, which is subsequently reacted with electrophiles, such as alkylhalides, like iodomethyl methylether or iodobutane. In Step 11, the thiazole lithium reagent is formed via deprotonation using *n*-, *sec*-, or *tert*-butyl lithium and then subsequently reacted with an electrophile, like iodomethyl methylether or iodobutane.

15 It will be appreciated by one skilled in the art that the thiazole ring system is readily functionalized and that thiazole intermediates such as 2-trimethylsilyl-thiazole (Dondoni, A.; et. al. *J. Org. Chem.* 1988, 53, 1748) can be readily prepared. 5-Bromo-4-methylthiazole is obtained by bromination of 4-methylthiazole with bromine in acetic acid (Collins, I. J., et. al. WO2003093252, 13 Nov 2003). 2,5-Dimethyl-4H-
20 pyrazolo[1,5-a]pyrimidine-7-one (1) is readily prepared by condensation of ethyl acetoacetate and 5-methyl-2H-pyrazol-3-ylamine in refluxing acetic acid.

Scheme 2



Formation of a compound of formula (9), (10), or (11) can be carried out in accordance with reactions as depicted in Scheme 2. An appropriate compound of formula (9), (10), or (11) is one in which R^1 , R^2 , R^a , and R^b are as defined for formula I and $R^{3b} =$ Br or CH_3 , and $R^{4a} = -\text{NR}^a\text{R}^b$ or $-\text{N-morpholinyl}$ and "het" is defined as depicted.

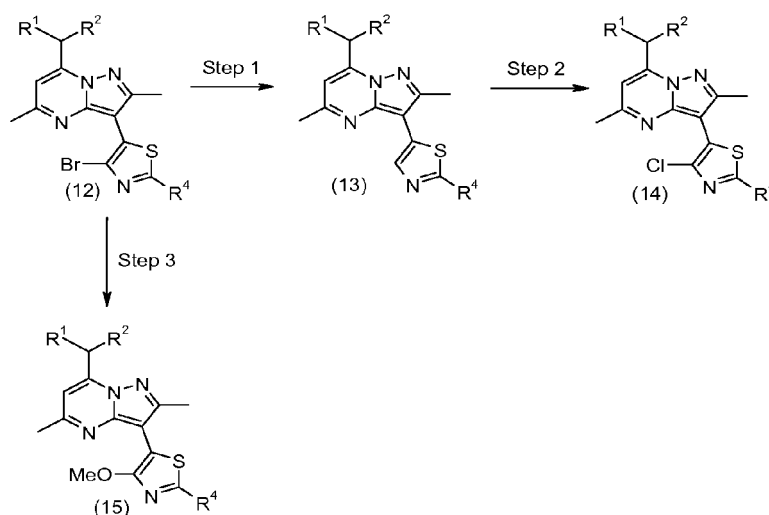
In Step 1, a bromothiazole of formula (7) is coupled with a heterocyclic zinc reagent in a Negishi cross-coupling reaction to provide a thiazole heterocycle of formula (9). For example, 1-methyl-1,2,4-triazole is treated with *n*-, *sec*-, or *tert*-butyl lithium, followed by zinc chloride, at about -80 to -65 $^\circ\text{C}$, and reacted *in situ* with a bromothiazole of formula (7). The reaction is performed preferentially in an inert solvent, such as THF, in the presence of a palladium catalyst, such as dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II) dichloromethane or tetrakis(triphenylphosphine)palladium (0). The reaction is warmed to reflux temperature. Alternatively, the heterocyclic zinc reagent is formed from a haloheterocycle, such as 4-iodopyridine and zinc metal.

In Scheme 2, Step 2, an intermediate 2-formyl thiazole is formed via halogen-lithium exchange using *n*-, *sec*-, or *t*-butyl lithium followed by reaction with N-formyl morpholine. The formyl thiazole is subjected to a reductive amination in the presence of an organic amine, such as morpholine, to provide a morpholinyl methyl thiazole of formula (10). Reductive aminations are well-known in the art typically using an

inorganic borohydride reagent such as sodium borohydride or sodium cyanoborohydride. Preferred conditions use sodium triacetoxyborohydride in an inert solvent such as dichloromethane or THF.

In Step 3, a bromothiazole of formula (7) undergoes a displacement reaction with an amine of formula $\text{-NR}^a\text{R}^b$ or with morpholine to provide an aminothiazole of formula (11). The reaction is preformed in an inert solvent, such as THF or dioxane, using an inorganic base, such as cesium carbonate at 70 to 110 °C.

Scheme 3



Formation of a compound of formula (13), (14), or (15) can be carried out in accordance with reactions as depicted in Scheme 3. An appropriate compound of formula (13), (14), or (15) is one in which R^1 , R^2 , and R^4 are as defined for formula I.

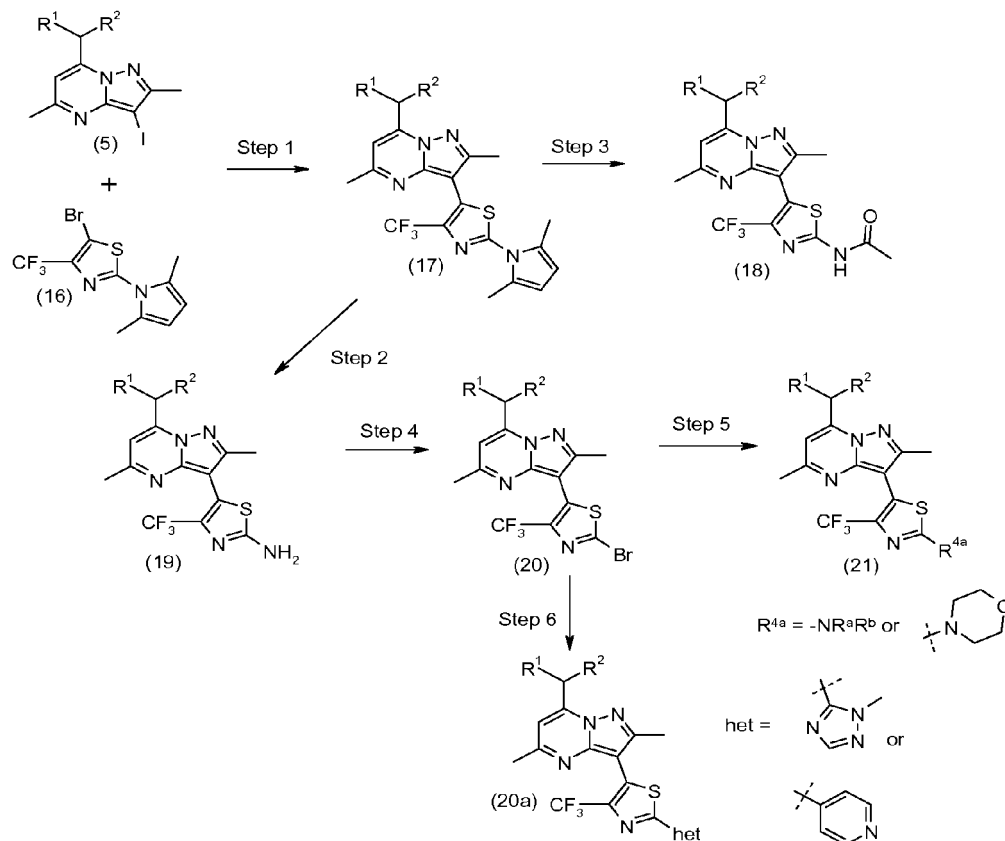
It will be recognized by one skilled in the art that a 4-bromothiazole, such as that of formula (12) is readily manipulated to other functionality. For example, in Step 1, the bromide can be dehalogenated with cuprous chloride to give a thiazole of formula (13) which is subsequently chlorinated with N-chlorosuccinimide to provide a 4-chlorothiazole of formula (14).

In Scheme 3, Step 3, a 4-bromothiazole of formula (12) is displaced with sodium methoxide in the presence of copper (I) iodide in methanol at about 100 to 120 °C in an

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inert solvent such as dimethylformamide, to provide a 4-methoxy thiazole of formula (15).

Scheme 4



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Formation of a compound of formula (17), (18), (19), (20), (20a), or (21) can be carried out in accordance with reactions as depicted in Scheme 4. An appropriate compound of formula (17), (18), (19), (20), (20a), or (21) is one in which R^1 , R^2 , R^a , and R^b are as defined for formula I and R^{4a} is $-NR^aR^b$ or $-N$ -morpholinyl and "het" is defined as depicted.

10

In Scheme 4, Step 1, an iodopyrazolopyrimidine of formula (5) and a 5-bromothiazole of formula (16) undergo a Negishi cross-coupling to form a dimethylpyrrolylthiazole of formula (17). For example, the 5-bromothiazole of formula

(16) is treated with *n*-, *sec*-, or *tert*-butyl lithium and then with zinc chloride at about -80 to -65 °C. The organozinc reagent is reacted *in situ* with an iodopyrazolopyrimidine of formula (5). The coupling reaction is performed preferentially in an inert solvent, such as THF, at reflux temperature, in the presence of a palladium catalyst, such as bis(tri-*t*-butylphosphine)palladium (0).

In Step 2, a dimethylpyrrolylthiazole of formula (17) is deprotected to provide an aminothiazole of formula (19). The dimethylpyrrole is treated with hydroxylamine in acetic acid at a temperature of about 60 to 100 °C, for about 4 to 8 h, preferably for about 6 h. In Step 3, to form the thiazole acetamide of formula (18), the same conditions are used as in Step 2, with the exception that the reaction is continued for about 72 h.

In Scheme 4, Step 4, a 2-aminothiazole of formula (19) is converted to a 2-bromothiazole of formula (20) using a modified Sandmeyer reaction. Preferred conditions use an alkyl nitrite, such as *t*-butyl nitrite, and copper (II) bromide, in acetonitrile at a temperature of about 60 to 80 °C.

In Step 5, a 2-bromothiazole of formula (20) undergoes a displacement reaction with an amine of formula $\text{-NR}^a\text{R}^b$ or with morpholine to provide an aminothiazole of formula (21). The reaction is performed in an inert solvent, such as methanol, THF, or dioxane, or is treated neat, with an excess of the amine at a temperature of about 70 to 110 °C. Alternatively the reaction is performed with the reacting amine and an excess of triethylamine, or an inorganic base, such as cesium carbonate. Also contemplated in the synthesis of aminothiazoles of formula (21) are various deprotection steps, such as removal of a *tert*-butyl ester carbamic acid (BOC), as may be required or beneficial for carrying out the reactions above wherein $\text{-NR}^a\text{R}^b$ carries additional amine functionality as defined for formula I. The selection and use of suitable protecting groups is well known and appreciated in the art (see for example, *Protecting Groups in Organic Synthesis*, Theodora Greene (Wiley-Interscience)).

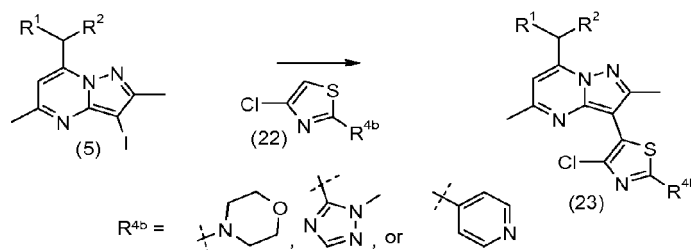
In Scheme 4, Step 6, a 2-bromothiazole of formula (20) is coupled with a heterocyclic zinc reagent in a Negishi cross-coupling reaction to provide a thiazole heterocycle of formula (20a) in a manner similar to that as described for Scheme 2, Step 1.

It will be appreciated by the skilled artisan that a functionalized thiazole of formula (16) can be prepared by means known in the art. For example, cyclization of

thiourea with a bromoketone, such as 3-bromo-1,1,1-trifluoropropan-2-one, provides 4-trifluoromethyl-thiazole-2-ylamine. Subsequent bromination and protection of the amine using hexane-2,5-dione provides (16).

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Scheme 5



Formation of a compound of formula (23) can be carried out in accordance with reactions as depicted in Scheme 5. An appropriate compound of formula (23) is one in which R^1 and R^2 are as defined for formula I and R^{4b} is defined as depicted.

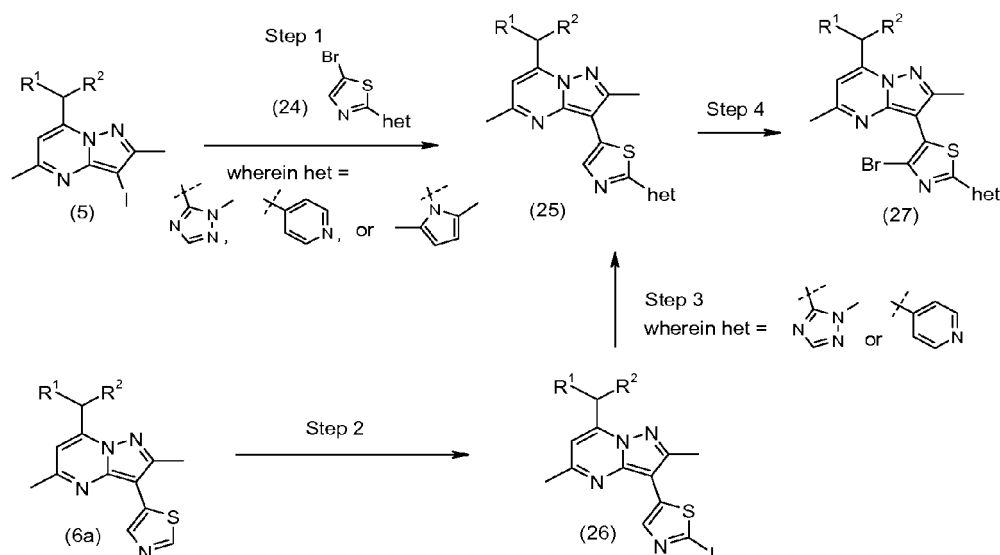
10 An iodopyrazolopyrimidine of formula (5) and a 4-chloro-2-morpholino-thiazole, for example, of formula (22) undergo cross-coupling to form a pyrazolopyrimidine thiazole of formula (23). For example, the reactants are coupled in the presence of copper iodide, palladium acetate, and triphenylphosphine, with a base such as cesium carbonate. The coupling reaction is performed preferentially in an inert solvent, such as DMF, at
15 about 100 – 150 °C for 4 – 24 h.

It will be appreciated by the skilled artisan that a functionalized thiazole of formula (22) can be prepared by means known in the art. For example, 2,4-dichlorothiazole can be reacted with morpholine to give the 2-morpholino-thiazole of formula (22). 2,4-Dichlorothiazole can also be exhaustively brominated to provide 2,5-dibromo-4-chlorothiazole. Subsequent bromine-lithium exchange with *n*-butyl lithium in THF at -90 °C and quench with water provides 2-bromo-4-chlorothiazole [*J. Chem. Soc. Perkin Trans I: Org Bioorg. Chem.* (1972-1999), (2):215-219 (1992)]. 2-Bromo-4-chlorothiazole can be subjected to a Negishi cross-coupling reaction with a heterocyclic zinc reagent to obtain the thiazol-2-yl triazole or pyridine.

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Scheme 6

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Formation of a compound of formula (27) can be carried out in accordance with reactions as depicted in Scheme 6. An appropriate compound of formula (27) is one in which R^1 and R^2 are as defined for formula I and "het" is defined as depicted for Step 1 or Step 3, respectively.

In Scheme 6, Step 1, a heterocyclic thiazole, for example, of formula (25) is obtained by reaction of an iodopyrazolopyrimidine of formula (5) with a bromothiazole of formula (24). The reaction is performed in the presence of N-butylammonium bromide and a base, such as potassium acetate with a palladium catalyst such as palladium acetate with tris(2,4-di-*tert*-butyl-phenyl)-phosphane, in an inert solvent such as N-methylpyrrolidinone at a temperature of about 100 – 150 °C.

Alternatively, in Step 2, a thiazolyl pyrazolopyrimidine of formula (6a) is iodinated to provide a 2-iodothiazole of formula (26). The thiazole is treated with lithium diisopropylamide at a temperature of -70 to -80 °C for about one hour and then treated with N-iodosuccinimide at about the same temperature in an inert solvent, such as THF. This is followed by Step 3, wherein a triazolyl or 4-pyridyl thiazole of formula (25) is formed using Negishi cross-coupling conditions similar to those described for Scheme 2, Step 1.

In Scheme 6, Step 4, a thiazolyl pyrazolopyrimidine of formula (25) is brominated to give a bromothiazole of formula (27). The bromination is effected using N-

bromosuccinimide in the presence of a small amount of acetic acid in an inert solvent, such as acetonitrile.

A compound of formula (27), wherein het = 2,5-dimethyl-pyrrol-1-yl, can be further elaborated to obtain compounds of the invention as described in Scheme 4, Steps 2, 3, 4, and 5.

It will be appreciated by the skilled artisan that heterocycle thiazoles of formula (24) can be readily prepared by means known in the art. For example, thiazole-2-carboxylic acid amide can be cyclized to the triazole with 1,1-dimethoxy-N,N-dimethyl-methanamine, followed by N-methyl-hydrazine to obtain the triazolyl thiazole, which can be subsequently brominated with N-bromosuccinimide. Literature procedures afford 4-thiazol-2-yl-pyridine which can be brominated to provide 4-(5-bromo-thiazol-2-yl)-pyridine. 5-Bromo-2-(2,5-dimethyl-pyrrol-1-yl)-thiazole can be readily obtained by reaction of 2-amino-5-bromothiazole with hexane-2,5-dione.

As used herein, "TLC" refers to thin layer chromatography; "HPLC" refers to high performance liquid chromatography; "LC/MS" refers to liquid chromatography/mass spectrometry; "GC/MS" refers to gas chromatography/mass spectrometry"; "HR-ToF" refers to high resolution time-of-flight; "APCI" refers to atmospheric pressure chemical ionization; "δ" refers to part per million down-field from tetramethylsilane; "THF" refers to tetrahydrofuran; "EtOAc" refers to ethyl acetate; "MeOH" refers to methanol; "EtOH" refers to ethanol; "DMF" refers to dimethylformamide.

EXAMPLES

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, practice the present invention to its fullest extent. The following preparations and examples are provided to describe the invention in further detail. They are intended to illustrate and not to limit the invention in any way whatsoever. The reagents and starting materials are readily available to, or may be readily synthesized by, one of ordinary skill in the art. Examples 1- 35 provide representative compounds and illustrate the preparation thereof. Examples A-D illustrates various biological assays that can be used for determining the biological properties of the compounds of the inventions. Those skilled in the art will promptly recognize appropriate variations from the procedures described in the examples. The names of the compounds of the present

invention are provided by ChemDraw Ultra® version 7.0.1. Salts are named as the free base plus the conjugate acid.

Preparation 1

5 2,5-Dimethyl-4H-pyrazolo[1,5-*a*]pyrimidin-7-one

 Add ethyl acetoacetate (128 g, 0.98 mol) dropwise to an acetic acid solution (500 mL) of 5-methyl-2*H*-pyrazol-3-ylamine (100 g, 0.95 mol), keeping the temperature at 25-28 °C. Heat the mixture at reflux for 10 h and then cool to room temperature. Add the reaction to *tert*-butyl methyl ether (5 L) cooled to 5 °C, keeping the temperature below 10 °C. Stir for 1 h at 5 °C, and filter. Dry the resulting material *in vacuo* overnight to provide a white solid (158 g, 96%).

Preparation 2

7-Chloro-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

15 To a suspension of 2,5-dimethyl-4*H*-pyrazolo[1,5-*a*]pyrimidin-7-one (10.0 g, 61.3 mmol) in toluene (150 mL) add *N,N*-dimethylaniline (9.7 mL, 76.7 mmol). Add phosphorus oxychloride (11.2 mL, 122.6 mmol) dropwise to this white suspension. Reflux for 3 h under an inert atmosphere, cool to room temperature, and concentrate the reaction to a brown oil using reduced pressure. Dissolve the oil in ethyl acetate (250 mL) and basify with 1.0 N NaOH. Separate the organic phase and extract the basic aqueous phase with ethyl acetate (2 × 100 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure to yield a brown solid. Purify the material using flash chromatography, eluting with 80% hexane/20% (30% THF/hexane) to 0% hexane/100% (30% THF/hexane) in a step gradient of 20% increments to provide a light green solid (6.65 g, 59%). ES/MS *m/z* (³⁵Cl) 182.3 (M+1)⁺.

Alternate Procedure:

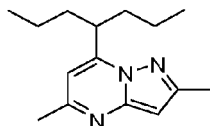
 Add 2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-one (20 g, 122 mmol) to 1,4-dioxane (60 mL). Stir the mixture at 22 °C for 10 min and then add *N,N*-diethylaniline (20.8 mL, 128 mmol). Stir for an additional 5 min and then add phosphorus oxychloride (11.7 mL, 126 mmol) over 15 min. Stir the mixture at 22 °C for 15 min, then heat to 80-85 °C over 35 min and hold the reaction at this temperature for 1.5 h. Add the cooled reaction mixture slowly to a solution of potassium phosphate dibasic (106.7 g, 612.82

22

mmol) in water (325 mL) cooled to 0-5 °C, keeping the temperature below 5 °C during the addition. Stir the mixture at 22 °C and then add methyl-*t*-butyl ether (150 mL). Separate the organic layer and extract the aqueous layer with methyl-*t*-butyl ether (2 × 100 mL). Combine the organic portions, dry over sodium sulfate, filter, and evaporate the solvent. Purify by silica gel chromatography, eluting with hexanes/ethyl acetate (2/1) to provide the title compound as a yellow solid (20.7 g, 88%). ES/MS m/z (^{35}Cl) 182 (M+1)⁺.

Preparation 3

7-(1-Propyl-butyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine



Charge an oven dried flask equipped with a reflux condenser, with anhydrous THF (40 mL), iodine (catalytic amount), magnesium ribbon (1.92 g, 78.9 mmol) and 4-bromoheptane (9.4 mL, 52 mmol). Heat the reaction to reflux in an oil bath. The temperature of the reaction spikes as the Grignard reaction is initiated. Stir the reaction an additional 4 h at 90 °C and cool to room temperature. Allow the magnesium metal to settle out (can also be centrifuged) and canulate off the Grignard reagent under positive argon pressure into a flask charged with 7-chloro-2,5-dimethylpyrazolo [1,5-*a*]pyrimidine (4.80 g, 26.3 mmol) in anhydrous toluene (20 mL). Reflux the reaction under an inert atmosphere overnight. Cool the reaction to room temperature and quench with water. Dilute with ethyl acetate (150 mL), then add water (100 mL) and saturated ammonium chloride (50 mL). Separate and extract the aqueous phase with dichloromethane (75 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 80% hexane/20% (20% ethyl acetate/hexane) to 0% hexane/100% (20% ethyl acetate/hexane) in a step gradient of 20% increments to give yellow crystals (3.08 g, 48%). ES/MS m/z 246.3 (M+1)⁺.

Alternate Procedure:

- Heat a mixture of magnesium turnings (3.5 g, 144 mmol) and a catalytic amount of iodine (100 mg) in THF (100 mL) to 65 °C under a nitrogen atmosphere. Add a few drops of neat 4-bromoheptane and heat the mixture until the reaction starts. Then add a solution of 4-bromoheptane (17.6 mL, 94.9 mmol) in THF (42 mL) keeping the
- 5 temperature at 65–70 °C over 2 h. Reflux the mixture for an additional hour and then cool the reaction to 22 °C. Add the prepared Grignard reagent to a solution of 7-chloro-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (10.2 g; 53.3 mmol) in THF (60 mL) cooled to 0 °C under a nitrogen atmosphere. Add the magnesium reagent solution via cannula over 45 min while keeping the temperature below 10 °C. Then stir the mixture for an
- 10 additional 30 min at 5 °C. Add to this mixture a 10% aqueous ammonium chloride solution (wt/wt) (125 mL) and stir at 22 °C for 30 min. Separate the organic layer and extract the aqueous layer with ethyl acetate (2 × 25 mL). Combine the organic layers and dry over sodium sulfate. Filter the mixture and evaporate the solvent. Purify the crude material by silica gel flash chromatography using an eluent of hexanes/ethyl acetate (5/1)
- 15 to provide the title compound (8 g, 62%). ES/MS *m/z* 246 (*M*+1)⁺.

Preparation 3a

7-(1-Propyl-butyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Alternate Route

Step 1: 3-Acetyl-3-oxo-4-propyl-heptanoic acid ethyl ester

- 20 Add magnesium chloride (14.63 g, 153.70 mmol) to dichloromethane (500 mL) followed by addition of ethyl acetoacetate (19.55 mL, 20.00 g, 153.79 mmol) all at once and stir at room temperature for one hour. Cool the mixture in an ice water bath and add pyridine (24.86 mL, 24.32 g, 307.39 mmol) dropwise. Add di-*n*-propylacetyl chloride (25.00 g, 153.70 mmoles) dropwise at 0 °C under nitrogen to the white slurry.
- 25 After the addition is complete remove the cooling bath, warm to ambient temperature, and stir for 16 h. Quench the reaction with 1 N HCl (400 mL) and separate the bottom layer. Dry the organic portion over magnesium sulfate, filter, and concentrate under vacuum to give a yellow oil (34 g, 86%). Use the material directly in the next step without further purification.

30 Step 2: 5-Propyl-octane-2,4-dione

Dissolve 3-acetyl-3-oxo-4-propyl-heptanoic acid ethyl ester (32.4 g, 126.39 mmoles) in dimethyl sulfoxide (100 mL) and water (5 mL). Heat the solution at 150 °C

for 6 to 8 h or follow the reaction by GC/MS. Cool the reaction and extract with heptane (3 × 100 mL). Wash the combined organic portions with water (100 mL) and brine (100 mL). Concentrate under vacuum at 50 °C to remove most of the heptane. Obtain 23.29g of an oil and use directly in the next step.

5 **Step 3:** 7-(1-Propyl-butyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Mix 5-propyl-octane-2,4-dione (15 g, 81.40 mmols) in acetic acid (15 mL) and cool in an ice bath. Add 5-amino-3-methylpyrazole (7.91 g, 81.40 mmol) portionwise and stir at ambient temperature. Check for completion of the reaction by GC/MS after 3 h. GC indicates correct region isomer in comparison with an authentic sample. Distill off the excess acetic acid. Add water (50 mL) and extract with heptane (50 mL). Wash the heptane with brine (50 mL). Dry the organic layer over MgSO₄, filter, and concentrate under vacuum to give a crude oil (15.8 g, 79%). ¹H NMR (CDCl₃): 6.39 (s, 1H); 6.31 (s, 1H); 3.75 (m, 1H); 2.55 (s, 3H); 2.45 (s, 3H); 1.71 (q, 4H); 1.23 (m, 4H); 0.85 (t, 6H).

15 Prepare the following compound essentially as described in Preparation 3, using either procedure. Use 3-bromopentane to prepare the Grignard reagent.

Prep. No.	Chemical name	Physical data
4	7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidine	ES/MS m/z 218.1 (M+1) ⁺

Preparation 5

7-(1-Propyl-butyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*] pyrimidine

20 Dissolve 7-(1-propyl-butyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (3.08 g, 12.5 mmol) in anhydrous acetonitrile (25 mL) and add 6 portions (0.70 g each) of N-iodosuccinimide (4.2 g, 18.7 mmol) at 10 minute intervals. Stir over the weekend at room temperature. Strip off the acetonitrile and dilute the oil with dichloromethane (100 mL). Wash with saturated ammonium chloride solution (2 × 50 mL). Collect the organic phase, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure to yield a dark red oil. Purify the oil using flash chromatography, eluting with 100% hexane/0% (20% ethyl acetate/hexane) to 0% hexane/100% (20% ethyl acetate/hexane) in a step gradient of 50% increments to give an orange oil (10.97 g, 87%).

25

¹H NMR (400 MHz, CDCl₃): 6.42 (s, 1H), 3.74-3.70 (m, 1H), 2.58 (s, 3H), 2.46 (s, 3H), 1.74-1.68 (m, 4H), 1.28-1.14 (m, 4H), 0.84 (t, *J* = 7.0 Hz, 6H).

Prepare the compound below essentially as described in Preparation 5.

Prep. No.	Chemical name	Physical data
6	7-(1-Ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5- <i>a</i>] pyrimidine	¹ H NMR (400 MHz, CDCl ₃): 6.44 (s, 1H), 3.59 (m, 1H), 2.61 (s, 3H), 2.49 (s, 3H), 1.86-1.76 (m, 4H), 0.85 (t, <i>J</i> = 7.5 Hz, 6H).

5

Alternate procedure for Preparation 6:

Add acetic acid (1 mL) and N-iodosuccinimide (6.7 g, 29.9 mmol) in one portion to a solution of 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (6 g, 27.5 mmol) in acetonitrile (60 mL). Stir the mixture at 22 °C for 2 h. Evaporate the solvent and take the residue up in water (50 mL) and methyl-*t*-butyl ether (100 mL). Separate the organic portions, dry over sodium sulfate, filter, and evaporate the solvent to afford the title compound (9.2 g, 96%). ES/MS *m/z* 344 (*M*+1)⁺.

10

Preparation 7

15

2-Trimethylsilylanyl-thiazole

Mix *n*-butyl lithium (20.4 mL, 51.0 mmol, 2.5 M in hexane) with diethyl ether (50 mL) in a three-necked flask, equipped with a dropping funnel and thermometer. Cool to -78 °C and add dropwise a solution of thiazole (4.25 g, 50.0 mmol) in diethyl ether (50 mL). After the addition is complete, stir the reaction mixture at -78 °C for 30 min, followed by addition of chlorotrimethylsilane (5.4 g, 50.0 mmol). Stir at -78 °C for an hour and then warm to room temperature. Quench the reaction by adding saturated sodium bicarbonate. Extract the aqueous layer with diethyl ether. Wash the combined organic portions with brine and dry over sodium sulfate. Filter and concentrate under reduced pressure to give a residue. Purify by distillation to give 8.33 g (52-56 °C/ 15 mm Hg) of title compound. ¹H NMR (400 MHz, CDCl₃) 8.13 (d, 1H, *J*=2.6 Hz), 7.54 (d, 1H, *J*= 2.6 Hz), 0.43 (s, 9H).

20

25

Preparation 8**5-Bromo-4-methylthiazole**

Add bromine (9.27 mL, 182 mmol) to a solution of 4-methylthiazole (15.0 g, 152 mmol) in acetic acid (30 mL) at 0 °C. Slowly warm the reaction mixture to room temperature and stir overnight. Dilute with dichloromethane and wash with 1 N NaOH and brine. Dry the organic layer over sodium sulfate, filter, and concentrate under vacuum. Purify the crude product by silica gel column chromatography, eluting with hexanes/ethyl acetate (5/1) to obtain the title compound (9.94 g, 37%). ¹H NMR (400 MHz, CDCl₃): δ 8.69 (s, 1H), 2.43 (s, 3H).

10

Preparation 9**Thiazole-2-carboxylic acid ethyl ester**

To a mixture of 2-trimethylsilylthiazole (135 g, 858.1 mmol) in toluene (1350 mL) add a solution of ethyl chloroformate (98.4 mL, 1.03 mol) in toluene (1350 mL) over 15 min. Stir the reaction at 22 °C for 2 h. Add the solution over aqueous sodium carbonate 25% (wt/wt) (5 L) and stir for 30 min. Separate the organic layer and re-extract the aqueous layer with methylene chloride (2 × 1 L). Combine the organic layers and evaporate the solvent to provide the title compound (134 g, 99%). ES/MS m/z 158 (M+1)⁺.

20

Preparation 10**Thiazole-2-carboxylic acid amide**

Add thiazole-2-carboxylic acid ethyl ester (150 g, 0.9 mol) to a mixture of methanol (75 mL) and 30% aqueous ammonium hydroxide (750 mL) and heat the mixture at reflux for 1 h. Then cool to 22 °C and evaporate the methanol under vacuum. Stir the mixture for 30 min at room temperature and filter the solid. Dry the isolated solid under vacuum to afford the title compound (98 g, 85%). ES/MS m/z 129 (M+1)⁺.

Preparation 11**1-Methyl-5-thiazol-2-yl-1H-[1,2,4]triazole**

Cool 1,1-dimethoxy-*N,N*-dimethyl-methanamine (240 mL) to 10 °C and add thiazole-2-carboxylic acid amide (60 g, 421 mmol) in three portions. Stir the mixture at

10 °C for 30 min. Then heat the mixture gradually to reflux in 45 min. Distill off the methanol formed and then heat the reaction to 100 °C for 1.5 h. Cool the mixture to 60 °C and remove the excess 1,1-dimethoxy-N,N-dimethyl-methanamine by vacuum distillation. Cool the residue to 22 °C and add hexanes (200 mL). Triturate the mixture
5 for 15 min, filter, and dry the solid to constant weight before using in the next step.

Add the solid isolated above (68 g) to acetic acid (680 mL) and cool the mixture to 10 °C. Add *N*-methyl-hydrazine (27 mL, 509 mmol) at such a rate as to keep the temperature below 15 °C. Warm the mixture to 20 °C in 30 min and then heat gradually to 90 °C. Stir at 90 °C for 30 min and then cool to 22 °C. Remove the acetic acid by
10 vacuum distillation. Add the residue over water and adjust to pH = 8 by adding 25% aqueous sodium hydroxide solution. Extract the aqueous layer with methyl-*t*-butyl ether (3 × 600 mL). Combine the organic layers and evaporate the solvent. Purify the resulting residue by silica gel chromatography using an eluent of hexanes/isopropanol (9/1) to provide the title compound (49 g, 70%). ES/MS *m/z* 167 (*M*+1)⁺.

15

Preparation 12

5-(5-Bromo-thiazol-2-yl)-1-methyl-1*H*-[1,2,4]triazole

To a mixture of methyl-5-thiazol-2-yl-1*H*-[1,2,4]triazole (6.55 g; 39.4 mmol) and dimethylformamide (32 mL), add *N*-bromosuccinimide (14 g, 78.8 mmol) in three
20 portions over 1 h. Stir the mixture at 22 °C for 18 h and then add to water (300 mL) chilled to 0–5 °C. Separate the aqueous layer and extract with methyl-*t*-butyl ether (2 × 200 mL). Combine the organic layers and wash with 7% aqueous sodium hydrogen carbonate (100 mL) and then dry over sodium sulfate. Filter and evaporate the solvent to afford the title compound (9.5 g, 93%). ES/MS *m/z* (⁷⁹Br/⁸¹Br) 245/247 (*M*+1)⁺.

25

Preparation 13

2,5-Dimethyl-3-[2-(2-methyl-2*H*-[1,2,4]triazol-3-yl)-thiazol-5-yl]-7-(1-propyl-butyl)-pyrazolo[1,5-*a*]pyrimidine

Combine 5-(5-bromo-thiazol-2-yl)-1-methyl-1*H*-[1,2,4]triazole (6.5 g, 23.8 mmol)
30 and 2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*]pyrimidine (6 g, 24.5 mmol) in *N*-methylpyrrolidinone (58 mL) and stir to complete solution under nitrogen. Then add tetra-*N*-butylammonium bromide (5.47 g, 16.7 mmol) and potassium acetate (11.8 g, 119

mmol) and heat the mixture to 100 °C under a nitrogen atmosphere. Degas the hot mixture by three cycles of vacuum/nitrogen purge. Then add palladium acetate (216 mg, 0.94 mmol) and tris(2,4-di-*tert*-butyl-phenyl)-phosphane (787 mg, 1.2 mmol). Heat the mixture 4 h at 125 °C under nitrogen. Cool the mixture to 22 °C and add to water (750 mL). Extract the aqueous layer with methyl-*t*-butyl ether (3 × 200 mL), combine the organic portions, and evaporate. Purify the residue by filtration through a silica gel pad eluting with hexanes/ethyl acetate (4/1). Combine the product containing fractions and evaporate the solvent to afford the title compound (7 g, 72%). ES/MS m/z 410 (M+1)⁺.

10

Preparation 14

2,5-Dimethyl-7-(1-propyl-butyl)-3-thiazol-5-yl-pyrazolo[1,5-*a*] pyrimidine

Charge an oven dried flask with 2-trimethylsilanylthiazole (1.765 g, 11.24 mmol) dissolved in anhydrous THF (30 mL) and chill under an inert atmosphere to -78 °C. Slowly add *n*-butyl lithium (2.5 M hexane solution, 4.5 mL, 11.24 mmol) and stir 30 min at -78 °C. Add anhydrous zinc chloride (2.26 g, 16.58 mmol) in one aliquot and stir 30 min at -78 °C. Allow the reaction to rise to room temperature, stir 30 min, and add 7-(1-propyl-butyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*] pyrimidine (1.624 g, 5.18 mmol) and dichloro[1,1'-bis(diphenylphosphino) ferrocene] palladium (II) dichloromethane (0.423 g, 0.518 mmol). Reflux overnight in an oil bath (90 °C) under an inert atmosphere. Cool the reaction to room temperature, quench with saturated sodium bicarbonate, and dilute with ethyl acetate (150 mL). Separate and extract the aqueous with ethyl acetate (75 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 100% hexane/0% (30% THF/hexane) to 0% hexane/100% (30% THF/hexane) in a step gradient of 10% increments to give a white solid (0.720 g, 42%). ES/MS m/z 329.0 (M+1)⁺.

20

25

Prepare the compound below essentially as described in Preparation 14.

Prep. No.	Chemical name	Physical data
15	7-(1-Ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5- <i>a</i>]pyrimidine	¹ H NMR (400 MHz, CDCl ₃): d 8.75 (s, 1H), 8.75 (s, 1H), 8.28 (s, 1H), 6.47 (s, 1H), 3.62-3.55 (m, 1H), 2.65 (s, 3H), 2.61 (s, 3H), 1.89-1.75 (m, 4H), 0.85 (t, 6H, <i>J</i> = 7.5 Hz), 0.85 (t, 6H, <i>J</i> = 7.5 Hz).

Alternate procedure for Preparation 15:

- Add n-butyl lithium (76.5 mL, 191 mmol, 2.5 M in hexanes) to a solution of 2-trimethylsilylthiazole (30 g, 191 mmol) in THF (450 mL) at -78 °C under nitrogen,
- 5 keeping the temperature below -74 °C during the addition. Stir the mixture at -78 °C for 30 min and then add zinc chloride, dry powder, (39.9 g, 286 mmol) in one portion and warm the mixture to 22 °C over one hour. Add 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine, (30 g, 87 mmol) and 1,1'-bis(diphenylphosphino)ferrocene palladium(II) chloride (6.5 g, 8 mmol) and heat the mixture to reflux under nitrogen for 8
- 10 h. Cool the mixture to 22 °C and add 10% aqueous ammonium chloride (450 mL). Separate the organic layer and wash the aqueous layer with methyl-*t*-butyl ether (2 × 100 mL). Combine the organic portions, dry over sodium sulfate, filter, and evaporate the solvent to afford the title compound (20.4 g, 78%). ES/MS *m/z* 301 (*M*+1)⁺.

15

Preparation 16

- 7-(1-Ethyl-propyl)-3-(2-iodo-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine
- Add a freshly prepared solution of lithium di-*i*-propylamide (150 ml; 62.4 mmol, 0.6 M in THF) to a mixture of 7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5-*a*]pyrimidine, (17.8 g, 62.4 mmol) in THF (100 mL) at -78 °C under a
- 20 nitrogen atmosphere, keeping the temperature below -74 °C during the addition. Stir the mixture at -78 °C for one hour and then add a solution of N-iodosuccinimide (15 g, 63 mmol) in THF (100 mL) keeping temperature below -74 °C. Warm the reaction gradually to 22 °C and then add a 10% aqueous solution of ammonium chloride (300 mL). Separate the organic layer and wash the aqueous layer with methyl-*t*-butyl ether (2
- 25 × 200 mL). Combine the organic layers, dry over sodium sulfate, filter, and evaporate the

solvent. Purify the resulting residue by silica gel chromatography, eluting with hexanes/acetone (5/1) to afford the title compound (15 g, 60%). ES/MS m/z 427 (M+1)⁺.

Example 1

- 5 7-(1-Ethyl-propyl)-2,5-dimethyl-3-(4-methyl-thiazol-5-yl)-pyrazolo[1,5-*a*]pyrimidine
- Add Rickc® zinc (10 g in 100 mL of THF, 13.2 mL, 18.48 mmol) to 5-bromo-4-methylthiazole (2.13 g, 18.48 mmol) and heat at reflux for 2 h. Cool the mixture to room temperature and settle the zinc down by centrifuge. Bubble nitrogen gas through 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (900 mg, 2.62 mmol) in dry
- 10 tetrahydrofuran (10 mL) and add the organozinc bromide solution followed by addition of [1,1'-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) (106 mg, 0.13 mmol). Stir the reaction mixture under reflux overnight and cool to room temperature. Add ammonium chloride solution to the reaction mixture and extract with dichloromethane. Dry the organic portion over sodium sulfate, filter, and remove the solvent under vacuum.
- 15 Purify the crude product by silica gel column chromatography, eluting with hexane/ethyl acetate (3/1) to obtain the title compound (652 mg, 79%). ¹H NMR (400 MHz, CDCl₃): δ 8.80 (s, 1H), 6.48 (s, 1H), 3.63 (m, 1H), 2.57 (s, 3H), 2.43 (s, 3H), 2.40 (s, 3H), 1.85 (m, 4H), 0.90 (t, 6H, *J* = 7.3Hz). ES/MS m/z 315 (M+1)⁺.

20

Preparation 17

- 3-(2,4-dibromo-thiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidine
- Dissolve 2,5-dimethyl-7-(1-propyl-butyl)-3-thiazol-5-yl-pyrazolo[1,5-*a*] pyrimidine (3.15 g, 9.59 mmol) in acetonitrile (100 mL) and add N-bromosuccinimide (4.27 g, 24.0 mmol) in one aliquot. Stir overnight under an inert atmosphere and confirm
- 25 the reaction is complete using TLC. Concentrate under reduced pressure, dilute the oil with dichloromethane (150 mL), and wash with water (75 mL). Dry the organic phase over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting oil using flash chromatography, eluting with 100% hexane/0% (30% THF/hexane) to 0% hexane/100% (30% THF/hexane) in a step gradient (0-10-15-20-25-
- 30 30-35-40-45-50-100% of 30% THF/hexane) to give yellow crystals (3.70 g, 79%). ES/MS m/z (⁷⁹Br⁸¹Br) 486.7 (M+1)⁺.

Prepare the compounds below essentially as described in Preparation 17, with the exception that dichloromethane is used as the solvent.

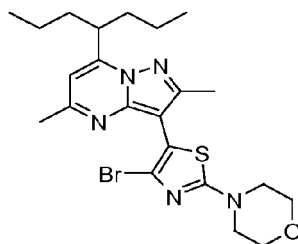
Ex. No.	Chemical name	Physical data
2	3-(2,4-Dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidine	MS (APCI) <i>m/z</i> (⁷⁹ Br ⁸¹ Br) 459.1 (M+1) ⁺
3*	3-(2-Bromo-4-methyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidine	ES/MS <i>m/z</i> (⁷⁹ Br) 393 (M+1) ⁺

*Use 1.1 eq NBS and stir for 3 days.

5

Example 4

3-(4-Bromo-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidine



Charge an oven dried flask with 3-(2,4-dibromothiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidine (0.973 g, 2.00 mmol), anhydrous dioxane (20 mL), morpholine (0.872 g, 10.0 mmol), and cesium carbonate (1.95 g, 6.00 mmol). Reflux in an oil bath (105 °C) under an inert atmosphere overnight. Confirm the reaction is complete using LC/MS. Dilute with ethyl acetate (100 mL), wash with water (50 mL), and back extract the aqueous with ethyl acetate (50 mL). Combine the organic phases, dry over magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 100% hexane/0% (40% dichloromethane/20% ethyl acetate/2% 7 N ammonia in methanol/38% hexane) to 0% hexane/100% (40% dichloromethane/20% ethyl acetate/2% 7 N ammonia in methanol/38% hexane) in a step gradient of 10% increments to yield an off-white solid (0.878 g, 89%). ES/MS *m/z* (⁷⁹Br) 491.7 (M+1)⁺.

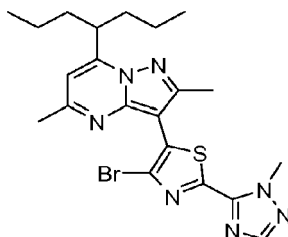
Prepare the following examples essentially as described in Example 4, using as the amine either 2.0 dimethylamine/THF or morpholine, and using THF or dioxane as the solvent. Run reactions in a sealed vessel or Schlenk tube.

Ex. No.	Chemical name	Physical data
5*	{4-Bromo-5-[2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5- <i>a</i>] pyrimidin-3-yl]-thiazol-2-yl} - dimethylamine	ES/MS (⁷⁹ Br) 449.8 (M+1) ⁺ .
6	3-(4-Bromo-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidine	MS (APCI) m/z (⁸¹ Br) 466.5 (M+1) ⁺
7	{4-Bromo-5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidin-3-yl]-thiazol-2-yl} - dimethyl-amine	MS (APCI) m/z (⁸¹ Br) 424.4 (M+1) ⁺
8	{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidin-3-yl]-4-methyl-thiazol-2-yl} -dimethyl-amine	ES/MS m/z 358 (M+1) ⁺
9	7-(1-Ethyl-propyl)-2,5-dimethyl-3-(4-methyl-2-morpholin-4-yl-thiazol-5-yl)-pyrazolo[1,5- <i>a</i>]pyrimidine	ES/MS m/z 400 (M+1) ⁺

5 *Use 6 eq of cesium carbonate.

Example 10

3-[4-Bromo-2-(2-methyl-2*H*-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*]pyrimidine



Charge an oven dried flask with 1-methyl-1,2,4-triazole (0.498, 6.00 mmol) and anhydrous THF (20 mL) and chill under an inert atmosphere to -78 °C. Slowly add *n*-butyl lithium (2.5 M hexane solution, 2.4 mL, 6.0 mmol) and stir 30 min. Add anhydrous zinc chloride (1.36 g, 10.0 mmol) in one aliquot and stir 30 min at -78 °C. Allow the
5 reaction to warm to room temperature, stir 30 min, and add 3-(2,4-dibromothiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidine (0.973 g, 2.00 mmol) and dichloro[1,1'-bis(diphenylphosphino) ferrocene] palladium (II) dichloromethane (0.163 g, 0.200 mmol). Reflux the reaction overnight in an oil bath (90 °C) under an inert atmosphere. Cool the reaction to room temperature, quench with water, and dilute with
10 ethyl acetate (100 mL). Separate and extract the aqueous portion with dichloromethane (50 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 100% hexane/0% (10% acetonitrile/40% THF/50% hexane) to 0% hexane/100% (10% acetonitrile/40% THF/50% hexane) in a step gradient of 10%
15 increments to give a white solid (0.090 g, 9%). ES/MS *m/z* (⁷⁹Br) 487.7 (M+1)⁺.

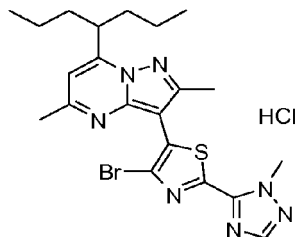
Alternate preparation:

To a solution of 2,5-dimethyl-3-[2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-7-(1-propyl-butyl)-pyrazolo[1,5-*a*]pyrimidine (6 g, 14.65 mmol) in acetonitrile (60 mL) add N-bromosuccinimide (2.74 g, 15.4 mmol) in one portion and stir at 22 °C for 10 h.
20 Evaporate the solvent and dissolve the residue in a mixture of water (50 mL) and methyl-*t*-butyl ether (100 mL). Separate the organic layer and extract the aqueous layer with additional methyl-*t*-butyl ether (2 × 50 mL). Combine the organic portions and evaporate the solvent. Purify the resulting material by filtration through a silica gel pad, eluting with hexanes/ethyl acetate (3/1). Combine the product containing fractions and
25 evaporate the solvent. Add heptanes (25 mL) and triturate the solid. Filter the solid and dry under vacuum to afford the title compound (5.5 g, 77%). ES/MS *m/z* (⁷⁹Br/⁸¹Br) 488/490 (M+1)⁺.

Example 10a

3-[4-Bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-
30 butyl)- pyrazolo[1,5-*a*]pyrimidine, hydrochloride

34



Dissolve 3-[4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*]pyrimidine (750 mg, 1.54 mmol) in acetone (5 mL) and add 1 M HCl in diethyl ether (1.84 mL, 1.84 mmol). Stir the mixture at room temperature for 3 h and concentrate *in vacuo*. Dissolve the residue in diethyl ether/hexane = 1/1 (5 mL) and crystallize the desired HCl salt (526 mg, 65%). ES/MS m/z (^{81}Br) 490 ($\text{M}+1$) $^{+}$; $^1\text{H-NMR}$ (CDCl_3): 8.20 (s, 1H), 6.82 (s, 1H), 4.21 (s, 3H), 3.64 (m, 1H), 2.49 (m, 3H), 2.44 (s, 3H), 1.75 (m, 4H), 1.96(m, 4H), 0.81 (m, 6H).

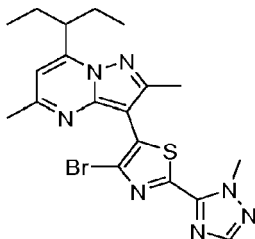
10 Prepare the following compound essentially as described in Example 10.

Ex. No.	Chemical name	Physical data
11*	7-(1-Ethyl-propyl)-2,5-dimethyl-3-[4-methyl-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-pyrazolo[1,5- <i>a</i>]pyrimidine	ES/MS m/z 396 (M+1) ⁺

* Use 0.5 M zinc chloride in THF instead of anhydrous zinc chloride. Heat at 80 °C for 3 days.

Example 12

15 3-(4-Bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine



Under nitrogen atmosphere, add *n*-butyl lithium (2.5 M in hexane, 0.6 mL, 1.5 mmol) to a solution of 1-methyl-1,2,4-triazole (124.5 mg, 1.5 mmol) in THF (3 mL) at –78 °C and stir for 30 min. Add anhydrous zinc chloride (409 mg, 3.0 mmol), continue stirring for 30 min, warm up to room temperature and stir for 2 h. Add 3-(2,4-dibromothiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (229 mg, 0.5 mmol), followed by tetrakis(triphenylphosphine)palladium (58 mg, 0.05 mmol) and reflux overnight. Cool to room temperature, dilute with ethyl acetate, and wash with saturated ammonia chloride. Dry the organic portion over sodium sulfate, filter, and concentrate to a residue. Purify the crude material by flash chromatography, eluting with hexanes:ethyl acetate (10:2.5) to give the title compound as a yellow foam (77 mg). MS (APCI) *m/z* (⁷⁹Br) 460.4 (M+1)⁺.

Alternate preparation from Example 21:

Add acetic acid (1 mL) and N-bromosuccinimide (4.1 g, 22 mmol) to a solution of 7-(1-ethyl-propyl)-2,5-dimethyl-3-[2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-pyrazolo[1,5-*a*]pyrimidine (8 g, 21 mmol) in acetonitrile (80 mL). Stir the mixture 2 h at 22 °C. Then evaporate the solvent and add water (50 mL) and methyl-*t*-butyl ether (100 mL) to the resulting residue. Separate the organic portion, dry over sodium sulfate, filter, and evaporate the solvent. Recrystallize the resulting residue from isopropyl alcohol to afford the title compound (8.7 g, 90%). ES/MS (*m/z*): (⁷⁹Br/⁸¹Br) 460/462 (M+1)⁺.

20

Example 12a

3-(4-Bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine, *p*-toluene sulfonic acid

Dissolve 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (50 mg, 0.109 mmol) in acetone (3 mL). Add a 0.25 M aqueous solution of *p*-toluene sulfonic acid (434.4 µL, 0.109 mmol) and evaporate the resulting mixture to dryness. Add ethyl acetate (12 mL) to get partial dissolution of the solids. Add methanol (1 mL) to achieve a clear solution. Concentrate the solution by slow evaporation until crystals are observed. Isolate the crystals by filtration and dry under vacuum at 25 °C to obtain about 50 mg of the title compound.

30

Determine the stoichiometry of the salt by ion chromatography using the following HPLC conditions: column: Phenomenex Phenosphere SAX, 4.6 × 150 mm at

30 °C; mobile phase: 50% acetonitrile/50% 0.025 M sodium phosphate buffer at pH = 4.5; flow rate = 1.5 mL/min; detection: UV at 205 nm; injection volume = 5 µL; run time = 3 min. Theoretical amount calc: 27.2% tosylate; found: 28.4% tosylate (average of three HPLC runs).

5

Example 13

{4-Chloro-5-[2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidin-3-yl]-thiazol-2-yl}-dimethylamine

Charge an oven dried flask with {4-bromo-5-[2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidin-3-yl]-thiazol-2-yl-dimethylamine (0.20, 0.44 mmol) and anhydrous THF (3.0 mL) and chill under an inert atmosphere to -78°C. Slowly add *n*-butyl lithium (1.6 M hexane solution, 0.42 mL, 0.67 mmol) and stir 30 min. Add *N*-chlorosuccinimide (0.120 g, 0.889 mmol) in one aliquot and stir 30 min at -78 °C. Allow the reaction to warm to room temperature, and stir for 5 h, checking the progress using LC/MS. Dilute with ethyl acetate (100 mL), wash with saturated ammonium chloride (50 mL), and back-extract the aqueous with dichloromethane (50 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 100% hexane/0% (25% THF/hexane) to 0% hexane/100% (25% THF/hexane) in a step gradient of 10% increments to give a white solid (0.087 g, 48%). ES/MS *m/z* (³⁵Cl) 406.0 (M+1)⁺.

20

Prepare the following examples essentially as described in Example 13 using the appropriate bromothiazole prepared above.

Ex. No.	Chemical name	Physical data
14	3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5- <i>a</i>] pyrimidine	ES/MS <i>m/z</i> (³⁵ Cl) 448.0 (M+1) ⁺
15	{4-Chloro-5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidin-3-yl]-thiazol-2-yl}-dimethyl-amine	MS (APCI) <i>m/z</i> (³⁵ Cl) 378.0 (M+1) ⁺

25

Preparation 18

37

2,4-dichlorothiazole

Cool to 5 °C a mixture of thiazolidine-2,4-dione (50 g, 0.43 mol) in phosphorus oxychloride (240 mL) and add pyridine (34 mL, 0.43 mol) over 15 min. Heat the mixture to 125 °C for 4 h and then cool to 22 °C. Remove the excess phosphorus oxychloride by vacuum distillation and add the residue to water (1 L) chilled to a temperature of 5 °C. Extract the mixture with methylene chloride (3 × 400 mL). Combine the organic portions and evaporate the solvent to afford the title compound (50 g, 76%). EI/MS m/z : ($^{35}\text{Cl}^{35}\text{Cl}/^{35}\text{Cl}^{37}\text{Cl}/^{37}\text{Cl}^{37}\text{Cl}$) 153/155/157 (M+1)⁺.

10

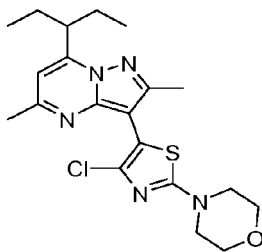
Preparation 19

4-chloro-2-morpholino-thiazole

To a mixture of 2,4-dichlorothiazole (34 g, 0.22 mol) in acetonitrile (425 mL) add potassium carbonate (60.9 g, 0.44 mol) and then morpholine (21.2 mL, 0.225 mol) dropwise over 30 min. Reflux the mixture at 40 °C and then cool to 22 °C. Filter the mixture and evaporate the filtrate. Triturate the residue with *i*-propyl alcohol (60 mL) at 22 °C for one hour. Filter the solids and dry under vacuum to a constant weight to afford the title compound (34.5 g, 76%). ES/MS m/z (^{35}Cl) 205 (M+1)⁺.

Example 16

20 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine



Under a nitrogen atmosphere dissolve 3-(4-bromo-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (116 mg, 0.25 mmol) in THF (1.5 mL) and chill to -78 °C. Add *n*-butyl lithium (0.1 mL, 2.5 M in hexane, 0.25 mmol) and stir at -78 °C for 30 min. Add *N*-chlorosuccinimide (33.4 mg, 0.25 mmol) and stir

for another 30 min, slowly warming to room temperature. After stirring overnight, quench the reaction by adding a solution of saturated ammonia chloride and extract with ethyl acetate. Wash the organic layer with brine, dry over sodium sulfate, filter, and concentrate to a residue. Purify the crude material by flash chromatography, eluting with
5 hexanes:dichloromethane:ethyl acetate (5:5:2) to provide the title compound (54 mg). MS (APCI) m/z (^{35}Cl) 420.6 ($M+1$)⁺; ^1H NMR (400 MHz, CDCl_3): 6.44 (s, 1H), 3.79 (t, 4H, $J=4.8$ Hz), 3.63-3.56 (m, 1H), 3.47 (t, 4H, $J=4.8$ Hz), 2.55 (s, 3H), 2.45 (s, 3H), 1.88-1.75 (m, 4H), 0.87 (t, 6H, $J=7.5$ Hz).

Alternate Preparation from Preparation 6:

10 Combine 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine, (9 g, 26.2 mmol) and 4-chloro-2-morpholino-thiazole (7.5 g, 36.7 mmol) in dimethylformamide (90 mL) previously degassed with nitrogen. Add cesium carbonate (17.8 g, 55 mmol), copper iodide (250 mg, 1.31 mmol), triphenylphosphine (550 mg, 2.09 mmol) and palladium acetate (117 mg, 0.52 mmol). Heat the mixture to 125 °C for 16 h
15 and then cool to 22 °C. Add water (900 mL) and extract with methyl-*t*-butyl ether (3 × 200 mL). Combine the organic portions and evaporate the solvent. Purify by silica gel chromatography eluting with hexanes/ethyl acetate (4/1) to afford the title compound (6.4 g, 62%). ES/MS m/z (^{35}Cl) 420 ($M+1$)⁺.

20

Example 16a

3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine, hydrochloride

25 Dissolve 3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (1.40 g, 3.33 mmol) in acetone (10 mL) at 50 °C and cool to room temperature. Add hydrogen chloride (2 M in diethyl ether, 2.0 mL, 4.0 mmol) and stir well in a sonicator. Concentrate the solution a little and add a minimal amount of diethyl ether to crystallize the HCl salt. Cool the mixture in a refrigerator
30 overnight. Add additional hydrogen chloride (2 M in diethyl ether, 2.0 mL, 4.0 mmol) and cool in a refrigerator. Filter the crystalline material and dry to obtain the title compound (1.15 g, 75%). ES/MS m/z (^{35}Cl) 420 ($M+1$)⁺; ^1H NMR(CDCl_3): 9.18 (br,

1H), 6.86 (s, 1H), 3.72 (m, 4H), 3.49(m, 1H), 3.39 (m, 4H), 2.48 (s, 3H), 2.38(s, 3H), 1.79 (m, 4H), 0.79 (m, 6H).

Example 17

5 3-(4-Bromo-2-butyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-
a]pyrimidine

Under a nitrogen atmosphere, add *n*-butyl lithium (2.5 M in hexane, 0.2 mL, 0.5 mmol) to a solution of 3-(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (230 mg, 0.5 mmol) in THF (3 mL) at -78°C . After 30 min, add 1-iodobutane (138 mg, 0.75 mmol) and continue stirring for 1 hour. Warm up to room temperature and stir for one hour. Quench the reaction by adding saturated ammonia chloride solution and extract with ethyl acetate. Dry the organic portion over sodium sulfate, filter, and concentrate to a residue. Purify the crude material by flash chromatography, eluting with hexanes/ethyl acetate (10/1.5) to give the title compound as orange foam (78 mg). ES/MS m/z (^{81}Br) 437.4 ($\text{M}+1$) $^{+}$.

Prepare the following example essentially as described in Example 17.

Ex. No.	Chemical name	Physical data
18	3-(4-Bromo-2-methoxymethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidine	MS (APCI) m/z (⁸¹ Br) 424.6 (M+1) ⁺

Example 19

20 7-(1-Ethyl-propyl)-3-(2-methoxymethyl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-
a]pyrimidine

Under a nitrogen atmosphere, add *n*-butyl lithium (2.5 M in hexane, 0.4 mL, 1.0 mmol) to a solution of 7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5-a]pyrimidine (300 mg, 1.0 mmol) in THF (3 mL) at -78 °C. Stir 30 min and add 1-iodomethyl methyl ether (205 mg, 1.2 mmol). Continue stirring for one hour and then slowly warm up to room temperature and stir overnight. Quench the reaction by adding saturated ammonium chloride solution and extract with ethyl acetate. Wash the organic layer with brine, dry over sodium sulfate, filter, and concentrate under vacuum to a

residue. Purify the crude material by flash chromatography, eluting with hexanes/ethyl acetate (10/2) to give the title compound as a yellow foam (184 mg). MS (APCI) m/z 345.3 (M+1)⁺.

5

Example 20

3-(4-Chloro-2-methoxymethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Add *n*-butyl lithium (2.5 M in hexanes, 174 mL, 0.43 mmol) to a stirred solution of 7-(1-ethyl-propyl)-3-(2-methoxymethyl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (150 mg, 0.43 mmol) in THF (3 mL) at -78 °C. Stir for 30 min and add *N*-chlorosuccinimide (87 mg, 0.653 mmol). Stir 30 min and then warm the reaction slowly to room temperature allowing the reaction to continue overnight. Quench the reaction by adding saturated ammonium chloride solution, extract with ethyl acetate, dry over sodium sulfate, filter, and concentrate to a residue. Purify by flash chromatography, eluting with hexanes/ethyl acetate (10/2) to give the title compound (7 mg). MS (APCI) m/z (35Cl) 379.3 (M+1)⁺.

10
15**Example 21**

7-(1-Ethyl-propyl)-2,5-dimethyl-3-[2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-pyrazolo[1,5-*a*]pyrimidine

20

Mix 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (175 mg, 0.38 mmol) and copper(I) chloride (132 mg, 1.33 mmol) in DMF (5 mL) and heat to 120 °C for 24 h. Cool to room temperature, dilute with ethyl acetate, and wash with brine. Dry the organic layer over sodium sulfate, filter, and concentrate under vacuum to a residue. Purify by flash chromatography, eluting with hexanes and then hexanes/EtOAc (10/1.8) to give a yellow-orange solid (45 mg). ES/MS m/z 382.0 (M+1)⁺.

25**Alternate Preparation:**

Add *n*-butyl lithium (2.5 M in hexanes, 57.6 mL, 144 mmol) to a solution of *N*-methyltriazole (11.95 g, 144 mmol) in THF (600 mL) at -78 °C under a nitrogen atmosphere, keeping the temperature below -74 °C during the addition. Then add zinc chloride, dry powder, (26 g, 192 mmol) in one portion and warm the mixture to 22 °C in

30

one hour. Add 7-(1-ethyl-propyl)-3-(2-iodo-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (12.5 g, 29 mmol) and tetrakis(triphenyl)phosphine palladium catalyst (1.15 g, 0.01 mol) in one portion and heat the mixture to reflux under nitrogen for 8 h. Cool the mixture to 22 °C and add water (300 mL). Separate the organic layer and extract the aqueous layer with methyl-*t*-butyl ether (2 × 200 mL). Combine the organic portions, dry over sodium sulfate, filter, and evaporate the solvent. Purify by passing over a silica gel pad eluting with hexanes/ethyl acetate (4/1) to afford the title compound (8 g, 72%). ES/MS *m/z* 382 (M+1)⁺.

Example 22

10 3-(4-Chloro-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Stir a mixture of 7-(1-ethyl-propyl)-2,5-dimethyl-3-[2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-pyrazolo[1,5-*a*]pyrimidine (20 mg, 0.052 mmol) and *N*-chlorosuccinimide (7.6 mg, 0.0569 mmol) in dichloromethane (0.5 mL) and acetonitrile (0.5 mL) at room temperature overnight in a vial. Concentrate to a residue. Purify by flash chromatography, eluting with hexanes and then with hexanes/ethyl acetate (10/1.5) to give the title compound (16 mg). ES/MS *m/z* (³⁵Cl) 416.0 (M+1)⁺.

Example 23

20 7-(1-Ethyl-propyl)-3-(4-methoxy-2-morpholin-4-yl-thiazol-5-yl)- 2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Stir a mixture of 3-(4-bromo-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (162 mg, 0.35 mmol), sodium methoxide (57 mg, 1.05 mmol) and copper(I) iodide (67 mg, 0.35 mmol) in methanol (3 mL) in a sealed 4-mL vial for 15 h at 120 °C. Cool to room temperature, remove the solid by filtration, and concentrate the filtrate under vacuum. Purify the residue by flash chromatography, eluting with hexanes/THF (10/2). Recrystallize the material from methanol to give the title compound (20 mg). ES/MS *m/z* 416.0 (M+1)⁺.

Preparation 20

30 4-Bromo-5-[7-(1-ethyl-propyl)- 2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-thiazole-2-carbaldehyde

- Under a nitrogen atmosphere, add *n*-butyl lithium (1.6 M in hexanes, 0.312 mL, 0.50 mmol) to a THF solution (2.5 mL) of 3-(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (230 mg, 0.50 mmol) at -78 °C and stir for 30 min. Add a THF (0.5 mL) solution of N-formyl morpholine (58 mg, 0.50 mmol).
- 5 Stir for one hour, then store the reaction at -20 °C overnight. Warm the reaction to room temperature, dilute with ether, and quench by adding 4 N HCl (4 mL). Separate and extract the organic phase with 4 N HCl (2 × 4 mL). Combine the aqueous portions, treat with solid sodium bicarbonate to pH = 8 to 9 and then extract with diethyl ether. Combine all the organic layers, wash with brine, dry over sodium sulfate, filter, and
- 10 concentrate to a residue. Purify the crude material by flash chromatography, eluting with hexanes/dichloromethane/ethyl acetate (5/5/1) to give the title compound (154 mg). MS (APCI) *m/z* (⁸¹Br) 409.0 (M+1)⁺.

15

Example 24

3-(4-Bromo-2-morpholin-4-yl-methyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

- Combine 4-bromo-5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-thiazole-2-carbaldehyde (150 mg, 0.368 mmol), morpholine (35 mg, 0.405 mmol) and sodium triacetoxyborohydride (97 mg, 0.46 mmol), in dichloromethane (3 mL) and methanol (0.5 mL). Stir overnight, add additional morpholine (35 mg, 0.405 mmol), and sodium triacetoxyborohydride (97 mg, 0.46 mmol), and stir 4 h more. Remove the
- 20 solvent under vacuum, dilute with dichloromethane, and wash with brine. Dry the organic phase over sodium sulfate, filter, and concentrate under vacuum. Purify the resulting material using flash chromatography, eluting with dichloromethane:2 M ammonia in methanol (10:1) to give a mixture. Purify the mixture using a reverse phase column, eluting with water/acetonitrile (80/20) to water/acetonitrile (10/90) to give the title compound (20 mg). ES/MS *m/z* (⁸¹Br) 480.0 (M+1)⁺.

30

Example 25

3-(4-Bromo-2-pyridin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-
a]pyrimidine

Under a nitrogen atmosphere, add *n*-butyl lithium (1.6 M in hexane, 0.312 mL, 0.5 mmol) to a solution of 3-(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (229 mg, 0.5 mmol) in THF (4 mL) at -78 °C. After stirring
5 for 30 min, add anhydrous zinc chloride (264 mg, 1.5 mmol) and continue stirring for 30 min. Warm the reaction to room temperature and stir for one hour. Add 4-iodopyridine (103 mg, 0.5 mmol), followed by 1,1'-bis (diphenylphosphino)ferrocene palladium (II) chloride (dichloromethane adduct) (0.408 mg, 0.05 mmol). Heat the reaction to reflux
10 overnight. Cool to room temperature, dilute with ethyl acetate, and wash with water and brine. Dry the organic layer over sodium sulfate, filter, and concentrate to a residue. Purify the crude material by flash chromatography, eluting with dichloromethane:2 N ammonia in methanol (10:0.75) to give a mixture. Purify the mixture using a reverse phase column, eluting with water:acetonitrile = 80:20 to water: acetonitrile = 10:90, to
15 give the title compound (19 mg). ES/MS m/z (^{79}Br) 456.0 ($M+1$)⁺.

Preparation 21

4-Trifluoromethyl-thiazole-2-ylamine

Add thiourea (4.0 g, 52.3 mmol) and 3-bromo-1,1,1-trifluoropropan-2-one (5.5 mL, 10 g, 52.3 mmol) to ethanol (100 mL) and heat at 50 °C for 2 h. Cool to room
20 temperature and concentrate to dryness. Dissolve the residue in water and adjust the pH to >12 with 2 M NaOH. Extract with diethyl ether (4×). Dry the combined organic extracts with sodium sulfate, filter, and concentrate under vacuum. Purify the resulting material by silica gel chromatography (CH₂Cl₂) to obtain the title compound (6.9 g, 79%).
25 ES/MS m/z 169 ($M+1$)⁺.

Preparation 22

5-Bromo-4-trifluoromethyl-thiazol-2-ylamine, hydrobromide

Add bromine (2.0 mL, 6.28 g, 39.3 mmol) dropwise to an ice-bath cooled solution
30 of 4-trifluoromethyl-thiazole-2-ylamine (6.0 g, 35.7 mmol) in diethyl ether (60 mL). Stir for one hour after the addition is complete and then warm to room temperature. Collect

44

the solids by filtration and wash with diethyl ether to obtain the title compound (10.5 g, 90%). ES/MS m/z ($^{79}\text{Br}/^{81}\text{Br}$) 247/249 ($M+1$)⁺.

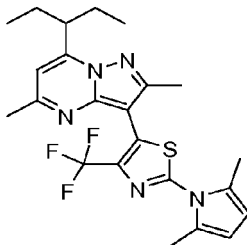
Preparation 23

5 5-Bromo-2-(2,5-dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazole

Add hexane-2,5-dione (3.5 mL, 3.4 g, 30.2 mmol) to a solution of 5-bromo-4-trifluoromethyl-thiazol-2-ylamine hydrobromide (9.0 g, 27.4 mmol) in methanol (60 mL). Stir at room temperature overnight. Add phosphate buffer (50 mL, pH = 7). Collect the resulting precipitate by filtration, washing with water. Dissolve the filter cake in CH_2Cl_2 and dry over sodium sulfate. Filter and concentrate under vacuum to obtain the title compound (8.2 g, 92%). ^1H NMR (400 MHz, CDCl_3) δ 5.91 (s, 2H), 2.27 (s, 6H).

Example 26

3-[2-(2,5-Dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazol-5-yl]-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine



Cool a solution of 5-bromo-2-(2,5-dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazole (2.2 g, 6.6 mmol) in THF (25 mL) in a dry ice bath. Add *t*-butyl lithium (1.7 M in pentane, 8.5 mL, 14.5 mmol) dropwise. Stir for 45 min and then add zinc chloride (0.5 M in THF, 14.6 mL, 7.3 mmol) dropwise. Stir 5 min and remove the cooling bath. Stir 30 min and then add 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (1.5 g, 4.4 mmol) and bis(tri-*t*-butylphosphine)palladium (0) (450 mg, 0.9 mmol). Reflux for 24 h. Cool the reaction, pour the mixture into diethyl ether, and wash with water (2 $\times$). Extract the combined water layers with diethyl ether. Dry the combined organic portions over sodium sulfate, filter, and concentrate to dryness under vacuum. Purify the resulting residue by silica gel chromatography (75-100% CH_2Cl_2 in hexanes) to give the title

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compound (1.47 g, 72%). HR-ToF-MS m/z calcd for $C_{23}H_{26}F_3N_5S+H^+$: 462.1939, found: 462.1915.

Example 27

5 5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-ylamine

Add hydroxylamine (2 mL, 50% in water) to a solution of 3-[2-(2,5-dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazol-5-yl]-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (1.1 g, 2.3 mmol) in acetic acid (10 mL). Heat the reaction to
10 80 °C for 6 h. Cool to room temperature. Pour into diethyl ether and wash with 2 M NaOH (2×) and then once with water. Dry the organic portion over sodium sulfate, filter, and concentrate to dryness under vacuum. Purify the resulting residue by silica gel chromatography (40% ethyl acetate in hexanes) to give the title compound (0.76 g, 87%). ES/MS m/z 384 (M+1)⁺.

15

Preparation 24

3-(2-Bromo-4-trifluoromethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

20 Heat a mixture of copper(II) bromide (540 mg, 2.4 mmol) and *t*-butylnitrite (0.36 mL, 310 mg, 3.0 mmol) in acetonitrile (20 mL) to 60 °C. Add 5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-ylamine (755 mg, 2.0 mmol) as a solid. Heat the reaction to 80 °C for 2 h. Cool the reaction, pour into diethyl ether and extract with water (3×). Dry the organic layer over sodium sulfate,
25 filter, and concentrate to dryness under vacuum. Purify the resulting residue by silica gel chromatography in CH_2Cl_2 to give the title compound (0.77 g, 87%). ES/MS m/z (⁷⁹Br/⁸¹Br) 447, 449 (M+1)⁺.

Example 28

30 {5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-dimethyl-amine

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Heat a sealed tube containing 3-(2-bromo-4-trifluoromethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (313 mg, 0.7 mmol), and dimethyl amine (2 M in THF, 4 mL, 8 mmol) in methanol (4 mL) to 80 °C for 2 h. Cool the reaction and concentrate under vacuum. Purify the resulting residue by silica gel chromatography, eluting with 0-30% ethyl acetate in CH₂Cl₂, to give the title compound (0.28 g, 97%). HR-ToF-MS m/z calcd for C₁₉H₂₄F₃N₅S+H⁺ 412.1770, found: 412.1783.

Prepare the following example essentially as described for Example 28 with the exception that the reaction is refluxed for 8 h.

Ex. No.	Chemical name	Physical data
29	7-(1-Ethyl-propyl)-2,5-dimethyl-3-(2-morpholin-4-yl-4-trifluoromethyl-thiazol-5-yl)-pyrazolo[1,5- <i>a</i>]pyrimidine	HR-ToF-MS m/z calcd for C ₂₁ H ₂₆ F ₃ N ₅ OS+H ⁺ : 454.1873, found: 454.1888

10

Example 30

N-{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-acetamide

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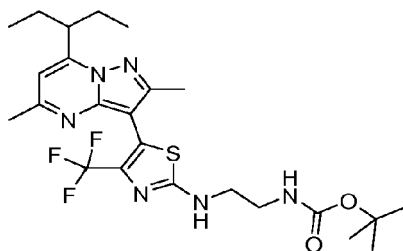
Add hydroxylamine (50% in water, 5 mL) to a solution of 3-[2-(2,5-dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazol-5-yl]-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (2.4 g, 5.2 mmol) in acetic acid (25 mL). Heat the reaction to 80 °C for 72 h. Cool the reaction to room temperature, pour into diethyl ether and wash with 2 M NaOH (2×) and then once with water. Dry the organic portion over sodium sulfate, filter, and concentrate to dryness. Purify the resulting residue by column chromatography, eluting with 40% ethyl acetate in hexanes to give the title compound (0.28 g, 13%). HR-ToF-MS m/z calcd for C₁₉H₂₂F₃N₅OS+H⁺ 426.1575, found: 426.1565.

20

25

Example 31

(2-({5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-ylamino}-ethyl)-carbamic acid *tert*-butyl ester



Add (2-amino-ethyl)-carbamic acid *tert*-butyl ester (0.5 mL, 506 mg, 3.2 mmol) to
 5 a solution of 3-(2-bromo-4-trifluoromethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-
 pyrazolo[1,5-*a*]pyrimidine (100 mg, 0.22 mmol) and triethylamine (0.3 mL, 222 mg, 2.2
 mmol) in methanol (1 mL). Evaporate off the methanol and heat to 80 °C overnight.
 Cool the reaction to room temperature and concentrate under vacuum. Purify the
 resulting residue by column chromatography, eluting with 10-50% ethyl acetate in
 10 CH₂Cl₂, to give the title compound (0.11 g, 97%). ES/MS *m/z* 527.2 (M+1)⁺.

Prepare the compounds below as essentially described in Example 31 using the
 appropriate amine.

Prep. or Ex. No.	Chemical name	Physical data
Prep. 25 [*]	[2-({5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-propyl-amino)-ethyl]-carbamic acid <i>tert</i> -butyl ester	ES/MS <i>m/z</i> 569.3 (M+1) ⁺
Prep. 26 ^{**}	(S)-(1-{5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-pyrrolidin-3-yl)-carbamic acid <i>tert</i> -butyl ester	ES/MS <i>m/z</i> 553.3 (M+1) ⁺

Ex. 32***	<i>N</i> -{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}- <i>N,N'</i> -dipropyl-ethane-1,2-diamine, hydrochloride	ES/MS <i>m/z</i> 511.2 (<i>M</i> +1) ⁺
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* Heat at reflux in EtOH overnight. Evaporate EtOH and heat at 110 °C for 24 h.

** Heat at 80 °C in EtOH overnight.

*** Make the HCl salt and recrystallize from EtOAc/hexanes.

5

Example 33

*N*¹-{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-ethane-1,2-diamine, hydrochloride

Add (2-{5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-ylamino}-ethyl)-carbamic acid *tert*-butyl ester (101 mg, 0.192 mmol) to 1 M HCl in methanol (1 mL). Heat the reaction at 70 °C overnight. Cool the reaction and concentrate under vacuum from methanol/ethyl acetate. Triturate the resulting residue with ethyl acetate to obtain the title compound (64 mg, 78%). ES/MS *m/z* 427.0 (*M*+1)⁺.

15

Prepare the following examples essentially as described for Example 33.

Ex. No.	Chemical name	Physical data
34	<i>N</i> ¹ -{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}- <i>N</i> ¹ -propyl-ethane-1,2-diamine, hydrochloride	ES/MS <i>m/z</i> 469.2 (<i>M</i> +1) ⁺
35	(<i>S</i>)-1-{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-pyrrolidin-3-ylamine, hydrochloride	ES/MS <i>m/z</i> 453.0 (<i>M</i> +1) ⁺

Example A

In Vivo Potency Assessment Using Ex Vivo Binding

To assess in vivo potency, a compound of the present invention is evaluated using ex vivo binding. Using the procedures as provided in D. R. Gehlert *et al.*, *EJP* 509: 145-153 (2005), a compound is administered to a rat via the oral route. The binding of ¹²⁵I-

sauvagine to the cerebellum is then assessed ex vivo as described in Gehlert *et al.* For example, Example 15 provides 65% inhibition at 10 mg/kg.

Example B

5

CRF1 Filter Binding Assay

The limitations of plasmid-based human CRF1 expression, in terms of generating a recombinant cell line with sufficient receptor density to develop a binding assay, are overcome by using a Phoenix retroviral expression system licensed from Stanford. The stable HEK-hCRF1 cell line is used to prepare membranes and binding reactions (200 μ L) are set up as follows: 50 μ L of ^{125} I-sauvagine (0.2 nM final), 50 μ L compound and 100 μ L CRF1 membrane (25 μ g/reaction). The reactions are incubated at room temperature for 2 h and then terminated by filtration through pre-treated FB Millipore glass fiber filter plates (96 well). The plates are washed twice with ice-cold assay buffer (50 mM tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM MgCl₂, 0.05% BSA, pH 7.2), air dried over night and counted with 100 μ L Microscint 40 in a MicroBeta counter. Non-specific binding (NSB) is determined in the presence of 0.5 μ M non-labeled sauvagine. Triplicate determinations are typically run and the median data points plotted by Graph Pad Prism.

Using this assay, the exemplified compounds of the present invention inhibit the binding of ^{125} I-Sauvagine (4 nM) in roller/adherent cells with a K_i (inhibition constant) below 1 μ M. For example, Example 15 exhibits a K_i of 6.2 nM.

20

Example C

CRF2 Filter Binding Assay

The limitations of plasmid-based human CRF2 expression, in terms of generating a recombinant cell line with sufficient receptor density to develop a binding assay, are overcome by using a Phoenix retroviral expression system licensed from Stanford. The stable HEK-hCRF2 cell line is used to prepare membranes and binding reactions (200 μ L) are set up as follows: 50 μ L of ^{125} I-sauvagine (0.2 nM final concentration), 50 μ L compound and 100 μ L CRF2 membrane (25 μ g/reaction). The reactions are incubated at room temperature for 2 hours and then terminated by filtration through pre-treated FB Millipore glass fiber filter plates (96 well). The plates are washed twice with ice-cold assay buffer (50 mM tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM MgCl₂, 0.05% BSA, pH

25

30

7.2), air dried over night and counted with 100 μ L Microscint 40 in a MicroBeta counter. Non-specific binding (NSB) is determined in the presence of 0.5 μ M non-labeled sauvagine. Alternatively, compounds are evaluated using a Scintillation Proximity assay. This assay is set up as follows: 50 μ L of ^{125} I-Sauvagine (0.2 nM final concentration), 50
5 μ L compound or non-labelled sauvagine (NSB) and 100 μ L containing 250 μ g wheat germ agglutinin (WGA) SPA beads and CRF2 membrane (1.5 μ g/reaction). Plates are incubated for 4-5 h at room temperature and then centrifuged at 200 X g for 10 min. Bound radioactivity is assessed using a Wallac Trilux scintillation counter. Binding is assessed typically using triplicate determinations and the median data points plotted by
10 Graph Pad Prism. Compounds are initially screened at a fixed concentration and, if sufficient activity is noted, subsequent concentration-response curves are generated.

Particular exemplified compounds of the present invention are tested in the CRF2 binding assay and exhibit weak affinity for the CRF2 receptor. For example, Example 15 exhibits 11% inhibition at a concentration of 50 μ M. This result suggests that the
15 compounds of the present invention are selective for the CRF1 receptor, (relative to CRF2).

Example D

Bioavailability and Pharmacokinetic Properties

20 The volume of distribution (Vdist) relates the amount of the drug in the body to the concentration of the drug in the blood or plasma. The volume of distribution refers to the fluid volume that would be required to contain the total amount of the drug in the body at the same concentration as in the blood or plasma: $V_{dist} = \text{amount of drug in the body} / \text{concentration of drug in blood or plasma}$ (Goodman and Gillman's). For a 10 mg
25 dose and a plasma concentration of 10 mg/L, the volume of distribution would be 1 liter. The volume of distribution reflects the extent to which the drug is present in the extravascular tissue. A large volume of distribution reflects the tendency of a compound to bind to the tissue components compared with plasma protein binding. In a clinical setting, Vdist can be used to determine a loading dose to achieve a steady state
30 concentration.

To test for volume of distribution, Male Sprague Dawley rats (N=3) are administered a single 1 mg/kg intravenous dose of compound. Multiple plasma samples

are collected at time points from 0.08 to 24 hours post-dose. The plasma samples are analyzed by LC/MS/MS to determine the plasma concentrations. Plasma pharmacokinetic calculations are performed to determine the pharmacokinetic parameters including Vdist and plasma clearance (CLp).

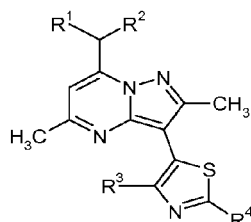
- 5 Compounds of the present invention preferably have favorable bioavailability profiles. For example, a majority of commercial CNS and cardiovascular drugs exhibit a human Vdist of <10 L/Kg. In comparison with CRF antagonists, CP154526 (Schulz et al., *Proc. Natl. Acad. Sci. (USA)*, 93:10477 (1996)) and NBI30775 (Chen et al., *Drug Development Research*, 65:216 (2005)), which exhibit a rat Vdist of 114 L/Kg and 76
- 10 L/Kg, respectively, when analyzed separately. Example 15 of the present invention, when analyzed separately, exhibits a rat Vdist of only 7.2 L/Kg following a single intravenous dose of 1 mg/kg.

- Further, the plasma clearance (CLp) is a measure of the rate of removal of the drug from the body. Following an intravenous dose and first-order kinetics, the plasma
- 15 clearance may be determined using the following equation: $CLp = \text{Dose} / AUC$, where AUC is the total area under the curve that describes the concentration of the drug in the plasma as a function of time from zero to infinity. Reference CRF antagonists CP154526 and NBI37582 exhibit rat clearance (CLp) of 83 and 306 mL/min/kg, respectively, when
- 20 analyzed separately, following a single intravenous dose, while Example 15 of the present invention, when analyzed separately, exhibits a rat CLp of only 23.6 mL/min/kg.

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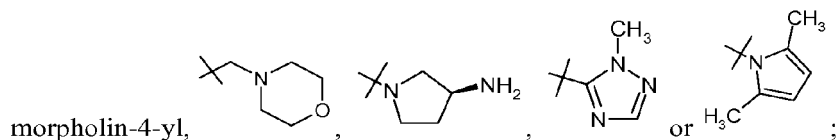
WE CLAIM:

1. A compound of Formula I



Formula I

5 wherein:

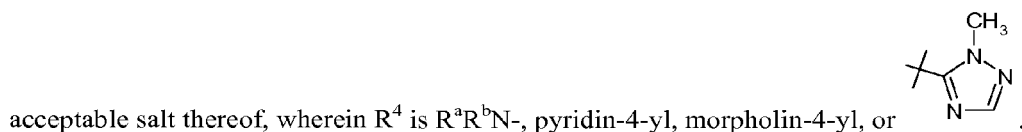
 R^1 and R^2 are independently ethyl or *n*-propyl; R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl or methoxy; R^4 is hydrogen, Br, $R^a R^b N$ -, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl,

10 R^a and R^b are independently hydrogen, C_1 - C_3 alkyl, $H_2NCH_2CH_2$ -,
 $(CH_3)_3COC(O)NHCH_2CH_2$ -, or $CH_3CH_2CH_2NHCH_2CH_2$ -,
 or a pharmaceutically acceptable salt thereof.

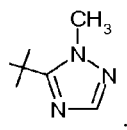
2. The compound according to claim 1, or a pharmaceutically acceptable salt
 15 thereof, wherein R^3 is Cl, Br, methyl or trifluoromethyl.

3. The compound according to claim 1 or 2, or a pharmaceutically acceptable salt
 thereof, wherein R^3 is Cl or Br.

20 4. The compound according to any one of claims 1 to 3, or a pharmaceutically



5. The compound according to any one of claims 1 to 4, or a pharmaceutically

acceptable salt thereof, wherein R⁴ is morpholin-4-yl or .

6. The compound according to any one of claims 1 to 4, or a pharmaceutically
5 acceptable salt thereof, wherein R⁴ is R^aR^bN- and R^a and R^b are independently C₁-
C₃alkyl.

7. The compound according to claim 1, or a pharmaceutically acceptable salt
thereof, which is 3-[4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-
10 dimethyl-7-(1-propyl-butyl)- pyrazolo[1,5-*a*]pyrimidine.

8. The compound according to claim 1, or a pharmaceutically acceptable salt
thereof, which is 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-
propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine.
15

9. The compound according to claim 1, or a pharmaceutically acceptable salt
thereof, which is 3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-
dimethyl-pyrazolo[1,5-*a*]pyrimidine.

20 10. A pharmaceutical composition comprising: a compound according to any
one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, and a pharmaceutically
acceptable excipient.

11. A method of treating depression, anxiety, alcohol or substance abuse, obesity,
25 hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep
disorders, allergy, migraine, premenstrual syndrome, infertility, sexual dysfunction,
congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric
ulceration, inflammatory disorders, pituitary or ectopic pituitary-derived tumors, chronic
fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis in a patient,
30 comprising: administering to a patient in need thereof a therapeutically effective amount

of a compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof.

12. A method of treating anxiety or depression in a patient, comprising:
5 administering to a patient in need thereof a therapeutically effective amount of a compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof.

13. A method of treating alcohol or substance abuse in a patient, comprising:
10 administering to a patient in need thereof a therapeutically effective amount of a compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof.

14. A compound according to any one of Claims 1 to 9, or a pharmaceutically
15 acceptable salt thereof, for use in therapy.

15. A compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, for use in the treatment of depression, anxiety, alcohol or substance abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep disorders, allergy, migraine, premenstrual syndrome, infertility,
20 sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric ulceration, inflammatory disorders, pituitary or ectopic pituitary-derived tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis.

25

16. A compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, for use in the treatment of anxiety or depression.

17. A compound according to any one of claims 1 to 9, or a pharmaceutically
30 acceptable salt thereof, for use in the treatment of alcohol or substance abuse.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/078605

A. CLASSIFICATION OF SUBJECT MATTER INV. C07D487/04 A61K31/505 A61P29/00 A61P25/22 A61P25/24 A61P25/30		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C07D Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, BEILSTEIN Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	GEHLERT ET AL: "3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism." THE JOURNAL OF NEUROSCIENCE, vol. 27, no. 10, 7 March 2007 (2007-03-07), pages 2718-2726, XP002465426 figure 1; table 1	1-17
Y	WO 01/23388 A (NEUROGEN CORP [US]; PFIZER [US]; DARROW JAMES W [US]; LOMBAERT STEPHAN) 5 April 2001 (2001-04-05) page 69; example 95 page 13, line 3	1-17
-/--		
☒ Further documents are listed in the continuation of Box C.		
☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "Z" document member of the same patent family		
Date of the actual completion of the international search 22 January 2008		Date of mailing of the international search report 31/01/2008
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Gettins, Marc

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/078605

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/072202 A (BRISTOL MYERS SQUIBB PHARMA CO [US]) 19 September 2002 (2002-09-19) claims 1,16 -----	1-17
A	HE LIQI ET AL: "4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)pyrazolo[1,5-a]-1,3,5-triazine: A Potent, Orally Bioavailable CRF1 Receptor Antagonist" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 43, 2000, pages 449-456, XP002196777 ISSN: 0022-2623 table 1 -----	1-17
A	WO 2005/063755 A (SB PHARMCO INC [US]; NEUROCRINE BIOSCIENCES INC [US]; LUO ZHIYONG [US]) 14 July 2005 (2005-07-14) claims 1,24 -----	1-17
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Y	CHEN C ET AL: "DESIGN OF 2,5-DIMETHYL-3-(6-DIMETHYL-4-METHYLPYRIDIN-3-YL)-7-DIPROPYL AMINOPYRAZOLO[1,5-ALPHA]PYRIMIDINE (NBI 30775/R121919) AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF POTENT AND ORALLY ACTIVE CORTICOTROPIN-RELEASING FACTOR RECEPTOR ANTAGONISTS" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 47, no. 19, 9 September 2004 (2004-09-09), pages 4787-4798, XP001206057 ISSN: 0022-2623 figure 1 -----	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/078605

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 11-13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2007/078605

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International application No

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ELECTRONIC PAYMENT RECEIPT

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Title of Invention

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	1040	FILED BY	Jennifer Vail
PATENT CENTER #	69068550	AUTHORIZED BY	Victoria Cheng
CUSTOMER #	21971	FILING DATE	-
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Alexis HOWERTON

Payment Information

PAYMENT METHOD DA / 232415	PAYMENT TRANSACTION ID E202525J20058604	PAYMENT AUTHORIZED BY Jennifer Vail
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07C 13/547, A61K 31/165, 31/22, 31/19	A1	(11) International Publication Number: WO 00/40529 (43) International Publication Date: 13 July 2000 (13.07.00)
(21) International Application Number: PCT/GB99/04436 (22) International Filing Date: 24 December 1999 (24.12.99) (30) Priority Data: 9900334.5 7 January 1999 (07.01.99) GB (71) Applicant (for all designated States except US): ANGIOGENE PHARMACEUTICALS LTD. [GB/GB]; Aston Rowant, Watlington, Oxfordshire OX9 5SX (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): DAVIS, Peter, David [GB/GB]; Aston Rowant, Watlington, Oxfordshire OX9 5SX (GB). ARNOULD, Jean-Claude [FR/FR]; Z.I. la Pompelle, BP 1050, F-51689 Reims Cedex 2 (FR). BOYLE, Francis, Thomas [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). (74) Agent: BILL, Kevin; Global Intellectual Property, Patents, Astrazeneca PLC, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS		
<div style="text-align: center;"> </div> <div style="text-align: right;">(I)</div>		
(57) Abstract <p>The invention relates to the use of compounds of formula (I): wherein X is -C(O)-, -C(S)-, -C=NOH, or -CH(R⁷)- wherein R⁷ is hydrogen, hydroxy, C₁₋₇alkoxy, -OR⁸ or -NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O, -C(O)NR¹¹-, -SO₂- or -SO₂NR¹²- (wherein R¹¹ and R¹², which may be the same or different, each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is as defined herein, and R⁹ includes hydrogen; R¹, R² and R³ are as defined herein and are preferably methyl; R⁴, R⁵ and R⁶ are as defined herein with the proviso that R⁵ is not hydroxy, alkoxy, substituted alkoxy, -OPO₃H₂, -O-C₁₋₇alkanoyl or benzyloxy; and salts thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans. The present invention further relates to compounds of the formula (I), pharmaceutical compositions containing them, processes for their preparation and to a method of treatment using the compounds to produce a vascular damaging effect in a warm-blooded animal such as a human. The compounds of formula (I) and the pharmaceutically acceptable salts thereof may be useful in the treatment of a number of disease states including cancer and rheumatoid arthritis.</p>		

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COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

The present invention relates to vascular damaging agents, in particular to the use of compounds of the invention in the manufacture of medicaments for use in the production of
5 antiangiogenic effects in warm-blooded animals such as humans, to processes for the preparation of such compounds, to pharmaceutical compositions containing such compounds as active ingredient, to methods for the treatment of disease states associated with angiogenesis and to the use of such compounds as medicaments.

Normal angiogenesis plays an important role in a variety of processes including
10 embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Formation of new vasculature by angiogenesis is a key
15 pathological feature of several diseases (J. Folkman, New England Journal of Medicine 333, 1757-1763 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of
20 rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy.

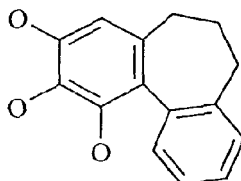
Reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect. The present invention is based on the discovery of tricyclic compounds that surprisingly specifically damage newly formed
25 vasculature without affecting the normal, established vascular endothelium of the host species, a property of value in the treatment of disease states associated with angiogenesis such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal
30 vessel proliferation.

Compounds of the present invention are colchinel derivatives. Colchinel derivatives for example *N*-acetyl-colchinel are known. Anti-tumour effects have been noted on animal

models (see for example - Jnl. Natl. Cancer Inst. 1952, 13, 379-392). However, the effect studied was that of gross damage (haemorrhage, softening and necrosis) and there is no suggestion of treatment of inappropriate angiogenesis by destruction of neovasculature.

A search of Chemical Abstracts (post 1955) based on the substructure:

5



revealed a number of colchicinol related structures. To the extent that any of these compounds have been studied for anti-cancer activity it is because tubulin-binding agents like colchicinol might be expected to be anti-mitotic and therefore to have a direct effect on tumour cells. Some compounds which bind tubulin have been shown to have anti-vascular effects when given at their maximum tolerated dose (MTD) (S.A. Hill et al. Eur. J Cancer, 29A, 1320-1324 (1993)) but other tubulin-binding agents have no vascular-damaging activity even when administered at the MTD, for example docetaxel (Lancet, 1994, 344, 1267-1271). Based on this information and in the course of the work on the present invention, the issue of the relevance of tubulin-binding properties to possible effectiveness as anti-vascular agent was studied but no predictability was found. No correlation between the potency of tubulin interaction and effectiveness as an anti-vascular agent is apparent. Certain compounds structurally related to those of the present invention but not of the present invention, have been found to have a therapeutic window (ratio of MTD to minimum effective dose (MED)) too small for potential clinical effectiveness.

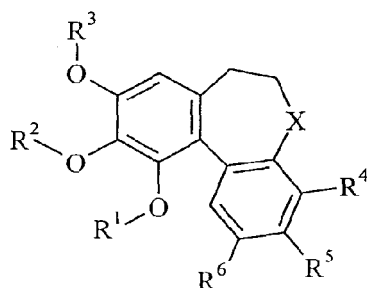
The presence of tubulin-binding properties is then not predictive for antivascular activity. Compounds which have strong tubulin-binding activity give rise to antimitotic effects *in vivo*. The effects of this are most noticeable on proliferating tissue and give rise to undesirable effects, for example on the proliferative tissue of the gut and bone marrow. Compounds which have vascular damaging activity but weak tubulin-binding activity would therefore be useful in the treatment of diseases involving angiogenesis.

It is believed, though this is not limiting on the invention, that the use of compounds of the invention damages newly-formed vasculature, for example the vasculature of tumours,

thus effectively reversing the process of angiogenesis as compared to known anti-angiogenic agents which tend to be less effective once the vasculature has formed.

According to one aspect of the present invention there is provided the use of a compound of the formula I:

5



(I)

wherein

X is

- 10 -C(O)-, -C(S)-, -C=NOH, or -CH(R⁷)- wherein R⁷ is hydrogen, hydroxy, C₁₋₇alkoxy, -OR⁸ or -NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR¹¹-, -SO₂- or -SO₂NR¹²- (wherein R¹¹ and R¹², which may be the same or different, each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is selected from one of the following nine groups:

- 15 1) hydrogen, C₁₋₇alkyl, C₃₋₇cycloalkyl, C₁₋₄alkylY⁸C₁₋₄alkyl wherein Y⁸ is as defined hereinafter, or phenyl,

(which alkyl, cycloalkyl, alkylY⁸alkyl or phenyl group may bear one or more substituents selected from:

- 20 halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, carboxy, carbamoyl, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, phenyl, nitro, sulphate, phosphate,

Z¹ (wherein Z¹ represents a 5-6 membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

- 25 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and Z² (wherein Z² is a 5-6-membered saturated

- 4 -

heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

- 5 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)),
- C₁₋₄alkylZ¹ (wherein Z¹ is as defined hereinbefore), and
- a group -Y²R¹³ (wherein Y² is -NR¹⁴C(O)- or -O-C(O)- (wherein R¹⁴ represents
- hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹³ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a
- 10 group R¹⁵ wherein R¹⁵ is a phenyl group or a 5-10-membered aromatic heterocyclic
- group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently
- from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more
- substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy,
- 15 carboxy, cyano, -CONR¹⁶R¹⁷ and -NR¹⁸COR¹⁹ (wherein R¹⁶, R¹⁷, R¹⁸ and R¹⁹, which
- may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))));
- 2) R¹⁵ wherein R¹⁵ is as defined hereinbefore;
- 3) C₂₋₇alkenylR¹⁵ (wherein R¹⁵ is as defined hereinbefore);
- 20 4) C₃₋₇alkynylR¹⁵ (wherein R¹⁵ is as defined hereinbefore));
- 5) Z¹ (wherein Z¹ is as defined hereinbefore);
- 6) C₁₋₇alkylZ¹ (wherein Z¹ is as defined hereinbefore);
- 7) C₁₋₇alkylY⁸Z¹ (wherein Z¹ is as defined hereinbefore and Y⁸ is -C(O)-, -NR⁵⁹C(O)-, -NR⁵⁹C(O)C₁₋₄alkyl-, -C(O)NR⁶⁰- or -C(O)NR⁶⁰C₁₋₄alkyl-, (wherein R⁵⁹ and R⁶⁰, which may be
- 25 the same or different, each represents hydrogen, C₁₋₃alkyl, C₁₋₃hydroxyalkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- 8) (C₁₋₇alkyl)_cY⁹Z³ (wherein c is 0 or 1, Z³ is an amino acid group and Y⁹ is a direct bond, -C(O)- or -NR⁶¹- (wherein R⁶¹ is hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)); and
- 9) C₁₋₇alkylR¹⁵ (wherein R¹⁵ is as defined hereinbefore);
- 30 and R⁹ is hydrogen, C₁₋₇alkyl or C₃₋₇cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C₁₋₄alkoxy and phenyl);

- R¹, R² and R³ are each independently hydrogen, PO₃H₂, sulphate, C₃₋₇cycloalkyl, C₂₋₇alkenyl, C₂₋₇alkynyl, C₁₋₇alkanoyl, a group R²⁰C₁₋₇alkyl (wherein R²⁰ is phenyl which may bear one or more substituents selected from C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl and C₁₋₄hydroxyalkoxy), C₁₋₇alkyl or C₁₋₇alkylsulphonyl (which alkyl or alkylsulphonyl group may bear one or more substituents selected from: halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y²R²¹ (wherein Y² is -NR²²C(O)- or -O-C(O)- (wherein R²² represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²¹ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R²³ wherein R²³ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR²⁴R²⁵ and -NR²⁶COR²⁷ (wherein R²⁴, R²⁵, R²⁶ and R²⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)));
- with the proviso that at least two of R¹, R² and R³ are C₁₋₇alkyl;

- R⁴, R⁵ and R⁶ are each independently selected from: hydrogen, -OPO₃H₂, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C₁₋₇alkoxy, C₁₋₇alkanoyl, C₁₋₇thioalkoxy, C₁₋₇alkyl, (which alkyl group may bear one or more substituents selected from: halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, sulphate, phosphate and a group -Y³R²⁸ (wherein Y³ is -NR²⁹C(O)- or -O-C(O)- (wherein R²⁹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²⁸ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R³⁰ wherein R³⁰ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR²⁴R²⁵ and -NR²⁶COR²⁷ (wherein R²⁴, R²⁵, R²⁶ and R²⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)));

- ₄hydroxyalkoxy, carboxy, cyano, -CONR³¹R³² and -NR³¹COR³² (wherein R³¹, R³², R³³ and R³⁴, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))), and
- a group -Y⁴R³⁵
- 5 (wherein Y⁴ is -C(O)-, -OC(O)-, -O-, -SO-, -SO₂-, -OSO₂-, -NR³⁶-, -C₁₋₄alkylNR³⁶-, -C₁₋₄alkylC(O)-, -NR³⁷C(O)-, -OC(O)O-, -C(O)NR³⁸- or -NR³⁹C(O)O- (wherein R³⁶, R³⁷, R³⁸ and R³⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and
- R³⁵ is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate,
- 10 hydroxy, amino, C₁₋₇alkyl, C₁₋₇alkoxy, C₁₋₇alkanoyl, C₁₋₇alkylamino, di(C₁₋₇alkyl)amino, aminoC₁₋₇alkylamino, C₁₋₇alkylaminoC₁₋₇alkylamino, C₁₋₇alkanoylaminoC₁₋₇alkyl, di(C₁₋₇alkyl)aminoC₁₋₇alkylamino, C₁₋₇alkylphosphate, C₁₋₇alkylphosphonate, C₁₋₇alkylcarbamoylC₁₋₇alkyl,
- (which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino,
- 15 alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:
- halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y⁵R⁴⁰ (wherein Y⁵
- 20 is -NR⁴¹C(O)-, -C(O)NR⁴²-, -C(O)-O- or -O-C(O)- (wherein R⁴¹ and R⁴² which may be the same or different each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R⁴⁰ is C₁₋₇alkyl, C₃₋₇cycloalkyl, carboxyC₁₋₇alkyl or a group R⁴³ wherein R⁴³ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via
- 25 carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR⁴⁴R⁴⁵ and -NR⁴⁶COR⁴⁷ (wherein R⁴⁴, R⁴⁵, R⁴⁶ and R⁴⁷, which
- 30 may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))),

R⁴⁸ (wherein R⁴⁸ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

- 5 hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, di(C₁₋₄hydroxyalkyl)aminoC₁₋₄alkyl, di(C₁₋₄aminoalkyl)aminoC₁₋₄alkyl, C₁₋₄hydroxyalkoxy, carboxy, C₁₋₄carboxyalkyl, phenyl, cyano, -CONR⁴⁹R⁵⁰, -NR⁵¹COR⁵² (wherein R⁴⁹, R⁵⁰, R⁵¹ and R⁵², which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and C₁₋₄alkylR⁵³ (wherein R⁵³ is as defined hereinafter),
- 10 C₁₋₇alkylR⁴⁸ (wherein R⁴⁸ is as defined hereinbefore),
R⁵³ (wherein R⁵³ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from
- 15 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄carboxyalkyl, C₁₋₄aminoalkyl, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and R⁵⁴ (wherein R⁵⁴ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from
- 20 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)), or
(CH₂)_aY⁶(CH₂)_bR⁵³ (wherein R⁵³ is as defined hereinbefore, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y⁶ represents a direct bond, -O-, -C(O)-, -NR⁵⁵-, -NR⁵⁶C(O)- or -C(O)NR⁵⁷- (wherein R⁵⁵, R⁵⁶, and R⁵⁷, which may be the same or different, each represents
- 25 hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl), and wherein one or more of the (CH₂)_a or (CH₂)_b groups may bear one or more substituents selected from hydroxy, amino and halogeno));

with the proviso that R⁵ is not hydroxy, alkoxy, substituted alkoxy (wherein R⁵ is Y⁴R³⁵ and Y⁴ is -O- and R³⁵ is C₁₋₇alkyl bearing one or more substituents selected from the list given

- 30 hereinbefore), -OPO₃H₂, -O-C₁₋₇alkanoyl or benzyloxy;

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.

According to a further aspect of the present invention there is provided the use of a
 5 compound of the formula I as defined hereinbefore and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof in the manufacture of a medicament for use in the production of a vascular damaging effect at less than the maximum tolerated dose in warm-blooded animals such as humans.

Conveniently X is -C(O)-, -C(S)- or -CH(R⁷)- wherein R⁷ is hydrogen, hydroxy, -OR⁸ or -
 10 NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR¹¹-, -SO₂- or -SO₂NR¹²- (wherein R¹¹ and R¹², which may be the same or different, each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is selected from one of the following seven groups:

1) hydrogen, C₁₋₇alkyl, C₃₋₇cycloalkyl, C₁₋₄alkylY⁸C₁₋₄alkyl wherein Y⁸ is as defined
 15 hereinafter, or phenyl,

(which alkyl, cycloalkyl, alkylY⁸alkyl or phenyl group may bear one or more substituents selected from:

halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, carboxy, carbamoyl, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, phenyl, nitro, sulphate, phosphate,
 20

Z¹ (wherein Z¹ represents a 5-6 membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and Z² (wherein Z² is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1
 25 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)),
 30

- $C_{1-4}alkylZ^1$ (wherein Z^1 is as defined hereinbefore), and
 a group $-Y^2R^{13}$ (wherein Y^2 is $-NR^{14}C(O)-$ or $-O-C(O)-$ (wherein R^{14} represents
 hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and R^{13} is $C_{1-7}alkyl$, $C_{3-7}cycloalkyl$ or a
 group R^{15} wherein R^{15} is a 5-10-membered aromatic heterocyclic group (linked via
 5 carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S,
 aromatic heterocyclic group may bear one or more substituents selected from hydroxy,
 nitro, halogeno, amino, $C_{1-4}alkyl$, $C_{1-4}haloalkyl$, $C_{1-4}alkoxy$, $C_{1-4}hydroxyalkyl$, C_{1-4}
 aminoalkyl, $C_{1-4}alkylamino$, $C_{1-4}hydroxyalkoxy$, carboxy, cyano, $-CONR^{16}R^{17}$ and $-$
 $NR^{18}COR^{19}$ (wherein R^{16} , R^{17} , R^{18} and R^{19} , which may be the same or different, each
 10 represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$));
- 2) R^{15} wherein R^{15} is as defined hereinbefore;
- 3) Z^1 (wherein Z^1 is as defined hereinbefore);
- 4) $C_{1-7}alkylZ^1$ (wherein Z^1 is as defined hereinbefore);
- 5) $C_{1-7}alkylY^8Z^1$ (wherein Z^1 is as defined hereinbefore and Y^8 is $-C(O)-$, $-NR^{59}C(O)-$, $-$
 15 $NR^{59}C(O)C_{1-4}alkyl-$, $-C(O)NR^{60}-$ or $-C(O)NR^{60}C_{1-4}alkyl-$, (wherein R^{59} and R^{60} , which may be
 the same or different, each represents hydrogen, $C_{1-3}alkyl$, $C_{1-3}hydroxyalkyl$ or $C_{1-3}alkoxyC_{2-3}$
 $alkyl$));
- 6) $(C_{1-7}alkyl)_cY^9Z^3$ (wherein c is 0 or 1, Z^3 is an amino acid group and Y^9 is a direct bond, $-$
 $C(O)-$ or $-NR^{61}-$ (wherein R^{61} is hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$)); and
- 20 7) $C_{1-7}alkylR^{15}$ (wherein R^{15} is as defined hereinbefore));
- and R^9 is hydrogen, $C_{1-7}alkyl$ or $C_{3-7}cycloalkyl$, which alkyl or cycloalkyl group may bear one
 or more substituents selected from $C_{1-4}alkoxy$ and phenyl).
- Advantageously X is $-CH(R^7)-$ wherein R^7 is $-OR^8$ or $-NR^8R^9$ (wherein R^8 is a group $-Y^1R^{10}$
 (wherein Y^1 is $-C(O)-$, $-C(O)O-$ or $-C(O)NR^{11}-$ (wherein R^{11} represents hydrogen, $C_{1-3}alkyl$ or
 25 $C_{1-3}alkoxyC_{2-3}alkyl$) and R^{10} is as defined hereinbefore) and R^9 is as defined hereinbefore).
 Preferably X is $-CH(R^7)-$ wherein R^7 is $-OR^8$ or $-NR^8R^9$ (wherein R^8 is a group $-Y^1R^{10}$
 (wherein Y^1 is $-C(O)-$ or $-C(O)O-$ and R^{10} is as defined hereinbefore) and R^9 is as defined
 hereinbefore).
- In one embodiment of the present invention preferably X is $-C(O)-$, $-CH_2-$, $-CH(OH)-$ or $-$
 30 $CH(NHC(O)CH_3)-$.
- In one embodiment of the present invention more preferably X is $-CH(NHC(O)CH_3)-$.
- Conveniently R^1 , R^2 and R^3 are each independently

hydrogen, PO_3H_2 , sulphate, C_{1-7} alkyl, C_{3-7} cycloalkyl, C_{2-7} alkenyl, C_{2-7} alkynyl, C_{1-7} alkanoyl, C_{1-7} alkylsulphonyl or a group $\text{R}^{20}\text{C}_{1-7}$ alkyl (wherein R^{20} is phenyl which may bear one or more substituents selected from C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} aminoalkyl and C_{1-4} hydroxyalkoxy); with the proviso that at least two of R^1 , R^2 and R^3 are C_{1-7} alkyl.

5 Preferably R^1 , R^2 and R^3 are each independently C_{1-4} alkyl.

More preferably R^1 , R^2 and R^3 are each methyl.

Conveniently R^4 is

hydrogen, cyano, halogeno, nitro, amino, hydroxy, C_{1-7} alkoxy, C_{1-7} thioalkoxy, C_{1-7} alkanoyl or C_{1-7} alkyl,

- 10 (which alkyl group may bear one or more substituents selected from halogeno, amino, C_{1-4} alkylamino, $\text{di}(\text{C}_{1-4}$ alkyl)amino, hydroxy, C_{1-4} alkoxy, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-\text{Y}^3\text{R}^{28}$ (wherein Y^3 is $-\text{NR}^{29}\text{C}(\text{O})-$ or $-\text{O}-\text{C}(\text{O})-$ (wherein R^{29} represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{28} is C_{1-7} alkyl, C_{3-7} cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, $-\text{CONR}^{31}\text{R}^{32}$ and $-\text{NR}^{31}\text{COR}^{32}$ (wherein R^{31} , R^{32} , R^{33} and R^{34} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl))).
- 15 20

Preferably R^4 is hydrogen, hydroxy, halogeno, cyano, amino or C_{1-7} alkanoyl.

More preferably R^4 is hydrogen.

25 Conveniently R^5 and R^6 are each independently selected from:

hydrogen, $-\text{OPO}_3\text{H}_2$, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C_{1-7} alkoxy, C_{1-7} alkanoyl, C_{1-7} thioalkoxy, C_{1-7} alkyl,

(which alkyl group may bear one or more substituents selected from:

- halogeno, amino, C_{1-4} alkylamino, $\text{di}(\text{C}_{1-4}$ alkyl)amino, hydroxy, C_{1-4} alkoxy, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy, phenyl, sulphate, phosphate and a group $-\text{Y}^3\text{R}^{28}$ (wherein Y^3 is $-\text{NR}^{29}\text{C}(\text{O})-$ or $-\text{O}-\text{C}(\text{O})-$ (wherein R^{29} represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{28} is C_{1-7} alkyl, C_{3-7} cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, $-\text{CONR}^{31}\text{R}^{32}$ and $-\text{NR}^{31}\text{COR}^{32}$ (wherein R^{31} , R^{32} , R^{33} and R^{34} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl))).
- 30

- 11 -

- cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, $-\text{CONR}^{31}\text{R}^{32}$ and $-\text{NR}^{31}\text{COR}^{32}$ (wherein R^{31} , R^{32} , R^{33} and R^{34} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl)), and
- a group $-\text{Y}^4\text{R}^{35}$
- (wherein Y^4 is $-\text{C}(\text{O})-$, $-\text{OC}(\text{O})-$, $-\text{O}-$, $-\text{SO}-$, $-\text{SO}_2-$, $-\text{OSO}_2-$, $-\text{NR}^{36}-$, $-\text{C}_{1-4}\text{alkylNR}^{36}-$, $-\text{C}_{1-4}\text{alkylC}(\text{O})-$, $-\text{NR}^{37}\text{C}(\text{O})-$, $-\text{OC}(\text{O})\text{O}-$, $-\text{C}(\text{O})\text{NR}^{38}-$ or $-\text{NR}^{39}\text{C}(\text{O})\text{O}-$ (wherein R^{36} , R^{37} , R^{38} and R^{39} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and
- R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, hydroxy, amino, C_{1-7} alkyl, C_{1-7} alkoxy, C_{1-7} alkanoyl, C_{1-7} alkylamino, $\text{di}(C_{1-7}\text{alkyl})\text{amino}$, $\text{aminoC}_{1-7}\text{alkylamino}$, $C_{1-7}\text{alkylaminoC}_{1-7}\text{alkylamino}$, $C_{1-7}\text{alkanoylaminoC}_{1-7}\text{alkyl}$, $\text{di}(C_{1-7}\text{alkyl})\text{aminoC}_{1-7}\text{alkylamino}$, $C_{1-7}\text{alkylphosphate}$, $C_{1-7}\text{alkylphosphonate}$, $C_{1-7}\text{alkylcarbamoylC}_{1-7}\text{alkyl}$,
- (which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino, alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:
- halogeno, amino, C_{1-4} alkylamino, $\text{di}(C_{1-4}\text{alkyl})\text{amino}$, hydroxy, C_{1-4} hydroxyalkyl, C_{1-4} alkoxy, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-\text{Y}^5\text{R}^{40}$ (wherein Y^5 is $-\text{NR}^{41}\text{C}(\text{O})-$, $-\text{C}(\text{O})\text{NR}^{42}-$, $-\text{C}(\text{O})-\text{O}-$ or $-\text{O}-\text{C}(\text{O})-$ (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{40} is C_{1-7} alkyl, C_{3-7} cycloalkyl, carboxy C_{1-7} alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4}

₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR⁴⁴R⁴⁵ and -NR⁴⁶COR⁴⁷ (wherein R⁴⁴, R⁴⁵, R⁴⁶ and R⁴⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))),

- 5 R⁴⁸ (wherein R⁴⁸ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, di(C₁₋₄hydroxyalkyl)aminoC₁₋₄alkyl, di(C₁₋₄aminoalkyl)aminoC₁₋₄alkyl, C₁₋₄hydroxyalkoxy, carboxy, C₁₋₄carboxyalkyl, phenyl, cyano, -CONR⁴⁹R⁵⁰, -NR⁵¹COR⁵² (wherein R⁴⁹, R⁵⁰, R⁵¹ and R⁵², which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and C₁₋₄alkylR⁵³ (wherein R⁵³ is as defined hereinafter),

- 15 C₁₋₇alkylR⁴⁸ (wherein R⁴⁸ is as defined hereinbefore),
R⁵³ (wherein R⁵³ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

20 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄carboxyalkyl, C₁₋₄aminoalkyl, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and R⁵⁴ (wherein R⁵⁴ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

25 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)), or

- (CH₂)_aY⁶(CH₂)_bR⁵³ (wherein R⁵³ is as defined hereinbefore, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y⁶ represents a direct bond, -O-, -C(O)-, -NR⁵⁵-, -NR⁵⁶C(O)- or -C(O)NR⁵⁷- (wherein R⁵⁵, R⁵⁶, and R⁵⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl), and wherein one or more of the (CH₂)_a or (CH₂)_b groups may bear one or more substituents selected from hydroxy, amino and halogeno));
- 30

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with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy (wherein R^5 is Y^4R^{35} and Y^4 is -O- and R^{35} is C_{1-7} alkyl bearing one or more substituents selected from the list given hereinbefore), - OPO_3H_2 , - $O-C_{1-7}$ alkanoyl or benzyloxy.

In another embodiment of the present invention conveniently R^5 and R^6 are each

5 independently selected from:

hydrogen, - OPO_3H_2 , cyano, halogeno, nitro, amino, carboxy, hydroxy, C_{1-7} alkoxy, C_{1-7} alkanoyl, C_{1-7} thioalkoxy, C_{1-7} alkyl,

(which alkyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-4} alkoxy, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy,

10 phenyl, sulphate, phosphate and a group - Y^3R^{28} (wherein Y^3 is - $NR^{29}C(O)-$ or - $O-C(O)-$ (wherein R^{29} represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{28} is C_{1-7} alkyl, C_{3-7} cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected

15 independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, - $CONR^{31}R^{32}$ and - $NR^{31}COR^{32}$ (wherein R^{31} , R^{32} , R^{33} and R^{34} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl))), and

20 a group - Y^4R^{35}

(wherein Y^4 is - $C(O)-$, - $OC(O)-$, -O-, -SO-, - SO_2- , - OSO_2- , - $NR^{36}-$, - $NR^{37}C(O)-$, - $OC(O)O-$, - $C(O)NR^{38}-$ or - $NR^{39}C(O)O-$ (wherein R^{36} , R^{37} , R^{38} and R^{39} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{35} is

25 a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, C_{1-7} alkyl, C_{1-7} alkoxy, C_{1-7} alkanoyl, amino C_{1-7} alkylamino, C_{1-7} alkylamino C_{1-7} alkylamino, di(C_{1-7} alkyl)amino C_{1-7} alkylamino, C_{1-7} alkylphosphate

(which alkyl, alkoxy, alkanoyl, aminoalkylamino, alkylaminoalkylamino, dialkylaminoalkylamino, or alkylphosphate may bear one or more substituents

30 selected from:

halogeno, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-4} alkoxy, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy,

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- phenyl, nitro, sulphate, phosphate and a group $-Y^5R^{40}$ (wherein Y^5 is $-NR^{41}C(O)-$, $-C(O)NR^{42}-$, $-C(O)-O-$ or $-O-C(O)-$ (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{40} is C_{1-7} alkyl, C_{3-7} cycloalkyl, carboxy C_{1-7} alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, $-CONR^{44}R^{45}$ and $-NR^{46}COR^{47}$ (wherein R^{44} , R^{45} , R^{46} and R^{47} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl))), R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, phenyl, cyano, $-CONR^{49}R^{50}$ and $-NR^{51}COR^{52}$ (wherein R^{49} , R^{50} , R^{51} and R^{52} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl)), or R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4} alkyl, C_{1-4} hydroxyalkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-4} alkyl, C_{1-4} alkylsulphonyl C_{1-4} alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4} alkyl, C_{1-4} hydroxyalkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-4} alkyl and C_{1-4} alkylsulphonyl C_{1-4} alkyl))));
- with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy, $-OPO_3H_2$, $-O-C_{1-7}$ alkanoyl or benzyloxy.
- Preferably R^6 is hydrogen, halogeno, amino, carboxy, hydroxy, C_{1-7} alkoxy or a group Y^4R^{35}

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(wherein Y^4 is $-C(O)-$, $-O-$ or $-OSO_2-$ and R^{35} is C_{1-7} alkyl, C_{1-7} alkoxy (which alkyl or alkoxy may bear one or more substituents selected from halogeno), R^{48} (wherein R^{48} is a benzyl group) or R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms selected independently from O, S and N)).

5 Particularly R^6 is hydrogen, $C(O)OCH_3$ or methoxy, especially $C(O)OCH_3$ or methoxy.

More preferably R^6 is hydrogen.

Preferably R^5 is hydrogen, halogeno, amino, carboxy, carbamoyl, C_{1-7} alkanoyl, C_{1-7} thioalkoxy, or

a group $-Y^4R^{35}$

10 (wherein Y^4 is $-C(O)-$, $-OC(O)-$, $-O-$, $-SO-$, $-OSO_2-$, $-NR^{36}-$, $-NR^{37}C(O)-$ or $-C(O)NR^{38}-$ (wherein R^{36} , R^{37} and R^{38} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, C_{1-7} alkyl, C_{1-7} alkoxy, C_{1-7} alkanoyl, C_{1-7} alkanoylamino C_{1-7} alkyl,

15 (which alkyl, alkoxy, alkanoyl, alkanoylaminoalkyl may bear one or more substituents selected from:

halogeno, amino, hydroxy, carboxy, and a group $-Y^5R^{40}$ (wherein Y^5 is $-C(O)-O-$ or $-O-C(O)-$ and R^{40} is C_{1-7} alkyl or a group R^{43} wherein R^{43} is a benzyl group),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic

20 heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, fluoro, amino, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, di(C_{1-4} alkyl)amino C_{1-4} alkyl, di(C_{1-4} hydroxyalkyl)amino C_{1-4} alkyl,

25 di(C_{1-4} aminoalkyl)amino C_{1-4} alkyl, C_{1-4} hydroxyalkoxy, carboxy, C_{1-4} carboxyalkyl, cyano, $-CONR^{49}R^{50}$, $-NR^{51}COR^{52}$ (wherein R^{49} , R^{50} , R^{51} and R^{52} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and C_{1-4} alkyl R^{53} (wherein R^{53} is as defined hereinafter),

C_{1-7} alkyl R^{48} (wherein R^{48} is as defined hereinbefore),

30 R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

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- oxo, hydroxy, fluoro, chloro, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄carboxyalkyl, C₁₋₄aminoalkyl, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and R⁵⁴ (wherein R⁵⁴ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N,
- 5 which heterocyclic group may bear 1 or 2 substituents selected from
- oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)), or
- (CH₂)_aY⁶(CH₂)_bR⁵³ (wherein R⁵³ is as defined hereinbefore, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y⁶ represents a direct bond, -O-, -C(O)-, -NR⁵⁵-, -NR⁵⁶C(O)- or -
- 10 C(O)NR⁵⁷- (wherein R⁵⁵, R⁵⁶, and R⁵⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl), and wherein one or more of the (CH₂)_a or (CH₂)_b groups may bear one or more substituents selected from hydroxy, amino and halogeno));

- with the proviso that R⁵ is not alkoxy, substituted alkoxy (wherein R⁵ is Y⁴R³⁵ and Y⁴ is -O- and R³⁵ is C₁₋₇alkyl bearing one or more substituents selected from the list given hereinbefore),
- 15 -O-C₁₋₇alkanoyl or benzyloxy.

Preferably R⁵³ is a group selected from morpholino, piperidinyl and piperazinyl which group may be substituted as hereinbefore defined.

Advantageous values for R⁵ include:

- 20 3-[(2*R*)-2,6-diaminohexanoyl]amino}propanoyloxy (such as in Example 4),
- 3-[(2-aminoacetyl)amino]propanoyloxy (such as in Example 5),
- 2-morpholinoacetylaminomethoxy (such as in Example 38),
- 2-carboxy-3,4,5-trihydroxytetrahydro-2*H*-pyran-6-yloxy (such as in Example 44),
- 4-(4-methylpiperazin-1-ylmethyl)phenylcarbonyloxy (such as in Example 16),
- 25 4-(morpholinomethyl)phenylcarbonyloxy (such as in Example 17),
- 3-(4-methylpiperazin-1-ylcarbonyl)propanoyloxy (such as in Example 40),
- 5-carboxypentanoyloxy (such as in Example 41),
- 3-(4-carboxyphenyl)propanoyloxy (such as in Example 18) and
- (3*R*)-2-amino-3-hydroxypropanoylamino (such as in Example 28).
- 30 Another advantageous value for R⁵ is
- (2*S*)-2-amino-5-[(2-nitroethanimidoyl)amino]pentanoylamino (such as in Example 52).
- Preferred values for R⁵ include

- 3-[[*(2R)*-2,6-diaminohexanoyl]amino]propanoyloxy (such as in Example 4),
 3-[(2-aminoacetyl)amino]propanoyloxy (such as in Example 5),
 4-(4-methylpiperazin-1-ylmethyl)phenylcarbonyloxy (such as in Example 16),
 4-(morpholinomethyl)phenylcarbonyloxy (such as in Example 17),
 5 3-(4-methylpiperazin-1-ylcarbonyl)propanoyloxy (such as in Example 40),
 5-carboxypentanoyloxy (such as in Example 41),
 3-(4-carboxyphenyl)propanoyloxy (such as in Example 18) and
 (3*R*)-2-amino-3-hydroxypropanoylamino (such as in Example 28).

More preferred values for R⁵ include

- 10 4-(4-methylpiperazin-1-ylmethyl)phenylcarbonyloxy (such as in Example 16) and
 (3*R*)-2-amino-3-hydroxypropanoylamino (such as in Example 28).

In another embodiment of the present invention preferred values for R⁵ include alanylamino, *N*-(benzyloxycarbonylalanyl)amino, and 4-(piperidino)piperidin-1-ylcarbonyloxy.

A more preferred value for R⁵ is alanylamino.

- 15 In another embodiment of the present invention particular values of R⁵ include amino, C₁,
 ,alkylamino and diC₁₋₇alkylamino, especially amino.

When R³⁵ is a sugar moiety it can be, for example a monosaccharide such as a glucuronyl, glucosyl or galactosyl group or a di- or trisaccharide.

When R³⁵ is a sugar moiety glucuronyl or a derivative thereof is preferred.

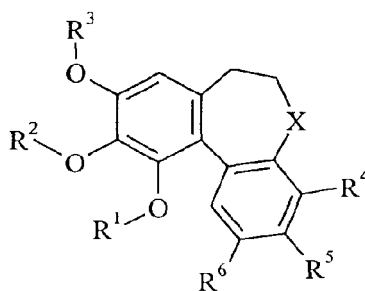
- 20 When R³⁵ is a mono-, di-, tri- or tetra- peptide it is preferably derived from a natural alpha amino acid for example such as glycine, valine, lysine, alanine or serine.

In another embodiment of the present invention R³⁵ is an amino acid group derived from serine, threonine, arginine, glycine, alanine, β-alanine or lysine.

According to another aspect of the present invention there is provided the use of a

- 25 compound of the formula I:

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(I)

wherein

5 X is

-C(O)-, -C(S)-, -C=NOH, or -CH(R⁷)- wherein R⁷ is hydrogen, hydroxy, C₁₋₇alkoxy or -NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR¹¹-, -SO₂- or -SO₂NR¹²- (wherein R¹¹ and R¹², which may be the same or different, each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is selected

10 from one of the following four groups:

1) hydrogen, C₁₋₇alkyl or C₃₋₇cycloalkyl

(which alkyl or cycloalkyl group may bear one or more substituents selected from:

halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, phenyl, nitro, sulphate, phosphate and a group -Y²R¹³ (wherein Y² is -NR¹⁴C(O)- or -O-C(O)- (wherein R¹⁴ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹³ is C₁₋₇alkyl, C₃₋

15 7-cycloalkyl or a group R¹⁵ wherein R¹⁵ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected

independently from O, N and S, which phenyl or aromatic heterocyclic group may bear

20 one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋

4haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋

4hydroxyalkoxy, carboxy, cyano, -CONR¹⁶R¹⁷ and -NR¹⁸COR¹⁹ (wherein R¹⁶, R¹⁷, R¹⁸ and R¹⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋

3alkoxyC₂₋₃alkyl));

25 2) R¹⁵ wherein R¹⁵ is as defined hereinbefore;

3) C₂₋₇alkenylR¹⁵ (wherein R¹⁵ is as defined hereinbefore); and

4) C₃₋₇alkynylR¹⁵ (wherein R¹⁵ is as defined hereinbefore));

and R⁹ is hydrogen, C₁₋₇alkyl or C₃₋₇cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C₁₋₄alkoxy and phenyl);

5 R¹, R² and R³ are each independently

hydrogen, PO₃H₂, sulphate, C₃₋₇cycloalkyl, C₂₋₇alkenyl, C₂₋₇alkynyl, C₁₋₇alkanoyl, a group R²⁰C₁₋₇alkyl (wherein R²⁰ is phenyl which may bear one or more substituents selected from C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl and C₁₋₄hydroxyalkoxy), C₁₋₇alkyl or C₁₋₇alkylsulphonyl (which alkyl or alkylsulphonyl group may bear one or more substituents selected from:

- 10 halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y²R²¹ (wherein Y² is -NR²²C(O)- or -O-C(O)- (wherein R²² represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²¹ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R²³ wherein R²³ is a phenyl group or a 5-10-membered
- 15 aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR²⁴R²⁵ and -NR²⁶COR²⁷ (wherein R²⁴, R²⁵, R²⁶ and
- 20 R²⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)));

with the proviso that at least two of R¹, R² and R³ are C₁₋₇alkyl;

R⁴, R⁵ and R⁶ are each independently selected from:

- 25 hydrogen, -OPO₃H₂, cyano, halogeno, nitro, amino, carboxy, hydroxy, C₁₋₇alkoxy, C₁₋₇alkanoyl, C₁₋₇thioalkoxy, C₁₋₇alkyl, (which alkyl group may bear one or more substituents selected from: halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy,
- 30 phenyl, sulphate, phosphate and a group -Y³R²⁸ (wherein Y³ is -NR²⁹C(O)- or -O-C(O)- (wherein R²⁹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²⁸ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R³⁰ wherein R³⁰ is a phenyl group or a 5-10-membered aromatic

- 20 -

- heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR³¹R³² and -NR³¹COR³² (wherein R³¹, R³², R³³ and R³⁴, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))), and
- 5 a group -Y⁴R³⁵ (wherein Y⁴ is -C(O)-, -OC(O)-, -O-, -SO-, -SO₂-, -OSO₂-, -NR³⁶-, -NR³⁷C(O)-, -OC(O)O-, -C(O)NR³⁸- or -NR³⁹C(O)O- (wherein R³⁶, R³⁷, R³⁸ and R³⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R³⁵ is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, C₁₋₇alkyl, C₁₋₇alkoxy, C₁₋₇alkanoyl, aminoC₁₋₇alkylamino, C₁₋₇alkylaminoC₁₋₇alkylamino, di(C₁₋₇alkyl)aminoC₁₋₇alkylamino, C₁₋₇alkylphosphate
- 10 (which alkyl, alkoxy, alkanoyl, aminoalkylamino, alkylaminoalkylamino, dialkylaminoalkylamino, or alkylphosphate may bear one or more substituents selected from: halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y⁵R⁴⁰ (wherein Y⁵ is -NR⁴¹C(O)-, -C(O)NR⁴²-, -C(O)-O- or -O-C(O)- (wherein R⁴¹ and R⁴² which may be the same or different each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R⁴⁰ is C₁₋₇alkyl, C₃₋₇cycloalkyl, carboxyC₁₋₇alkyl or a group R⁴³ wherein R⁴³ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR⁴⁴R⁴⁵ and -NR⁴⁶COR⁴⁷ (wherein R⁴⁴, R⁴⁵, R⁴⁶ and R⁴⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))),
- 15 R⁴⁸ (wherein R⁴⁸ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected
- 20
- 25
- 30

independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, phenyl, cyano, -

5 CONR⁴⁹R⁵⁰ and -NR⁵¹COR⁵² (wherein R⁴⁹, R⁵⁰, R⁵¹ and R⁵², which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)), or

R⁵³ (wherein R⁵³ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

10 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and R⁵⁴ (wherein R⁵⁴ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋
15 ₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl))));

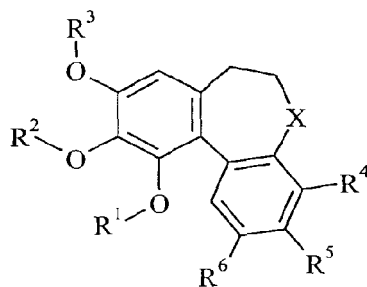
with the proviso that R⁵ is not hydroxy, alkoxy, substituted alkoxy, -OPO₃H₂,

-O-C₁₋₇alkanoyl or benzyloxy;

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof for example esters, amides and sulphides, in the manufacture of a

20 medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.

According to another aspect of the present invention there is provided a compound of the formula IIa:



(IIa)

wherein

X is

-C(O)-, -C(S)-, -C=NOH, or -CH(R⁷)- wherein R⁷ is hydrogen, hydroxy, C₁₋₇alkoxy, -OR⁸ or -NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is a direct bond, -C(O)-, -C(S)-, -S-, -

- 5 C(O)O-, -C(O)NR¹¹-, -SO₂- or -SO₂NR¹²- (wherein R¹¹ and R¹², which may be the same or different, each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is selected from one of the following nine groups:

1) hydrogen, C₁₋₇alkyl, C₃₋₇cycloalkyl, C₁₋₄alkylY⁸C₁₋₄alkyl wherein Y⁸ is as defined hereinafter, or phenyl,

- 10 (which alkyl, cycloalkyl, alkylY⁸alkyl or phenyl group may bear one or more substituents selected from:

halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, carboxy, carbamoyl, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, phenyl, nitro, sulphate, phosphate,

- 15 Z¹ (wherein Z¹ represents a 5-6 membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and Z² (wherein Z² is a 5-6-membered saturated

- 20 heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

25 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)),

C₁₋₄alkylZ¹ (wherein Z¹ is as defined hereinbefore), and

a group -Y²R¹³ (wherein Y² is -NR¹⁴C(O)- or -O-C(O)- (wherein R¹⁴ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹³ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a

- 30 group R¹⁵ wherein R¹⁵ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more

- substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR¹⁶R¹⁷ and -NR¹⁸COR¹⁹ (wherein R¹⁶, R¹⁷, R¹⁸ and R¹⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- 5 2) R¹⁵ wherein R¹⁵ is as defined hereinbefore;
- 3) C₂₋₇alkenylR¹⁵ (wherein R¹⁵ is as defined hereinbefore);
- 4) C₃₋₇alkynylR¹⁵ (wherein R¹⁵ is as defined hereinbefore));
- 5) Z¹ (wherein Z¹ is as defined hereinbefore);
- 10 6) C₁₋₇alkylZ¹ (wherein Z¹ is as defined hereinbefore);
- 7) C₁₋₇alkylY⁸Z¹ (wherein Z¹ is as defined hereinbefore and Y⁸ is -C(O)-, -NR⁵⁹C(O)-, -NR⁵⁹C(O)C₁₋₄alkyl-, -C(O)NR⁶⁰- or -C(O)NR⁶⁰C₁₋₄alkyl-, (wherein R⁵⁹ and R⁶⁰, which may be the same or different, each represents hydrogen, C₁₋₃alkyl, C₁₋₃hydroxyalkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- 15 8) (C₁₋₇alkyl)_cY⁹Z³ (wherein c is 0 or 1, Z³ is an amino acid group and Y⁹ is a direct bond, -C(O)- or -NR⁶¹- (wherein R⁶¹ is hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)); and
- 9) C₁₋₇alkylR¹⁵ (wherein R¹⁵ is as defined hereinbefore);
- and R⁹ is hydrogen, C₁₋₇alkyl or C₃₋₇cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C₁₋₄alkoxy and phenyl);
- 20 R¹, R² and R³ are each independently
- hydrogen, PO₃H₂, sulphate, C₃₋₇cycloalkyl, C₂₋₇alkenyl, C₂₋₇alkynyl, C₁₋₇alkanoyl, a group R²⁰C₁₋₇alkyl (wherein R²⁰ is phenyl which may bear one or more substituents selected from C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl and C₁₋₄hydroxyalkoxy), C₁₋₇alkyl or C₁₋₇alkylsulphonyl (which alkyl or alkylsulphonyl group may bear one or more substituents selected from:
- 25 halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y²R²¹ (wherein Y² is -NR²²C(O)- or -O-C(O)- (wherein R²² represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²¹ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R²³ wherein R²³ is a phenyl group or a 5-10-membered
- 30 aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋

₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR²⁴R²⁵ and -NR²⁶COR²⁷ (wherein R²⁴, R²⁵, R²⁶ and R²⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl));

5 with the proviso that at least two of R¹, R² and R³ are C₁₋₇alkyl;

R⁴ is

hydrogen, cyano, halogeno, nitro, amino, hydroxy, C₁₋₇alkoxy, C₁₋₇thioalkoxy, C₁₋₇alkanoyl or C₁₋₇alkyl,

(which alkyl group may bear one or more substituents selected from:

- 10 halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y³R²⁸ (wherein Y³ is -NR²⁹C(O)- or -O-C(O)- (wherein R²⁹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²⁸ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R³⁰ wherein R³⁰ is a phenyl group or a 5-10-membered
- 15 aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR³¹R³² and -NR³¹COR³² (wherein R³¹, R³², R³³ and
- 20 R³⁴, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl));

R⁵ and R⁶ are each independently selected from

hydrogen, -OPO₃H₂, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C₁₋₇alkoxy, C₁₋₇alkanoyl, C₁₋₇thioalkoxy, C₁₋₇alkyl,

- 25 (which alkyl group may bear one or more substituents selected from: halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, sulphate, phosphate and a group -Y³R²⁸ (wherein Y³ is -NR²⁹C(O)- or -O-C(O)- (wherein R²⁹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²⁸ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R³⁰ wherein R³⁰ is a phenyl group or a 5-10-membered aromatic
- 30 heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear

- one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR³¹R³² and -NR³¹COR³² (wherein R³¹, R³², R³³ and R³⁴, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))), and
- 5 a group -Y⁴R³⁵
- (wherein Y⁴ is -C(O)-, -OC(O)-, -O-, -SO-, -SO₂-, -OSO₂-, -NR³⁶-, -C₁₋₄alkylNR³⁶-, -C₁₋₄alkylC(O)-, -NR³⁷C(O)-, -OC(O)O-, -C(O)NR³⁸- or -NR³⁹C(O)O- (wherein R³⁶, R³⁷, R³⁸ and R³⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and
- 10 R³⁵ is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, hydroxy, amino, C₁₋₇alkyl, C₁₋₇alkoxy, C₁₋₇alkanoyl, C₁₋₇alkylamino, di(C₁₋₇alkyl)amino, aminoC₁₋₇alkylamino, C₁₋₇alkylaminoC₁₋₇alkylamino, C₁₋₇alkanoylaminoC₁₋₇alkyl, di(C₁₋₇alkyl)aminoC₁₋₇alkylamino, C₁₋₇alkylphosphate, C₁₋₇alkylphosphonate, C₁₋₇alkylcarbamoylC₁₋₇alkyl,
- 15 (which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino, alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:
- 20 halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y⁵R⁴⁰ (wherein Y⁵ is -NR⁴¹C(O)-, -C(O)NR⁴²-, -C(O)-O- or -O-C(O)- (wherein R⁴¹ and R⁴² which may be the same or different each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and
- 25 R⁴⁰ is C₁₋₇alkyl, C₃₋₇cycloalkyl, carboxyC₁₋₇alkyl or a group R⁴³ wherein R⁴³ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy,
- 30 carboxy, cyano, -CONR⁴⁴R⁴⁵ and -NR⁴⁶COR⁴⁷ (wherein R⁴⁴, R⁴⁵, R⁴⁶ and R⁴⁷, which

may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))),

R⁴⁸ (wherein R⁴⁸ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected

5 independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, di(C₁₋₄hydroxyalkyl)aminoC₁₋₄alkyl, di(C₁₋₄aminoalkyl)aminoC₁₋₄alkyl, C₁₋₄hydroxyalkoxy, 10 carboxy, C₁₋₄carboxyalkyl, phenyl, cyano, -CONR⁴⁹R⁵⁰, -NR⁵¹COR⁵² (wherein R⁴⁹, R⁵⁰, R⁵¹ and R⁵², which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and C₁₋₄alkylR⁵³ (wherein R⁵³ is as defined hereinafter),

C₁₋₇alkylR⁴⁸ (wherein R⁴⁸ is as defined hereinbefore),

R⁵³ (wherein R⁵³ is a 5-6-membered saturated heterocyclic group (linked via carbon or 15 nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄carboxyalkyl, C₁₋₄aminoalkyl, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and R⁵⁴ (wherein R⁵⁴ is a 5-6-membered saturated heterocyclic group (linked via 20 carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)), or

(CH₂)_aY⁶(CH₂)_bR⁵³ (wherein R⁵³ is as defined hereinbefore, a is 0, or an integer 1-4, b is 0

25 or an integer 1-4 and Y⁶ represents a direct bond, -O-, -C(O)-, -NR⁵⁵-, -NR⁵⁶C(O)- or -C(O)NR⁵⁷- (wherein R⁵⁵, R⁵⁶, and R⁵⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl), and wherein one or more of the (CH₂)_a or (CH₂)_b groups may bear one or more substituents selected from hydroxy, amino and halogeno));

30 with the proviso that R⁵ is not hydroxy, alkoxy, substituted alkoxy (wherein R⁵ is Y⁴R³⁵ and Y⁴ is -O- and R³⁵ is C₁₋₇alkyl bearing one or more substituents selected from the list given hereinbefore), -OPO₃H₂, -O-C₁₋₇alkanoyl or benzyloxy;

with the further proviso that at least one of R^5 or R^6 is a group $-Y^4R^{35}$ (wherein Y^4 and R^{35} are as defined hereinbefore) but with the further provisos

that when R^5 is $-Y^4R^{35}$ and R^6 is hydrogen, hydroxy, methoxy or methoxycarbonyl, $-Y^4R^{35}$ is not selected from cases wherein:

- 5 Y^4 is $-C(O)-$, $-OC(O)-$, $-O-$, $-SO-$, $-OSO_2-$, $-NR^{36}-$, $-NR^{37}C(O)-$ or $-C(O)NR^{38}-$ (wherein R^{36} , R^{37} and R^{38} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{35} is a glycine, valine or lysine group, a dipeptide of glycine and valine groups, C_{1-7} alkyl, C_{1-7} alkoxy, C_{1-7} alkanoyl,

- 10 (which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno, hydroxy, and a group $-Y^5R^{40}$ (wherein Y^5 is $-O-C(O)-$ and R^{40} is C_{1-7} alkyl)), or

- R^{48} (wherein R^{48} is a tetrazolyl group (which may or may not be substituted as hereinbefore defined), a phenyl group or a benzyl group which phenyl or benzyl group may bear one or more substituents selected from C_{1-4} alkyl); and
- 15 that when R^6 is $-Y^4R^{35}$ and R^5 is hydrogen, hydroxy, methoxy or methoxycarbonyl, $-Y^4R^{35}$ is not selected from cases wherein:

Y^4 is $-C(O)-$, $-O-$ or $-OSO_2-$ and R^{35} is C_{1-7} alkyl, C_{1-7} alkoxy

- 20 (which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno),

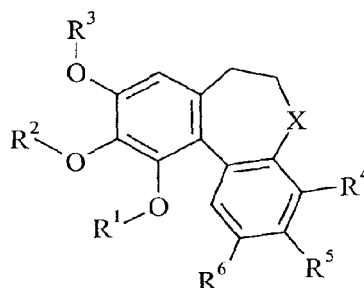
R^{48} (wherein R^{48} is a benzyl group which benzyl group may bear one or more substituents selected from C_{1-4} alkyl), or

R^{53} (wherein R^{53} is piperidinyl);

- 25 and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

According to a further aspect of the present invention there is provided a compound of the formula IIa:

- 28 -



(IIa)

wherein

X is

- 5 -C(O)-, -C(S)-, -C=NOH, or -CH(R⁷)- wherein R⁷ is hydrogen, hydroxy, C₁₋₇alkoxy, -OR⁸ or -NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR¹¹-, -SO₂- or -SO₂NR¹²- (wherein R¹¹ and R¹², which may be the same or different, each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is selected from one of the following nine groups:
- 10 1) hydrogen, C₁₋₇alkyl, C₃₋₇cycloalkyl, C₁₋₄alkylY⁸C₁₋₄alkyl wherein Y⁸ is as defined hereinafter, or phenyl,
- (which alkyl, cycloalkyl, alkylY⁸alkyl or phenyl group may bear one or more substituents selected from:
- halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, carboxy, carbamoyl,
- 15 C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, phenyl, nitro, sulphate, phosphate,
- Z¹ (wherein Z¹ represents a 5-6 membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from
- 20 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and Z² (wherein Z² is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1
- 25 or 2 substituents selected from

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- oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)),
- C₁₋₄alkylZ¹ (wherein Z¹ is as defined hereinbefore), and
- 5 a group -Y²R¹³ (wherein Y² is -NR¹⁴C(O)- or -O-C(O)- (wherein R¹⁴ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹³ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R¹⁵ wherein R¹⁵ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more
- 10 substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR¹⁶R¹⁷ and -NR¹⁸COR¹⁹ (wherein R¹⁶, R¹⁷, R¹⁸ and R¹⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- 15 2) R¹⁵ wherein R¹⁵ is as defined hereinbefore;
- 3) C₂₋₇alkenylR¹⁵ (wherein R¹⁵ is as defined hereinbefore);
- 4) C₃₋₇alkynylR¹⁵ (wherein R¹⁵ is as defined hereinbefore));
- 5) Z¹ (wherein Z¹ is as defined hereinbefore);
- 6) C₁₋₇alkylZ¹ (wherein Z¹ is as defined hereinbefore);
- 20 7) C₁₋₇alkylY⁸Z¹ (wherein Z¹ is as defined hereinbefore and Y⁸ is -C(O)-, -NR⁵⁹C(O)-, -NR⁵⁹C(O)C₁₋₄alkyl-, -C(O)NR⁶⁰- or -C(O)NR⁶⁰C₁₋₄alkyl-, (wherein R⁵⁹ and R⁶⁰, which may be the same or different, each represents hydrogen, C₁₋₃alkyl, C₁₋₃hydroxyalkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- 8) (C₁₋₇alkyl)_cY⁹Z³ (wherein c is 0 or 1, Z³ is an amino acid group and Y⁹ is a direct bond, -C(O)- or -NR⁶¹- (wherein R⁶¹ is hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)); and
- 25 9) C₁₋₇alkylR¹⁵ (wherein R¹⁵ is as defined hereinbefore);
- and R⁹ is hydrogen, C₁₋₇alkyl or C₃₋₇cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C₁₋₄alkoxy and phenyl);
- R¹, R² and R³ are each independently
- 30 hydrogen, PO₃H₂, sulphate, C₃₋₇cycloalkyl, C₂₋₇alkenyl, C₂₋₇alkynyl, C₁₋₇alkanoyl, a group R²⁰C₁₋₇alkyl (wherein R²⁰ is phenyl which may bear one or more substituents selected from C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl and C₁₋₄hydroxyalkoxy), C₁₋₇alkyl or C₁₋₇alkylsulphonyl

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(which alkyl or alkylsulphonyl group may bear one or more substituents selected from: halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y²R²¹ (wherein Y² is -NR²²C(O)- or -O-C(O)- (wherein R²² represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²¹ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R²³ wherein R²³ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR²⁴R²⁵ and -NR²⁶COR²⁷ (wherein R²⁴, R²⁵, R²⁶ and R²⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))));

with the proviso that at least two of R¹, R² and R³ are C₁₋₇alkyl;

15 R⁴ is

hydrogen, cyano, halogeno, nitro, amino, hydroxy, C₁₋₇alkoxy, C₁₋₇thioalkoxy, C₁₋₇alkanoyl or C₁₋₇alkyl,

(which alkyl group may bear one or more substituents selected from: halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y³R²⁸ (wherein Y³ is -NR²⁹C(O)- or -O-C(O)- (wherein R²⁹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²⁸ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R³⁰ wherein R³⁰ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR³¹R³² and -NR³¹COR³² (wherein R³¹, R³², R³³ and R³⁴, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))));

R⁵ and R⁶ are each independently selected from

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hydrogen, $-\text{OPO}_3\text{H}_2$, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C_{1-7} alkoxy, C_{1-7} alkanoyl, C_{1-7} thioalkoxy, C_{1-7} alkyl,

(which alkyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4} alkylamino, $\text{di}(\text{C}_{1-4}$ alkyl)amino, hydroxy, C_{1-4} alkoxy, C_{1-}

- 5 C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy, phenyl, sulphate, phosphate and a group $-\text{Y}^3\text{R}^{28}$ (wherein Y^3 is $-\text{NR}^{29}\text{C}(\text{O})-$ or $-\text{O}-\text{C}(\text{O})-$ (wherein R^{29} represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{28} is C_{1-7} alkyl, C_{3-7} cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected
- 10 independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, $-\text{CONR}^{31}\text{R}^{32}$ and $-\text{NR}^{31}\text{COR}^{32}$ (wherein R^{31} , R^{32} , R^{33} and R^{34} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl))), and
- 15 a group $-\text{Y}^4\text{R}^{35}$

(wherein Y^4 is $-\text{C}(\text{O})-$, $-\text{OC}(\text{O})-$, $-\text{O}-$, $-\text{SO}-$, $-\text{SO}_2-$, $-\text{OSO}_2-$, $-\text{NR}^{36}-$, $-\text{C}_{1-4}$ alkyl $\text{NR}^{36}-$, $-\text{C}_{1-4}$ alkyl $\text{C}(\text{O})-$, $-\text{NR}^{37}\text{C}(\text{O})-$, $-\text{OC}(\text{O})\text{O}-$, $-\text{C}(\text{O})\text{NR}^{38}-$ or $-\text{NR}^{39}\text{C}(\text{O})\text{O}-$ (wherein R^{36} , R^{37} , R^{38} and R^{39} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and

- 20 R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, hydroxy, amino, C_{1-7} alkyl, C_{1-7} alkoxy, C_{1-7} alkanoyl, C_{1-7} alkylamino, $\text{di}(\text{C}_{1-7}$ alkyl)amino, amino C_{1-7} alkylamino, C_{1-7} alkylamino C_{1-7} alkylamino, C_{1-7} alkanoylamino C_{1-7} alkyl, $\text{di}(\text{C}_{1-7}$ alkyl)amino C_{1-7} alkylamino, C_{1-7} alkylphosphate, C_{1-7} alkylphosphonate, C_{1-7} alkylcarbamoyl C_{1-7} alkyl,
- 25

(which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino, alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:

- 30 halogeno, amino, C_{1-4} alkylamino, $\text{di}(\text{C}_{1-4}$ alkyl)amino, hydroxy, C_{1-4} hydroxyalkyl, C_{1-4} alkoxy, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-\text{Y}^5\text{R}^{40}$ (wherein Y^5

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is $-NR^{41}C(O)-$, $-C(O)NR^{42}-$, $-C(O)-O-$ or $-O-C(O)-$ (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{40} is C_{1-7} alkyl, C_{3-7} cycloalkyl, carboxy C_{1-7} alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, $-CONR^{44}R^{45}$ and $-NR^{46}COR^{47}$ (wherein R^{44} , R^{45} , R^{46} and R^{47} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl))),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, di(C_{1-4} alkyl)amino C_{1-4} alkyl, di(C_{1-4} hydroxyalkyl)amino C_{1-4} alkyl, di(C_{1-4} aminoalkyl)amino C_{1-4} alkyl, C_{1-4} hydroxyalkoxy, carboxy, C_{1-4} carboxyalkyl, phenyl, cyano, $-CONR^{49}R^{50}$, $-NR^{51}COR^{52}$ (wherein R^{49} , R^{50} , R^{51} and R^{52} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and C_{1-4} alkyl R^{53} (wherein R^{53} is as defined hereinafter), C_{1-7} alkyl R^{48} (wherein R^{48} is as defined hereinbefore),

R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4} alkyl, C_{1-4} hydroxyalkyl, C_{1-4} alkoxy, C_{1-4} carboxyalkyl, C_{1-4} aminoalkyl, di(C_{1-4} alkyl)amino C_{1-4} alkyl, C_{1-4} alkoxy C_{1-4} alkyl, C_{1-4} alkylsulphonyl C_{1-4} alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4} alkyl, C_{1-4} hydroxyalkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-4} alkyl and C_{1-4} alkylsulphonyl C_{1-4} alkyl))), or

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- (CH₂)_aY⁶(CH₂)_bR⁵³ (wherein R⁵³ is as defined hereinbefore, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y⁶ represents a direct bond, -O-, -C(O)-, -NR⁵⁵-, -NR⁵⁶C(O)- or -C(O)NR⁵⁷- (wherein R⁵⁵, R⁵⁶, and R⁵⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl), and wherein one or more of the (CH₂)_a or (CH₂)_b groups may bear one or more substituents selected from hydroxy, amino and halogeno));

with the proviso that R⁵ is not hydroxy, alkoxy, substituted alkoxy (wherein R⁵ is Y⁴R³⁵ and Y⁴ is -O- and R³⁵ is C₁₋₇alkyl bearing one or more substituents selected from the list given hereinbefore), -OPO₃H₂, -O-C₁₋₇alkanoyl or benzyloxy;

- 10 with the further proviso that at least one of R⁵ or R⁶ is a group -Y⁴R³⁵ (wherein Y⁴ and R³⁵ are as defined hereinbefore) but with the further provisos that when R⁵ is -Y⁴R³⁵ and R⁶ is hydrogen, hydroxy, methoxy or methoxycarbonyl, -Y⁴R³⁵ is not selected from cases wherein:

- 15 Y⁴ is -C(O)-, -OC(O)-, -O-, -SO-, -OSO₂-, -NR³⁶-, -NR³⁷C(O)- or -C(O)NR³⁸- (wherein R³⁶, R³⁷ and R³⁸, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R³⁵ is a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, C₁₋₇alkyl, C₁₋₇alkoxy, C₁₋₇alkanoyl,

- 20 (which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno, hydroxy, and a group -Y⁵R⁴⁰ (wherein Y⁵ is -O-C(O)- and R⁴⁰ is C₁₋₇alkyl)), or

R⁴⁸ (wherein R⁴⁸ is a tetrazolyl group (which may or may not be substituted as hereinbefore defined), a phenyl group or a benzyl group which phenyl or benzyl group may bear one or more substituents selected from C₁₋₄alkyl); and

- 25 that when R⁶ is -Y⁴R³⁵ and R⁵ is hydrogen, hydroxy, methoxy or methoxycarbonyl, -Y⁴R³⁵ is not selected from cases wherein:

Y⁴ is -C(O)-, -O- or -OSO₂- and R³⁵ is C₁₋₇alkyl, C₁₋₇alkoxy

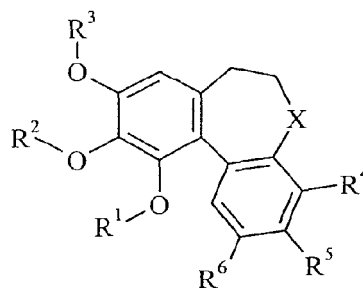
- 30 (which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno),

R⁴⁸ (wherein R⁴⁸ is a benzyl group which benzyl group may bear one or more substituents selected from C₁₋₄alkyl), or

R^{53} (wherein R^{53} is piperidiny);
and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

According to a further aspect of the present invention there is provided the use of a
5 compound of the formula IIa as defined hereinbefore, and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.

According to another aspect of the present invention there is provided the use of a
10 compound of the formula IIb:



(IIb)

wherein

15 X is
-C(O)-, -C(S)-, or -CH(R⁷)- wherein R⁷ is hydrogen, hydroxy or -NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is a direct bond, -C(O)-, -C(S)-, -C(O)O-, -C(O)NR¹¹-, -SO₂- or -SO₂NR¹²- (wherein R¹¹ and R¹², which may be the same or different, each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is selected from one of the
20 following two groups:

1) hydrogen, C₁₋₇alkyl or C₃₋₇cycloalkyl

(which alkyl or cycloalkyl group may bear one or more substituents selected from:

halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, phenyl, nitro,
25 sulphate, phosphate and a group -Y²R¹³ (wherein Y² is -NR¹⁴C(O)- or -O-C(O)- (wherein R¹⁴ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹³ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R¹⁵ wherein R¹⁵ is a phenyl group or a 5-10-membered aromatic

- heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR¹⁶R¹⁷ and -NR¹⁸COR¹⁹ (wherein R¹⁶, R¹⁷, R¹⁸ and R¹⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)); and
- 5 2) R¹⁵ wherein R¹⁵ is as defined hereinbefore;
- and R⁹ is hydrogen, C₁₋₇alkyl or C₃₋₇cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C₁₋₄alkoxy and phenyl);
- 10 R¹, R² and R³ are each independently hydrogen, PO₃H₂, sulphate, C₃₋₇cycloalkyl, C₂₋₇alkenyl, C₂₋₇alkynyl, C₁₋₇alkanoyl, a group R²⁰C₁₋₇alkyl (wherein R²⁰ is phenyl which may bear one or more substituents selected from C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl and C₁₋₄hydroxyalkoxy), C₁₋₇alkyl or C₁₋₇alkylsulphonyl
- 15 (which alkyl or alkylsulphonyl group may bear one or more substituents selected from: halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y²R²¹ (wherein Y² is -NR²²C(O)- or -O-C(O)- (wherein R²² represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²¹ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R²³ wherein R²³ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR²⁴R²⁵ and -NR²⁶COR²⁷ (wherein R²⁴, R²⁵, R²⁶ and R²⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- 20 with the proviso that at least two of R¹, R² and R³ are C₁₋₇alkyl;
- R⁴ is
- 30 hydrogen, cyano, halogeno, nitro, amino, hydroxy, C₁₋₇alkoxy, C₁₋₇thioalkoxy, C₁₋₇alkanoyl or C₁₋₇alkyl,
- (which alkyl group may bear one or more substituents selected from:

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- halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y³R²⁸ (wherein Y³ is -NR²⁹C(O)- or -O-C(O)- (wherein R²⁹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²⁸ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R³⁰ wherein R³⁰ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR³¹R³² and -NR³¹COR³² (wherein R³¹, R³², R³³ and R³⁴, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))));

R⁵ and R⁶ are each independently selected from

- hydrogen, -OPO₃H₂, cyano, halogeno, nitro, amino, carboxy, hydroxy, C₁₋₇alkoxy, C₁₋₇alkanoyl, C₁₋₇thioalkoxy, C₁₋₇alkyl, (which alkyl group may bear one or more substituents selected from: halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, sulphate, phosphate and a group -Y³R²⁸ (wherein Y³ is -NR²⁹C(O)- or -O-C(O)- (wherein R²⁹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²⁸ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R³⁰ wherein R³⁰ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR³¹R³² and -NR³¹COR³² (wherein R³¹, R³², R³³ and R³⁴, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))), and

a group -Y⁴R³⁵

- (wherein Y⁴ is -C(O)-, -OC(O)-, -O-, -SO-, -SO₂-, -OSO₂-, -NR³⁶-, -NR³⁷C(O)-, -OC(O)O-, -C(O)NR³⁸- or -NR³⁹C(O)O- (wherein R³⁶, R³⁷, R³⁸ and R³⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R³⁵ is

a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, C₁₋₇alkyl, C₁₋₇alkoxy, C₁₋₇alkanoyl, aminoC₁₋₇alkylamino, C₁₋₇alkylaminoC₁₋₇alkylamino, di(C₁₋₇alkyl)aminoC₁₋₇alkylamino, C₁₋₇alkylphosphate

(which alkyl, alkoxy, alkanoyl, aminoalkylamino, alkylaminoalkylamino,

5 dialkylaminoalkylamino, or alkylphosphate may bear one or more substituents selected from:

halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y⁵R⁴⁰ (wherein Y⁵ is -NR⁴¹C(O)-, -C(O)NR⁴²-, -C(O)-O- or -O-C(O)- (wherein R⁴¹ and R⁴² which may be the same or different each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R⁴⁰ is C₁₋₇alkyl, C₃₋₇cycloalkyl, carboxyC₁₋₇alkyl or a group R⁴³ wherein R⁴³ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which

15 phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR⁴⁴R⁴⁵ and -NR⁴⁶COR⁴⁷ (wherein R⁴⁴, R⁴⁵, R⁴⁶ and R⁴⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))),

20 R⁴⁸ (wherein R⁴⁸ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, phenyl, cyano, -CONR⁴⁹R⁵⁰ and -NR⁵¹COR⁵² (wherein R⁴⁹, R⁵⁰, R⁵¹ and R⁵², which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)), or

25 R⁵³ (wherein R⁵³ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which

30 heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and R⁵⁴ (wherein R⁵⁴ is a 5-6-membered saturated heterocyclic group (linked via carbon or

- nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl));
- 5 with the proviso that R⁵ is not hydroxy, alkoxy, substituted alkoxy, -OPO₃H₂, -O-C₁₋₇alkanoyl or benzyloxy;
- with the further proviso that at least one of R⁵ or R⁶ is a group -Y⁴R³⁵ (wherein Y⁴ and R³⁵ are as defined hereinbefore) but with the further provisos
- that when R⁵ is -Y⁴R³⁵ and R⁶ is hydrogen, hydroxy or methoxy -Y⁴R³⁵ is not selected from
- 10 cases wherein:
- Y⁴ is -C(O)-, -OC(O)-, -O-, -SO-, -OSO₂-, -NR³⁶-, -NR³⁷C(O)- or -C(O)NR³⁸- (wherein R³⁶, R³⁷ and R³⁸, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R³⁵ is
- a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, C₁₋₇alkyl, C₁₋₇alkoxy, C₁₋₇alkanoyl,
- 15 (which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno, hydroxy, and a group -Y⁵R⁴⁰ (wherein Y⁵ is -C(O)-O- or -O-C(O)- and R⁴⁰ is C₁₋₇alkyl)), or
- R⁴⁸ (wherein R⁴⁸ is a phenyl group or a benzyl group which phenyl or benzyl group may
- 20 bear one or more substituents selected from C₁₋₇alkyl); and
- that when R⁶ is -Y⁴R³⁵ and R⁵ is hydrogen, hydroxy or methoxy -Y⁴R³⁵ is not selected from cases wherein:
- Y⁴ is -C(O)-, -O- or -OSO₂- and R³⁵ is
- C₁₋₇alkyl, C₁₋₇alkoxy
- 25 (which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno),
- R⁴⁸ (wherein R⁴⁸ is a benzyl group which phenyl or benzyl group may bear one or more substituents selected from C₁₋₇alkyl), or
- R⁵³ (wherein R⁵³ is piperidinyl);
- 30 and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof for example esters, amides and sulphides, in the manufacture of a

medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.

According to a further aspect of the present invention there is provided a compound of the formula IIb as defined hereinbefore, and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof for example esters, amides and sulphides.

Preferred compounds of the present invention include:

- (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-
 {[(2*R*)-2,6-diaminohexanoyl]amino}propanoate,
- 10 (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[(2-aminoacetyl)amino]propanoate,
N-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]oxymethyl}-2-morpholinoacetamide,
 (2*S*,3*S*,4*S*,5*R*,6*R*)-6-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
 15 dibenzo[*a,c*]cyclohepten-3-yl]oxy}-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylic acid,
N-{[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide,
N-{[(5*S*)-3-(4-{morpholinomethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
 dibenzo[*a,c*]cyclohepten-5-yl]acetamide,
- 20 (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[4-methylpiperazin-1-ylcarbonyl]propanoate,
 5-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl}oxycarbonyl]pentanoic acid,
 4-(3-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-
 25 yl]oxy-3-oxopropyl}benzoic acid and
 (2*S*)-*N*-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-amino-3-hydroxypropanamide,
 and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.
- 30 More preferred compounds of the present invention include:
 (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-
 {[(2*R*)-2,6-diaminohexanoyl]amino}propanoate,

- (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[(2-aminoacetyl)amino]propanoate,
N-[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide,
 5 *N*-[(5*S*)-3-(4-{morpholinomethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide,
 (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[4-methylpiperazin-1-ylcarbonyl]propanoate,
 5-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]oxycarbonyl]pentanoic acid,
 10 4-(3-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]oxy-3-oxopropyl)benzoic acid and
 (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-amino-3-hydroxypropanamide,
 15 and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

Especially preferred compounds of the present invention include:

- N*-[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide and
 20 (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-amino-3-hydroxypropanamide,
 and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

- In another embodiment of the present invention preferred compounds include (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-amino-5-[(2-nitroethanimidoyl)amino]pentanamide, and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

- In one embodiment of the invention preferred compounds include those in which R¹, R² and R³ are each alkyl, Y⁴ is NH and R³⁵ is an acyl group derived from a natural alpha-amino acid such as glycine, L-alanine or L-serine.

In one embodiment of the invention more preferred compounds include compounds wherein R¹, R² and R³ are each methyl, R⁴ is hydrogen and X is -CH(NHC(O)CH₃)-.

In another embodiment of the invention particular compounds include compounds wherein R^1 , R^2 and R^3 are each alkyl and R^{35} is amino C_{1-7} alkylamino, C_{1-7} alkylamino C_{1-7} alkylamino, di(C_{1-7} alkyl)amino C_{1-7} alkylamino, 1-piperazinyl or 4-(piperidino)piperidin-1-yl.

In another embodiment of the invention further particular compounds include
5 compounds wherein R^1 , R^2 and R^3 are each alkyl, R^4 is hydrogen and X is $-CH(NHC(O)CH_3)-$.

In another embodiment of the invention more particular compounds include compounds wherein R^1 , R^2 and R^3 are each alkyl and R^4 and R^6 are each hydrogen.

In another embodiment of the invention especially preferred compounds include compounds wherein R^1 , R^2 and R^3 are each methyl and R^{35} is a substituted acyl group.

10 Preferred compounds of the present invention include:

N-[3-(alanyl-amino)-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]acetamide and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof for example esters, amides and sulphides.

A preferred compound for use in the manufacture of a medicament for use in the production
15 of a vascular damaging effect in warm-blooded animals such as humans is

N-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]acetamide.

For the avoidance of doubt it is to be understood that where in this specification a group is qualified by 'hereinbefore defined' or 'defined hereinbefore', or 'hereinafter defined' or 'defined hereinafter', the said group encompasses the first occurring and broadest definition
20 as well as each and all of the preferred definitions for that group.

In this specification unless stated otherwise the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. An analogous convention applies to other generic terms. Unless otherwise stated the term "alkyl" advantageously refers to chains with 1-7
25 carbon atoms, preferably 1-4 carbon atoms. The term "alkoxy" as used herein, unless stated otherwise includes "alkyl"-O- groups in which "alkyl" is as hereinbefore defined. The term "aryl" as used herein unless stated otherwise includes reference to a C_{6-10} aryl group which may, if desired, carry one or more substituents selected from halogeno, alkyl, haloalkyl, alkoxy, hydroxy, amino, nitro and cyano, (wherein alkyl, haloalkyl and alkoxy are as
30 hereinbefore and hereinafter defined). The term "aryloxy" as used herein unless otherwise stated includes "aryl"-O-groups in which "aryl" is as hereinbefore defined. The term "sulphonyloxy" as used herein refers to alkylsulphonyloxy and arylsulphonyloxy groups in

which "alkyl" and "aryl" are as hereinbefore defined. The term "heteroaryl" as used herein unless stated otherwise includes reference to a C₆₋₁₀ aryl group containing one or more ring heteroatoms selected from O, N and S which heteroaryl group may, if desired, carry one or more substituents selected from halogeno, alkyl, haloalkyl, alkoxy, hydroxy, amino, nitro and cyano, (wherein alkyl, haloalkyl and alkoxy are as hereinbefore and hereinafter defined). The term "alkanoyl" as used herein unless otherwise stated includes formyl and alkylC=O groups in which "alkyl" is as defined hereinbefore, for example C₂alkanoyl is ethanoyl and refers to CH₃C=O, C₁alkanoyl is formyl and refers to CHO. In this specification unless stated otherwise the term "alkenyl" includes both straight and branched chain alkenyl groups but references to individual alkenyl groups such as 2-butenyl are specific for the straight chain version only. Unless otherwise stated the term "alkenyl" advantageously refers to chains with 2-7 carbon atoms, preferably 2-4 carbon atoms. In this specification unless stated otherwise the term "alkynyl" includes both straight and branched chain alkynyl groups but references to individual alkynyl groups such as 2-butyne are specific for the straight chain version only. Unless otherwise stated the term "alkynyl" advantageously refers to chains with 2-7 carbon atoms, preferably 2-4 carbon atoms. The term "halogeno" means fluoro, chloro, bromo or iodo unless otherwise stated. A haloalkyl group is an alkyl group as defined hereinbefore substituted with one or more halogeno groups for example trifluoromethyl and dichloromethyl. A hydroxyalkyl group is an alkyl group as defined hereinbefore substituted with one or more hydroxy groups.

In this specification unless stated otherwise the term "acyl" refers to a group linked via a carbonyl group. "Acyl" includes a group -C(O)-R⁵⁸ wherein R⁵⁸ is an alkyl, aryl or heteroaryl group as hereinbefore defined, or -C(O)-R⁵⁸ is derived from an amino acid.

In this specification mono-peptide means an amino acid including α -amino acids β -amino acids and γ -amino acids. The amino acids may be L-isomers or D-isomers, preferably L-isomers. Preferred amino acids include glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, β -alanine and ornithine. More preferred amino acids include serine, threonine, arginine, glycine, alanine, β -alanine and lysine. Especially preferred amino acids include serine, threonine, arginine, alanine and β -alanine.

An aromatic heterocyclic group includes those selected from pyridyl, pyrimidyl, furyl, thienyl, pyrrolyl, pyrazolyl, indolyl, benzofuryl, benzothienyl, benzothiazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, quinolyl and isoquinolyl.

For the avoidance of any doubt, it is to be understood that when Y^1 is, for example, a group of formula $-C(O)NR^{11}-$, it is the nitrogen atom bearing the R^{11} group which is attached to the group R^{10} and the carbonyl group ($C(O)$) is attached to the nitrogen atom which is linked to the hepten ring. A similar convention applies to the other two atom Y^1 linking groups such as $-SO_2NR^{12}-$. An analogous convention applies to other groups. It is further to be understood that when Y^1 represents $-C(O)NR^{11}-$ and R^{11} is C_{1-3} alkoxy C_{2-3} alkyl it is the C_{2-3} alkyl moiety which is linked to the nitrogen atom of Y^1 and an analogous convention applies to other groups.

For the avoidance of any doubt it is to be understood that when Y^2 is, for example, a group of formula $-OC(O)-$ it is the oxygen atom which is bound to the substituted group and the carbonyl group ($C(O)$) which is bound to R^{13} and an analogous convention applies to other groups.

For the avoidance of any doubt it is to be understood that when Y^4 is, for example, a group of formula $-NR^{39}C(O)O-$ it is the nitrogen atom which is bound to the benz ring and the oxygen atom which is bound to R^{35} and an analogous convention applies to other groups.

For the avoidance of any doubt it is to be understood that when R^{35} is a group C_{1-4} alkyl R^{48} it is the alkyl chain which is linked to Y^4 , similarly when R^{35} is a group $(CH_2)_aY^6(CH_2)_bR^{53}$ it is the $(CH_2)_a$ group which is linked to Y^4 and a similar convention applies to other groups.

For the avoidance of any doubt, it is to be understood that when a group carries a C_{1-4} alkylamino substituent it is the amino moiety which is attached to the group whereas when a group carries a C_{1-4} aminoalkyl substituent it is the C_{1-4} alkyl moiety which is attached to the group and an analogous convention applies to other substituents.

Within the present invention it is to be understood that a compound of the formula I or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which has vascular damaging activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings. The formulae drawings within this specification can represent only one of

the possible tautomeric forms and it is to be understood that the specification encompasses all possible tautomeric forms of the compounds drawn not just those forms which it has been possible to show graphically herein.

It will be appreciated that compounds of the formula I or a salt thereof may possess an asymmetric carbon atom. Such an asymmetric carbon atom is also involved in the tautomerism described above, and it is to be understood that the present invention encompasses any chiral form (including both pure enantiomers and racemic mixtures), as well as any tautomeric form, which has vascular damaging activity, and is not to be limited merely to any one tautomeric form or chiral form utilised within the formulae drawings. It is to be understood that the invention encompasses all optical and diastereomers which have vascular damaging activity.

It is also to be understood that certain compounds of the formula I and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which have vascular damaging activity.

The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula I and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula I as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. Suitable salts include hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates. In addition where the compounds of formula I are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for

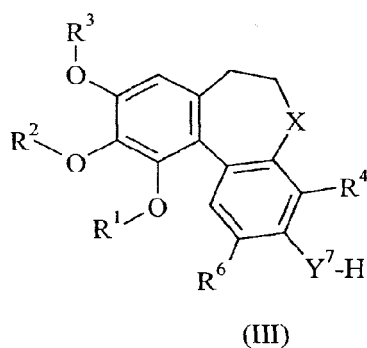
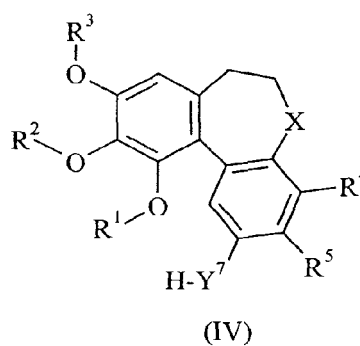
example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

Compounds of Formula I may be prepared by any process known to a person skilled in the art. Such processes include, for example, solid phase synthesis. Compounds of Formula I
 5 may be prepared by a number of processes as generally described hereinbelow and more specifically in the Examples hereinafter. In the general preparations described hereinafter it may be necessary to employ protecting groups which are then removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art. Processes for the preparation of
 10 novel compounds of formula I, are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of
 15 an organic chemist.

Thus, the following processes (a) to (i) and (i) to (iii) constitute further features of the present invention.

Synthesis of Compounds of Formula I

(a) Thus according to a further aspect of the invention a compound of formula I, in which R⁵
 20 or R⁶ is a group Y⁴R³⁵ (wherein R³⁵ is as defined hereinbefore and Y⁴ is a group -OC(O)- or -NHC(O)-), can be prepared from a compound of formula III or IV:



25 (wherein X, R¹, R², R³, R⁴, R⁵, R⁶ are as defined hereinbefore and Y⁷ is -O- or -NH-), as appropriate, by standard acylation or coupling conditions including, for example, treatment of a compound of formula III or IV with a substituted carboxylic acid in the presence of a

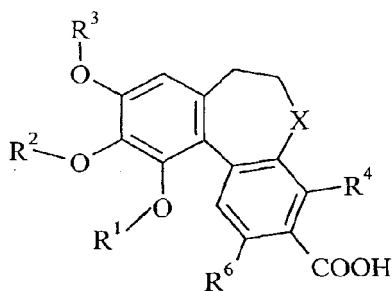
coupling agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and, optionally, a base such as an organic base for example triethylamine in a solvent such as an aprotic solvent for example dimethylformamide or in a chlorinated solvent for example trichloromethane or dichloromethane at a temperature in the range from
5 about -30°C to about 60°C, conveniently at or near ambient temperature.

(b) In another general example a compound of formula I, in which R⁵ or R⁶ is a group Y⁴R³⁵ (wherein R³⁵ is C₁₋₇alkoxy which may be substituted as defined hereinbefore and Y⁴ is a group -OC(O)- or -NHC(O)-), can be prepared from a compound of formula III and IV, as appropriate, by standard acylation reactions including, for example, treatment of a compound
10 of formula III or IV with a substituted alkylchloroformate in the presence of a base such as an organic base for example, triethylamine or *N*-methylmorpholine in a solvent such as an ether solvent for example tetrahydrofuran or in a chlorinated solvent for example dichloromethane at a temperature in the range from about -20°C to the reflux temperature of the solvent.

(c) In a further general example a compound of formula I, in which R⁵ or R⁶ is a group Y⁴R³⁵ (wherein R³⁵ is aminoC₁₋₇alkylamino, C₁₋₇alkylaminoC₁₋₇alkylamino, di(C₁₋₇alkyl)aminoC₁₋₇alkylamino and may be substituted as defined hereinbefore, or is R⁵³ (wherein R⁵³ is as defined hereinbefore) and Y⁴ is a group -OC(O)- or -NHC(O)-), can be prepared from a compound of formula III or IV, as appropriate, by standard acylation reactions including, for example, treatment of a compound of formula III or IV with a substituted alkylisocyanate or a
15 carbamoyl chloride in the presence of a base such as an organic base for example triethylamine, pyridine or *N*-methylmorpholine in a solvent such as an ether solvent for example tetrahydrofuran or in a chlorinated solvent for example dichloromethane at a temperature in the range from about -20°C to the reflux temperature of the solvent.

(d) In a further example a compound of formula I, in which R⁵ or R⁶ is a group Y⁴R³⁵ (wherein R³⁵ is a sugar moiety and Y⁴ is a group -O- or -NH-), can be prepared from a compound of formula III or IV, as appropriate, by standard glycosylation reactions including for example treatment of a compound of formula III or IV with a suitably protected 1-bromo sugar in a solvent such as a chlorinated solvent for example trichloromethane or an aromatic solvent for example toluene at a temperature in the range from about 0°C to the reflux
25 temperature of the solvent, followed by deprotection. Suitable protecting groups include acetyl groups for the sugar hydroxyl groups and esters for sugar carboxylic acids.
30

- (e) In a further example a compound of formula I in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is sulphate and Y^4 is a group -O- or -NH-), can be prepared from a compound of formula III or IV, as appropriate, by standard sulphonylation reactions including for example treatment of a compound of formula III or IV with chlorosulphonic acid in the presence of a base such as dimethylaniline in a chlorinated solvent such as trichloromethane at a temperature in the range from about -20°C to about 60°C, or more preferably with chlorosulphonic acid in pyridine at a temperature in the range from about -20°C to about 60°C.
- (f) In a further example a compound of formula I in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is C_{1-7} alkylphosphate and may be substituted as defined hereinbefore and Y^4 is a group -O- or -NH-), can be prepared from a compound of formula III or IV, as appropriate, by standard phosphorylation reactions including for example treatment of a compound of formula III or IV with phosphorus oxychloride in the presence of a base such as triethylamine in a chlorinated solvent such as trichloromethane at a temperature in the range from about -20°C to about 60°C, followed by treatment with an alcohol, or more preferably with alkyl dichlorophosphate in the presence of a base such as lithiumHMDS in THF at a temperature in the range from about -20°C to about 60°C, followed by treatment with water.
- (g) Compounds of formula I in which R^5 is amino can be prepared from carboxylic acids of formula V:



(V)

(wherein X, R^1 , R^2 , R^3 , R^4 and R^6 are as defined hereinbefore) via Curtius rearrangement and hydrolysis (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72).

- (h) Compounds of formula I may also be prepared from other compounds of formula I by chemical modification. Examples of such chemical modifications that may be applied are standard alkylation, arylation, heteroarylation, acylation, thioacylation, sulphonylation, sulphation, phosphorylation, aromatic halogenation and coupling reactions. These reactions

may be used to add new substituents or to modify existing substituents. Alternatively, existing substituents in compounds of formula I may be modified by, for example, oxidation, reduction, elimination, hydrolysis or other cleavage reaction to yield other compounds of formula I.

- 5 (i) A compound of formula I in which R⁵ or R⁶ is chloro may be prepared from a compound of formula III or IV by standard processes such as the Sandmeyer reaction.

Thus for example a compound of formula I containing an amino group may be acylated on the amino group by treatment with, for example, an acyl halide or anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for
10 example, a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In another general example of an interconversion process an amino group in a compound of formula I may be sulphonylated by treatment with, for example, an alkyl or aryl sulphonyl chloride or an alkyl or aryl sulphonic anhydride in the presence of a base, for
15 example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In a further general example a compound of formula I containing a hydroxy group can be converted into the corresponding dihydrogenphosphate ester by treatment with for example
20 di-tert-butyl diisopropylphosphoramidite or di-tert-butyl diethylphosphoramidite in the presence of a suitable catalyst for example tetrazole in a solvent such as an ether solvent for example tetrahydrofuran at a temperature in the range -40°C to 40°C, conveniently at or near ambient temperature, followed by treatment with an oxidising agent for example 3-chloroperoxy benzoic acid at a temperature in the range -78°C to 40°C preferably -40°C to
25 10°C. The resulting intermediate phosphate triester is treated with an acid for example trifluoroacetic acid in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30°C to 40°C conveniently at or near 0°C to give the compound of formula I containing a dihydrogenphosphate ester.

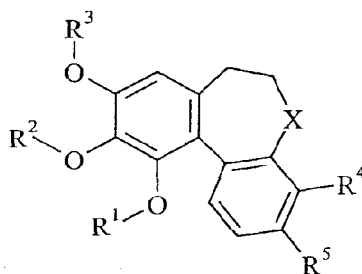
In a further general example a compound of formula I containing an amide can be
30 hydrolysed by treatment with for example an acid such as hydrochloric acid in a solvent such as an alcohol, for example methanol at an elevated temperature conveniently at the reflux temperature.

In another general example an O-alkyl group may be cleaved to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C.

In a further general example compounds of formula I may be alkylated by reaction
5 with a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether
10 solvent such as tetrahydrofuran at a temperature of around -10°C to 80°C.

Synthesis of Intermediates

(i) Compounds of formula III or IV, used as starting materials for the preparation of compounds of the invention are either known or can be prepared from known compounds by the application of standard procedures of organic synthesis known in the art. For example a
15 compound of formula VI:



(VI)

20 (wherein X, R¹, R², R³, R⁴ and R⁵ are as defined hereinbefore), can be converted into a compound of formula IV where Y⁷ is NH by the sequential application of standard nitration conditions followed by reduction of the incorporated nitro group under standard reduction conditions. Suitable nitration conditions include, for example, treatment with concentrated nitric acid in a solvent such as glacial acetic acid at a temperature from about -40°C to about
25 40°C. Suitable reduction conditions include, for example, treatment with tin(II) chloride in a solvent such as hydrochloric acid, with or without an alcoholic cosolvent, at a temperature between ambient temperature and about 100°C.

(ii) Compounds of formulae III or IV can also be prepared from other compounds of formulae III or IV by chemical modification. For example a compound of formula IV in which Y⁷ is NH can be converted into the corresponding compound in which Y⁷ is O by treatment with sodium nitrite in sulphuric acid at around 0°C followed by heating to around 100°C.

5 Preparation of a compound of formula I as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure starting material or intermediate or by resolution of the final product in a conventional manner.

Acid addition salts of the compounds of formula I are prepared in a conventional manner by treating a solution or suspension of the free base I with about one equivalent of a
10 pharmaceutically acceptable acid. Salts of compounds of formula I derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension of the free acid I with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard
15 fashion. Conventional concentration and recrystallisation techniques are employed in isolating the salts.

(iii) Compounds of formula V may be prepared from the corresponding colchicine derivatives by treatment with sodium methoxide in methanol followed by ester hydrolysis with aqueous acid or aqueous base (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72). Compounds of
20 formula VI may be prepared by any of the methods described for compounds of formula I.

Many of the intermediates defined herein, for example, those of the formulae III, IV, V, and VI are novel and these are provided as a further feature of the invention. The preparation of these compounds is as described herein and/or is by methods well known to persons skilled in the art of organic chemistry.

25 Compounds according to the invention are able to destroy vasculature that has been newly formed such as tumour vasculature while leaving unaffected normal, mature vasculature. The identification of compounds which selectively, and preferably potently, damage newly-formed vasculature is desirable and is the subject of the present invention. The ability of the compounds to act in this way may be assessed, for example, using one or more
30 of the procedures set out below:

(a) Activity against tumour vasculature measured by radioactive tracer

This assay demonstrates the ability of compounds to damage selectively tumour vasculature.

Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5 mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour, kidney, liver, skin, muscle, gut and brain by the $^{86}\text{RbCl}$ extraction technique (Sapirstein, Amer. Jnl. Physiol., 1958, 193, 161-168). Tissue radioactivity measured 1 minute after an intravenous injection of $^{86}\text{RbCl}$ was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit. Jnl. Radiol., 1982, 55, 905-913). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

15 (b) Activity against tumour vasculature measured by fluorescent dye

This assay demonstrates the ability of compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the fluorescent dye Hoechst 33342 according to the method of Smith et al (Brit. Jnl. Cancer 1988, 57, 247-253). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25mg/ml and injected intravenously at 10mg/kg 24 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; $10\mu\text{m}$ sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (Jnl. Natl. Cancer Inst., 1943, 4, 47-53). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels.

The ability of the compounds to bind to preparations of mammalian tubulin can be evaluated by a number of methods available in the literature, for example by following temperature initiated tubulin polymerisation by turbidity in the absence and presence of the compound (for example O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

The activity of *N*-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide, (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72), against tumour vasculature was measured by the fluorescent dye method described above. This compound decreased perfused vascular volume by 88% relative to control when dosed at 50mg/kg intraperitoneally. The IC₅₀ of this compound in a tubulin polymerisation assay was 58 micromolar (O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

The activity of compounds Examples 2 and 3 (described hereinafter) against tumour vasculature was measured by the fluorescent dye method described hereinbefore.

10 Compound of Example	% Decrease in vascular volume
2	95
3	45

15 (c) HUVEC detachment assay

This assay examined the effects of compounds on the adherence of HUVECs to tissue culture plasticware.

HUVECs were plated in 0.2% gelatin-coated 12 well tissue culture plates at a concentration of 3×10^4 cells per well in 1ml TCS medium. After 24 hours, when the cells were at ~30% confluency, the cells were dosed with compound for 40 minutes at 37°C, 5% CO₂. After this incubation the medium containing drug was pipetted off, and the cells were then gently washed in 2mls of HBSS (Hanks' Balanced Salt Solution purchased from Life Technologies Ltd, Paisley UK; Catalogue # 24020-083) to remove any detached cells. The washing solution was then removed, and the adherent cells remaining were trypsinised using 300µl of 1x Trypsin-EDTA solution (Life Technologies Ltd, Paisley, UK; Catalogue # 43500-019) at ambient temperature for 2 minutes. The trypsinised cells were then made up to 1ml with TCS Biologicals medium, then centrifuged at 2000rpm for 2 minutes. The cell pellet was then resuspended in a volume of 50µl of TCS Biologicals medium. Total cell counts were obtained by counting the cells on a haemocytometer. The amount of cell detachment was calculated by comparing the number of cells remaining attached following treatment with the number in undosed control wells.

(d) Hras5 necrosis model

NIH 3T3 fibroblasts transfected with Harvey ras, clone 5, (Hras5 cells) were kept in continual passage in Dulbecco's modified Eagles medium (DMEM) containing 10% foetal bovine serum (FBS) and 1% glutamine, at 37°C in a humidified incubator gassed with 7.5% carbon dioxide and 92.5% oxygen. Cells were implanted subcutaneously into the left flank of male nude mice (8-10 weeks of age) at an inoculum of 2×10^5 cells/mouse. Tumours were measured using calipers and randomised into groups of 2-4 mice between days 9-14 after implant. Mice were dosed with compounds, either intravenously or intraperitoneally, once on day of randomisation and culled 24 hours after dosing. Compounds were dissolved in 20% hydroxypropyl beta cyclodextrin in physiological saline at pH 7 and dosed in a volume of 0.1ml per 10g body weight. Tumours were excised, weighed and placed in buffered formalin. Area of necrosis in individual tumours was assessed from a haematoxylin/eosin stained-slide by a pathologist and scored from 0, meaning no significant change, to 10, meaning 91-100% necrosis.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula I as defined hereinbefore or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for nasal administration or administration by inhalation, for example as a powder or solution, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be
5 varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

According to a further aspect of the present invention there is provided a compound of the formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore for use
10 in a method of treatment of the human or animal body by therapy.

A further feature of the present invention is a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament, conveniently a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament for producing a vascular damaging effect in a warm-blooded animal such as a human being.

15 Thus according to a further aspect of the invention there is provided the use of a compound of the formula I, or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for
20 producing a vascular damaging effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore.

The antiangiogenic treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances
25 and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic treatment defined hereinbefore may
30 be: surgery, radiotherapy or chemotherapy. Such chemotherapy may include the following categories of therapeutic agent:

- (i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v \beta 3$ function, angiostatin, endostatin, razoxin, thalidomide) and including vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors (RTKIs) (for example those described in International Patent Applications Publication Nos. WO 97/22596, WO 97/30035, WO 97/32856 and WO 98/13354 the entire disclosure of which documents is incorporated herein by reference);
- (ii) cytostatic agents such as antioestrogens (for example tamoxifen, toremifene, raloxifene, droloxifene, idoxifene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrozole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5 α -dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example epidermal growth factor (EGF), platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors);
- (iii) biological response modifiers (for example interferon);
- (iv) antibodies (for example edrecolomab); and
- (v) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimetotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); enzymes (for example asparaginase); thymidylate synthase inhibitors (for example raltitrexed); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan, irinotecan).

As stated above the compounds defined in the present invention are of interest for their vascular damaging effects. Such compounds of the invention are expected to be useful

in the prophylaxis and treatment of a wide range of disease states where inappropriate angiogenesis occurs including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and
5 ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin.

In addition to their use in therapeutic medicine, the compounds of formula I and their pharmaceutically acceptable salts are also useful as pharmacological tools in the
10 development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of vascular damaging agents in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

15 The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:

(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

20 (ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon or nitrogen;

(iii) yields are given for illustration only and are not necessarily the maximum attainable;

(iv) the structures of the end-products of the formula I were confirmed by nuclear
25 (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet, quin, quintet;

(v) intermediates were not generally fully characterised and purity was assessed by
30 thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

Abbreviations

- 57 -

1,3-Dicyclohexylcarbodiimide	DCCI
4-Dimethylaminopyridine	DMAP
Tetrahydrofuran	THF
Diethyl azodicarboxylate	DEAD
5 <i>N,N</i> -Dimethylformamide	DMF
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	EDCI
Dimethyl sulphoxide	DMSO
Trifluoroacetic acid	TFA
10 1,1,1,3,3,3-hexamethyldisilazane	HMDS

Example 1

N-[3-((*N*-benzyloxycarbonyl)alanyl)amino]-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide

- 15 A solution of *N*-benzyloxycarbonyl-(L)-alanine (63mg, 0.28mmol) in dichloromethane (4ml) at -20°C was treated with *N*-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (100mg, 0.28mmol), (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72), and 1,3-dicyclohexylcarbodiimide (134mg, 0.31mmol) and the solution stirred for 16 hours at ambient temperature. Solvent was evaporated under reduced
- 20 pressure and the residue chromatographed on silica gel, eluting with ethyl acetate to give a white solid which was triturated with diethyl ether. The title compound (85mg) was obtained as a white solid.
- m.p. 140-141°C
- m/e 561

25

Example 2

N-[3-(alanyl)amino]-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide

- 30 A solution of *N*-[3-((*N*-benzyloxycarbonyl)alanyl)amino]-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (70mg, 0.125mmol), (prepared as described in Example 1), in ethanol (2ml) was hydrogenated at atmospheric pressure over 5% palladium on carbon (10mg) for 2 hours. Ethanol (3ml) was added and the solution was

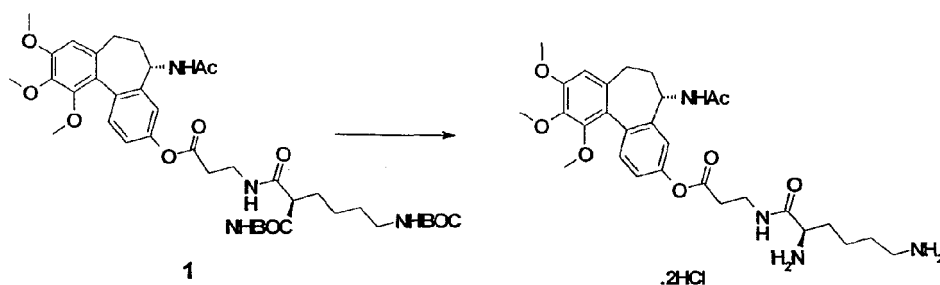
filtered through diatomaceous earth and the filtrate concentrated under reduced pressure.
 Trituration with ethyl acetate/diethyl ether gave the title compound (35mg) as a white solid.
 m.p. 170-173°C
 m/e 427

5

Example 3

N-[3-(4-(1-piperidinyl)piperidinylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5yl]acetamide

A solution of *N*-acetyl-colchicol (300mg, 0.84mmol), (J. Cech F. Santacy Collect.
 10 Czech Comm 1949, 4, 532-539), in pyridine (5ml) was treated with 4-piperidinopiperidine
 carbamoyl chloride (346mg, 1.5mmol), (K.H.Henegar et al. J.Org. Chem., 1997, 62, 6588-
 6597) and the solution heated at reflux for 1 hour. The cooled mixture was filtered and the
 filtrate concentrated under reduced pressure. The residue was purified on silica gel eluting
 with methanol to give the title compound (180mg) as a white solid.
 15 m.p. 168-175°C
 m/e 551

Example 4

20

A solution of (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
 dibenzo[*a,c*]cyclohepten-3-yl 3-{[(2*R*)-2,6-
 di(*tert*butoxycarbonylamino)hexanoyl]amino}propanoate (1) (0.123 g ; 0.162 mmol) in
 dichloromethane (10 ml) was treated with a 4.8M solution of hydrogen chloride in ether (170
 25 μ l ; 0.81 mmol). The mixture was stirred at ambient temperature for 1 hour and the resulting
 precipitate was filtered, washed with ether and dried to give (5*S*)-5-(acetylamino)-9,10,11-

- 59 -

trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[[*(2R)*-2,6-diaminohexanoyl]amino}propanoate as a white solid.

Yield : 84%

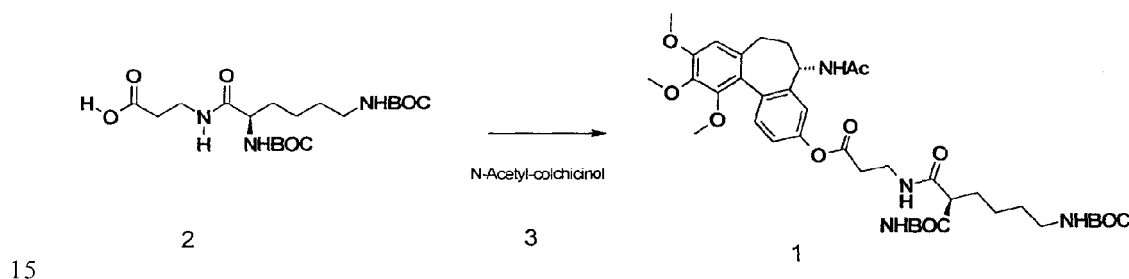
¹H NMR spectrum (DMSO-d₆) : 1.39 (m, 2H) ; 1.58 (m, 2H) ; 1.74 (m, 2H) ; 1.89 (s, 3H) ;

5 1.89 (m, 1H) ; 2.02 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 2.74 (m, 2H) ; 2.84 (t, 2H) ; 3.52 (m, 5H) ; 3.78 (s, 3H) ; 3.78 (m, 1H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 6.80 (s, 1H) ; 7.1-7.15 (m, 2H) ; 7.35 (dd, 1H) ; 8.01 (br s, 2H) ; 8.32 (m, 2H) ; 8.53 (d, 1H) ; 8.96 (t, 1H).

MS-ESI : 557 [MH]⁺

10	Elemental analysis :	Found	C 53.8	H 6.8	N 8.7
	C ₂₉ H ₄₀ N ₄ O ₇ ; 0.8 H ₂ O, 2 HCl	Requires	C 53.9	H 7.2	N 8.1%

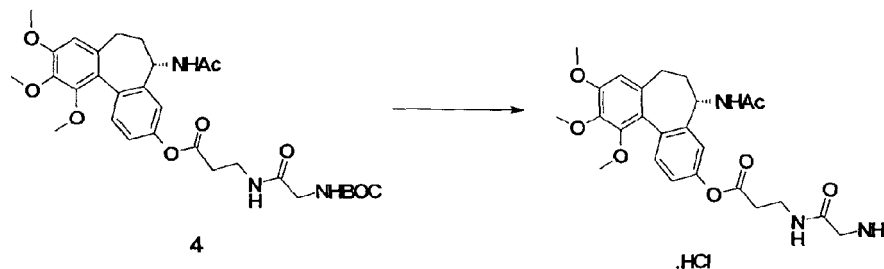
The starting material was prepared as follows:



A mixture of 3-[[*(2R)*-2,6-di(*tert*butoxycarbonylamino)hexanoyl]amino}propanoic acid (2) (0.178 g ; 0.5 mmol), DCCl (0.124 g ; 0.6 mmol), DMAP (0.013 g ; 0.1mmol) and *N*-acetyl-colchicinol (0.25 g ; 1.2 mmol) in dichloromethane was stirred under argon atmosphere
20 at ambient temperature for 5 hours. After filtration of the insoluble material the residue was purified by flash chromatography eluting with dichloromethane/ethanol (95/5) to give (1).

Yield : 32%

¹H NMR spectrum (DMSO-d₆) : 1.15-1.6 (m, 6H) ; 1.36 (s, 18H) ; 1.87 (s, 3H) ; 1.87 (m, 1H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.50 (m, 1H, signal obscured partially by DMSO peak) ; 2.75 (t, 2H) ; 2.86 (m, 2H) ; 3.85 (m, 2H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 3.85 (m, 1H) ;
25 4.55 (m, 1H) ; 3.7-3.8 (m, 2H) ; 6.8 (s, 1H) ; 7.08 (s, 1H) ; 7.1 (m, 1H) ; 7.32 (dd, 1H) ; 8.01 (t, 1H) ; 8.35 (d, 1H).

Example 5

5

A solution of (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[(2-*tert*butoxycarbonylaminoacetyl)amino]propanoate (4) (0.36 g ; 0.61 mmol) in dichloromethane (5 ml) was treated with a 4.8M solution of hydrogen chloride in ether (1 ml). The mixture was stirred at ambient temperature for 1 hour. After
 10 dilution with ether, the resulting precipitate was filtered, washed with ether and dried to give (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[(2-aminoacetyl)amino]propanoate.

Yield : 98%

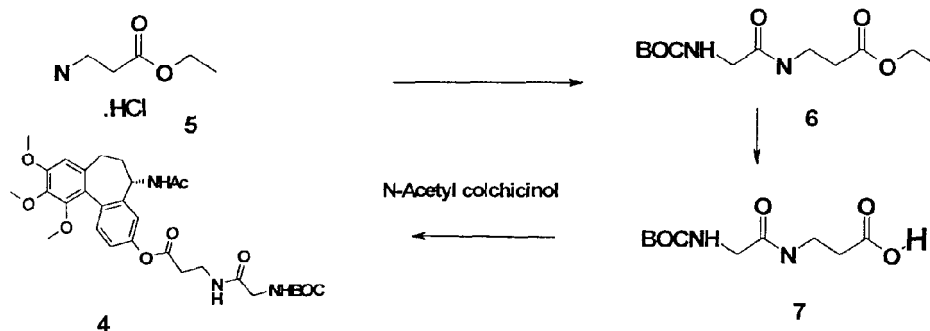
¹H NMR spectrum (DMSO-d₆) : 1.90 (m, 1H) ; 1.90 (s, 3H) ; 2.05 (m, 1H) ; 2.3 (m, 1H) ; 2.9
 15 (m, 1H, signal obscured partially by DMSO peak) ; 2.84 (t, 2H) ; 3.52 (s, 3H) ; 3.52 (m, 2H) ; 3.6 (m, 2H, signal obscured partially by H₂O peak) ; 3.8 (s, 3H) ; 3.86 (s, 3H) ; 4.55 (m, 1H) ; 6.82 (s, 1H) ; 7.13 (m, 2H) ; 7.37 (dd, 1H) ; 8.1 (br s, 2H) ; 8.46 (d, 1H) ; 8.67 (t, 1H).

MS-ESI : 486 [MH]⁺

Elemental analysis :	Found	C 54.8	H 6.2	N 7.7
20 C ₂₅ H ₃₁ N ₃ O ₇ ; 0.8 H ₂ O, 1.3 HCl	Requires	C 54.8	H 6.3	N 7.6%

The starting material was prepared as follows:

- 61 -



A solution of β -alanine ethyl ester hydrochloride salt (5) (3.07 g ; 0.02 mmol), *N*-(*tert*butoxycarbonyl)glycine (3.5 g ; 0.02 mmol), DCCI (4.12 g ; 0.02mmol) and 4-methylmorpholine (2.2 ml) in dichloromethane (60 ml) was stirred overnight under argon atmosphere at ambient temperature. After filtration the residue was purified by flash chromatography eluting with dichloromethane/ethanol (96/4) to give ethyl 3-[(2-*tert*butoxycarbonylaminoacetyl)amino]propanoate (6).

Yield : 62%

¹H NMR spectrum (CDCl₃) : 1.27 (t, 3H) ; 1.45 (s, 9H) ; 3.55 (m, 2H) ; 3.77 (d, 2H) ; 4.15 (q, 2H) ; 5.3 (br s, 2H) ; 6.56 (br s, 2H).

A solution of (6) at 0°C (3.43 g ; 0.012 mmol) in methanol (40 ml) was treated with 2N sodium hydroxide (6.9 ml ; 0.013 mmol). The mixture was stirred at ambient temperature for 90 minutes. After evaporation of the methanol and removal of the insoluble material by filtration, the solution was adjusted to pH5 with 6N hydrochloric acid. The mixture was extracted with dichloromethane and the organic layer evaporated to dryness to give 3-[(2-*tert*butoxycarbonylaminoacetyl)amino]propanoic acid (7).

Yield : 16%

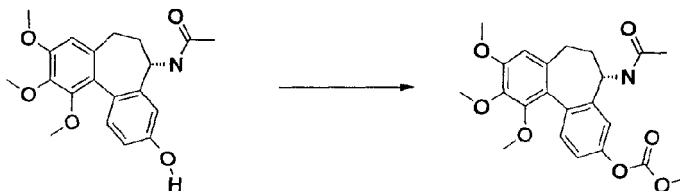
¹H NMR spectrum (CDCl₃ + CD₃CO₂D) : 1.44 (s, 9H) ; 2.61 (t, 2H) ; 3.5 (m, 2H) ; 3.80 (m, 2H).

A mixture of *N*-acetyl-colchicinol (0.357 g ; 1 mmol), DCCI (0.248 g ; 1.2 mmol), DMAP (0.025 g ; 0.2 mmol) and (7) (0.246 g ; 1 mmol) in dichloromethane (7 ml) was stirred under argon atmosphere for 5 hours. After removal of the insoluble material by filtration the residue was purified by flash chromatography eluting with dichloromethane/ethanol (92/8) to give (4) as a foam.

- 62 -

Yield : 61%

¹H NMR spectrum (DMSO-d₆) : 1.4 (s, 9H) ; 1.89 (s, 3H) ; 1.89 (m, 1H) ; 2.07 (m, 1H) ; 2.18 (m, 1H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 2.77 (t, 2H) ; 3.45 (m, 2H) ; 3.52 (s, 3H) ; 3.5 (m, 2H) ; 3.8 (s, 3H) ; 3.85 (s, 3H) ; 4.55 (m, 1H) ; 6.81 (s, 1H) ; 6.97 (t, 1H) ; 7.11 (m, 2H) ; 7.35 (dd, 1H) ; 8.01 (t, 1H) ; 8.38 (d, 1H).

Example 6

10

Triethylamine (70 μ l ; 0.5 mmol) and methyl chloroformate (39 μ l ; 0.5 mmol) were added to a solution of *N*-acetyl-colchicinol (0.178 g ; 0.5 mmol) in THF (3 ml). The mixture was stirred at ambient temperature for 90 minutes. After removal of the insoluble material by filtration the residue was purified by flash chromatography eluting with dichloromethane/ethanol (98/2) to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl methyl carbonate**.

Yield : 75%

¹H NMR spectrum (DMSO-d₆) : 1.87 (s, 3H) ; 1.87 (m, 1H) ; 2.05 (m, 1H) ; 2.17 (m, 1H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.52 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 3.85 (s, 3H) ; 4.52 (m, 1H) ; 6.8 (s, 1H) ; 7.16-7.18 (m, 2H) ; 7.36 (dd, 1H) ; 8.38 (d, 1H).

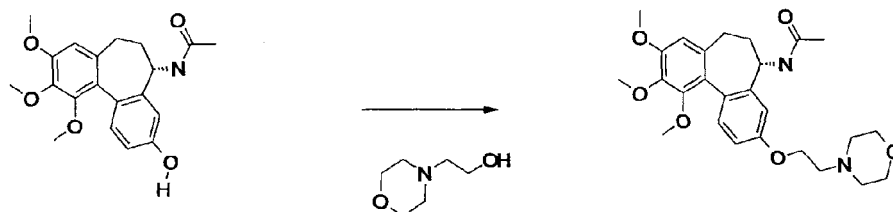
MS-ESI : 438 [MH]⁺

Elemental analysis :	Found	C 61.7	H 6.2	N 3.3
C ₂₂ H ₂₅ NO ₇ ; 0.7 H ₂ O	Requires	C 61.6	H 6.2	N 3.4%

25

Example 7

- 63 -



DEAD (0.118 g ; 0.75 mmol), triphenylphosphine (0.196 g ; 0.75 mmol) and 4-(2-hydroxyethyl)morpholine (61 μ l ; 0.5 mmol) were added to a solution of *N*-acetyl-colchicinol (0.178 g ; 0.5 mmol) in dichloromethane (5 ml) under argon atmosphere. The mixture was stirred at ambient temperature for 6 hours. After evaporation the residue was purified by flash chromatography eluting with a gradient of 2-10% ethanol/dichloromethane to give *N*-[(5*S*)-3-(2-morpholinoethoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide.

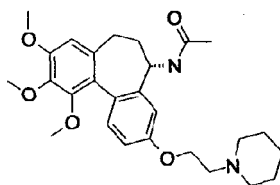
Yield : 37%

¹H NMR spectrum (DMSO-d₆) : 1.85 (m, 1H) ; 1.87 (s, 3H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.39 (m, 2H) ; 2.5 (m, 3H, signal obscured partially by DMSO peak) ; 2.72 (t, 2H) ; 3.46 (s, 3H) ; 3.54-3.6 (s, 4H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.09-4.12 (m, 2H) ; 4.55 (m, 1H) ; 6.76 (s, 1H) ; 6.86-6.90 (m, 2H) ; 7.23 (dd, 2H) ; 8.35 (d, 1H).

MS-ESI : 471 [MH]⁺

Elemental analysis :	Found	C 66.4	H 7.3	N 6.0
C ₂₆ H ₃₄ N ₂ O ₆	Requires	C 66.6	H 7.3	N 6.3%

20 Example 8



Using an analogous procedure to that described for Example 7, *N*-acetyl-colchicinol was reacted with 4-(2-hydroxyethyl)piperidine to give *N*-[(5*S*)-3-(2-piperidinoethoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide.

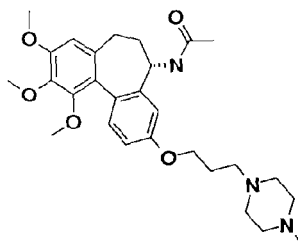
Yield : 20%

¹H NMR spectrum (DMSO-d₆) : 1.39 (m, 2H) ; 1.49 (m, 4H) ; 1.80 (m, 1H) ; 1.88 (s, 3H) ;
2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.45 (m, 4H) ; 2.5 (m, 1H, signal obscured partially by DMSO
peak) ; 2.67 (m, 2H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.08 (t, 2H) ; 4.55 (m, 1H) ;
5 6.76 (s, 1H) ; 6.86-6.9 (m, 2H) ; 7.22 (dd, 1H) ; 8.35 (d, 1H).

MS-ESI : 469 [MH]⁺

Elemental analysis :	Found	C 68.7	H 7.8	N 5.9
C ₂₇ H ₃₆ N ₂ O ₅ ; 0.2 H ₂ O	Requires	C 68.5	H 8.0	N 6.1%

10 Example 9



Using an analogous procedure to that described for Example 7, *N*-acetyl-colchicinol
was reacted with 4-(3-hydroxypropyl)-1-methylpiperazine to give *N*-[(5*S*)-3-(3-(4-
15 methylpiperazin-1-yl)propoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
dibenzo[*a,c*]cyclohepten-5-yl]acetamide.

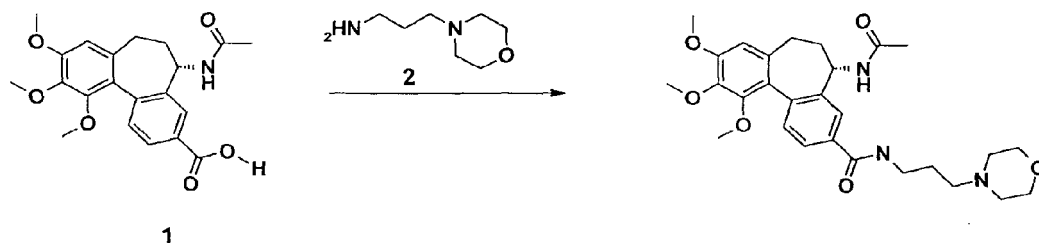
Yield : 22%

¹H NMR spectrum (DMSO d₆) : 1.88 (s, 3H) ; 1.85-1.9 (m, 3H) ; 2.04-2.16 (m, 5H) ; 2.32-
2.53 (m, 11H, signals obscured partially by DMSO peak) ; 3.47 (s, 3H) ; 3.78 (s, 3H) ; 3.83 (s,
20 3H) ; 4.03 (t, 2H) ; 4.55 (m, 1H) ; 6.72 (s, 1H) ; 6.85 (dd, 1H) ; 6.9 (m, 1H) ; 7.23 (d, 1H) ;
8.23 (d, 1H).

MS-ESI : 498 [MH]⁺

Elemental analysis :	Found	C 64.8	H 8.0	N 8.1
C ₂₈ H ₃₉ N ₃ O ₅ ; 0.8 H ₂ O,	Requires	C 64.7	H 7.7	N 8.2%

25 0.1 dichloromethane

Example 10

5 A solution of *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (0.3 g ; 0.779 mmol), (Med. Chem. Res. 1991, 142), DCCI (0.322 g ; 1.55 mmol), DMAP (0.069 g ; 0.389mmol) and 4-(3-aminopropyl)morpholine (170 μ l ; 1.17 mmol) in dichloromethane (6 ml) was stirred under argon atmosphere overnight. After removal of the insoluble material by filtration, the residue
 10 was purified on reverse phase silica eluting with a gradient of 40-50% methanol/ammonium carbonate buffer (2 g/l, pH7). The appropriate fractions were evaporated to dryness and triturated in ether to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-*N*-(3-morpholinopropyl)-6,7-dihydro-5*H*-dibenzo[*a,c*]cycloheptene-3-carboxamide** as a white solid.

15 Yield : 30%

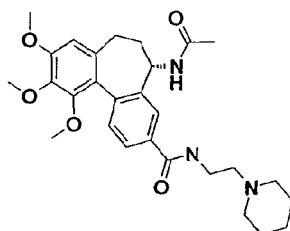
¹H NMR spectrum (DMSO-d₆) : 1.7 (m, 2H) ; 1.91 (s, 3H) ; 1.91 (m, 1H) ; 2.05 (m, 1H) ; 2.2 (m, 1H) ; 2.37 (m, 6H) ; 2.5 (m, 3H, signal obscured partially by DMSO peak) ; 3.51 (s, 3H) ; 3.58 (m, 4H) ; 3.8 (s, 3H) ; 3.86 (s, 3H) ; 4.58 (m, 1H) ; 6.83 (s, 1H) ; 7.39 (dd, 1H) ; 7.74 (m, 1H) ; 7.84 (s, 1H) ; 8.51 (m, 2H).

20 MS-ESI : 512 [MH]⁺

Elemental analysis :	Found	C 64.8	H 7.3	N 8.1
C ₂₈ H ₃₇ N ₃ O ₆ ; 0.4 H ₂ O	Requires	C 64.5	H 7.3	N 8.0%

Example 11

- 66 -



Using an analogous procedure to that described for Example 10, *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide was reacted with 1-(2-aminoethyl)piperidine to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-*N*-(2-piperidinoethyl)-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-carboxamide**.

Yield : 43%

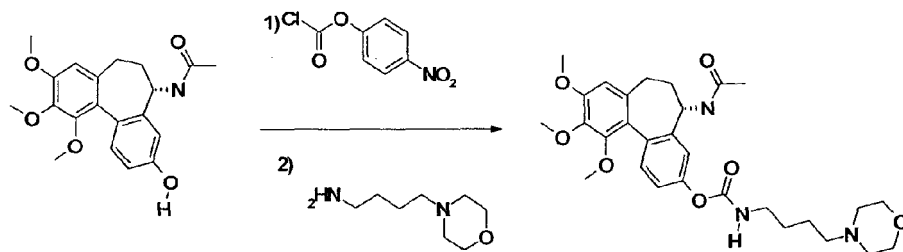
¹H NMR spectrum (DMSO-d₆) : 1.38 (m, 2H) ; 1.49 (m, 4H) ; 1.89 (s, 3H) ; 1.89 (m, 1H) ;
2.05 (m, 1H) ; 2.18 (m, 1H) ; 2.4-2.5 (m, 4H) ; 2.5 (m, 1H, signal obscured partially by
10 DMSO peak) ; 3.38 (m, 4H) ; 3.49 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.58 (m, 1H) ; 6.81
(s, 1H) ; 7.37 (d, 1H) ; 7.71 (m, 1H) ; 7.81 (s, 1H) ; 8.35 (t, 1H) ; 8.49 (d, 1H).

MS-ESI : 496 [MH]⁺

Elemental analysis :	Found	C 66.4	H 7.6	N 8.3
$C_{28}H_{37}N_3O_5$; 0.6 H_2O	Requires	C 66.1	H 7.7	N 8.3%

15

Example 12



20 A solution of *N*-acetyl-colchicinol (0.357 g ; 1 mmol), 4-nitrophenyl chloroformate (0.262g; 1.3 mmol) and triethylamine (182 μ l ; 1.3 mmol) in dichloromethane (10 ml) was stirred, under argon atmosphere, at ambient temperature for 90 minutes. 4-(4-Aminobutyl)morpholine (0.237g ; 1.5 mmol) was then added and the mixture was further

stirred for 4 hours. After evaporation to dryness the residue was purified by flash chromatography eluting with a gradient of 0-12% ethanol/dichloromethane to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl *N*-(4-morpholinobutyl)carbamate** as a white foam.

5 Yield : 24%

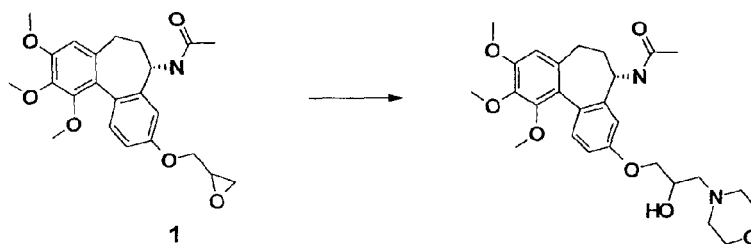
¹H NMR spectrum (DMSO-d₆) : 1.49 (m, 4H) ; 1.86 (s, 3H) ; 1.86 (m, 1H) ; 2.05 (m, 1H) ; 2.18 (m, 1H) ; 2.26-2.34 (m, 6H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.08 (m, 2H) ; 3.51 (s, 3H) ; 3.57 (m, 4H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 6.79 (s, 1H) ; 7.02 (m, 2H) ; 7.29 (d, 1H) ; 7.78 (t, 1H) ; 8.39 (d, 1H).

10 MS-ESI : 542 [MH]⁺

Elemental analysis :	Found	C 63.5	H 7.3	N 7.7
C ₂₉ H ₃₉ N ₃ O ₇ ; 0.4 H ₂ O	Requires	C 63.4	H 7.3	N 7.7%

Example 13

15



A solution of *N*-[(5*S*)-3-(2,3-epoxypropoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (**1**) (0.092 g ; 0.22 mmol) and morpholine (40 μl ; 0.44 mmol) in methanol was heated at reflux for 4 hours. After evaporation to dryness the residue was purified by flash chromatography eluting with dichloromethane/ethanol (90/10) to give *N*-[(5*S*)-3-(2-hydroxy-3-morpholinopropoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide as a yellow foam.

Yield : 55%

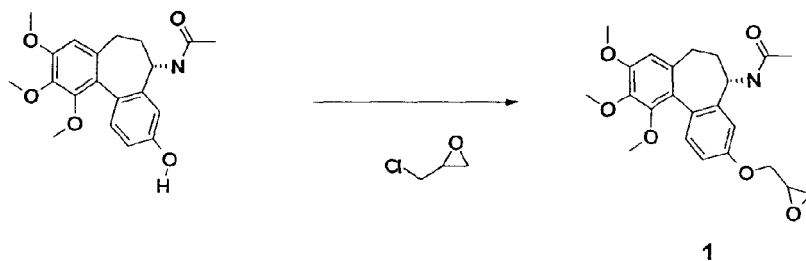
25 ¹H NMR spectrum (DMSO-d₆) : 1.88 (s, 3H) ; 1.88 (m, 1H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.42-2.5 (m, 4H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.33-3.4 (m, 4H) ;

3.46 (s, 3H) ; 3.57 (m, 2H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 3.90 (m, 1H) ; 3.99 (m, 2H) ; 4.52 (m, 1H) ; 4.9 (t, 1H) ; 6.76 (s, 1H) ; 6.86-6.9 (m, 2H) ; 7.23 (d, 1H) ; 8.37 (d, 1H).

MS-ESI : 501 [MH]⁺

Elemental analysis :	Found	C 64.8	H 7.3	N 5.6
5 C ₂₇ H ₃₆ N ₂ O ₇	Requires	C 64.5	H 7.5	N 5.5%

The starting material was prepared as follows:



10

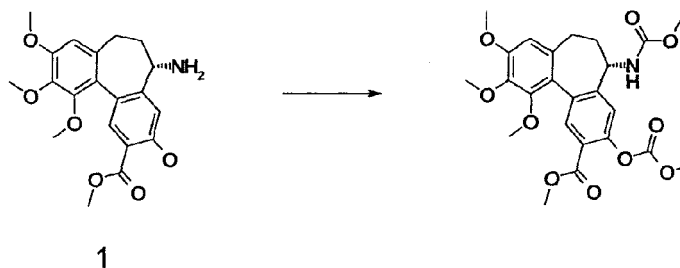
A solution of *N*-acetyl-colchicinol (0.179 g ; 0.5 mmol), potassium carbonate (0.083g; 0.6 mmol) and epichlorohydrin (0.059 g ; 0.75 mmol) in DMF (2 ml) was heated at 80°C for 5 hours. The mixture was poured into saturated sodium hydrogen carbonate and extracted with ethyl acetate. The organic phase was washed with brine, dried (MgSO₄) and evaporated to
 15 give an oil which was purified by flash chromatography eluting with a 2-4% gradient of ethanol/dichloromethane to give (1).

Yield : 46%

¹H NMR spectrum (DMSO-d₆) : 1.88 (s, 3H) ; 1.84 (m, 1H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H partially obscured by DMSO peak) ; 2.75 (m, 1H) ; 2.88 (m, 1H) ; 3.42 (m, 1H) ; 3.46
 20 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 3.87 (m, 1H) ; 4.35 (m, 1H) ; 4.52 (m, 1H) ; 6.76 (s, 1H) ; 6.88-6.94 (m, 2H) ; 7.24 (d, 1H) ; 8.35 (d, 1H).

Example 14

- 69 -



A solution of methyl (5*S*)-9,10,11-trimethoxy-5-amino-3-hydroxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cycloheptene-2-carboxylate (1) (0.373 g; 1 mmol) methyl chloroformate (0.17 ml ; 2.2 mmol) and triethylamine was stirred at ambient temperature overnight. After

5 evaporation to dryness, the residue was purified by flash chromatography, eluting with ethanol/dichloromethane (2/98) and further purified by preparative HPLC on reverse phase silica eluting with methanol/ammonium carbonate buffer (2 g/l pH7) (50/50) to give **methyl (5*S*)-9,10,11-trimethoxy-5-[(methoxycarbonyl)amino]-3-[(methoxycarbonyl)oxy]-6,7-dihydro-5*H*-dibenzo[*a,c*]cycloheptene-2-carboxylate**.

10 Yield : 48 %

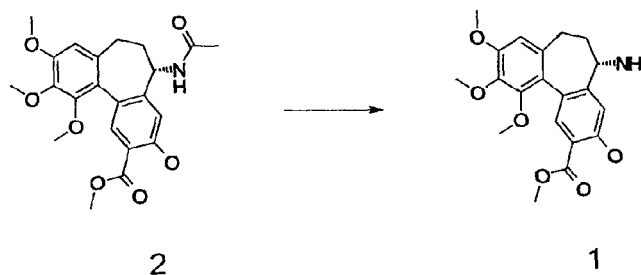
¹H NMR spectrum (DMSO-d₆) : 1.84-2.09 (m, 2H) ; 2.19-2.31 (m, 1H) ; 2.57 (m, 1H, partially obscured by DMSO peak) ; 3.50 (s, 3H) ; 3.55 (s, 3H) ; 3.81 (s, 3H) ; 3.82 (s, 3H) ; 3.86 (s, 3H) ; 3.87 (s, 3H) ; 4.28-4.389 (m, 1H) ; 6.85 (s, 1H) ; 7.24 (s, 1H) ; 7.88-7.97 (m, 1H) ; 7.91 (s, 1H).

15 MS-ESI : 512 [MNa]⁺

Elemental analysis	Found	C 58.7	H 5.7	N 3.0
C ₂₄ H ₂₇ NO ₁₀	Requires	C 58.9	H 5.6	N 2.9%

The starting material was prepared as follows:

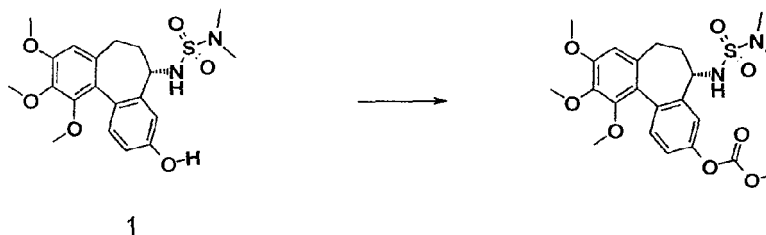
20



A solution of methyl (5*S*)-9,10,11-trimethoxy-5-acetylamino-3-hydroxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cycloheptene-2-carboxylate (2) (Collect. Czech. Chem. Communi. **64**, 217 (1999)) in a mixture of 6*N* hydrochloric acid and methanol (30/70) was heated at reflux for 8 hours. The mixture was adjusted to pH8 by addition of sodium carbonate. Extraction with dichloromethane and purification by flash chromatography (elution with dichloromethane /methanol (94/6)) gave (1) as a foam.

¹H NMR spectrum (DMSO-*d*₆) : 1.61 (m, 1H) ; 2.04 (m, 1H) ; 2.28 (m, 1H) ; 2.45 (m, 1H) ; 3.50 (s, 3H) ; 3.54 (m, 1H) ; 3.77 (s, 3H) ; 3.83 (s, 3H) ; 3.90 (s, 3H) ; 6.77 (s, 1H) ; 7.30 (s, 1H) ; 7.73 (s, 1H) ; 1.57 (br s, 1H).

Example 15



15

A solution of (5*S*)-5-[[[(dimethylamino)sulphonyl]amino]-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-ol (1) (0.9 ; 0.71 mmol), methyl chloroformate (0.061 ml ; 0.782 mmol) and triethylamine (0.109 ml ; 0.782 mmol) in acetonitrile (8 ml) was stirred under argon atmosphere at 40°C for 4 hours. After evaporation to dryness, the residue was purified by flash chromatography eluting with ethanol/dichloromethane (2/98) to give (5*S*)-5-[[[(dimethylamino)sulphonyl]amino]-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl methyl carbonate.

Yield : 32 %

¹H NMR spectrum (DMSO-*d*₆) : 1.93-2.03 (m, 2H) ; 2.12-2.17 (m, 1H) ; 2.46 (s, 6H) ; 2.45-2.55 (m, 1H) ; 3.46 (s, 3H) ; 3.79 (s, 3H) ; 3.85 (s, 3H) ; 3.87 (s, 3H) ; 3.94-4.10 (m, 1H) ; 6.82 (s, 1H) ; 7.21 (d, 1H) ; 7.37 (d, 1H) ; 7.43 (s, 1H) ; 7.93 (br s, 1H).

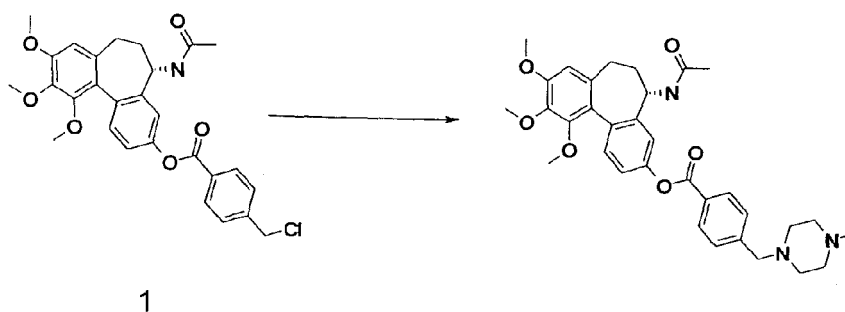
MS-ESI : 503 [MNa]⁺

- 71 -

Elemental analysis	Found	C 55.2	H 6.1	N 5.8	S 6.3
$C_{22}H_{28}N_2O_8S$	Requires	C 55.0	H 5.9	N 5.8	S 6.7%

Example 16

5



A solution of *N*-[(5*S*)-3-(4-chloromethylphenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) (0.308 g ; 0.604 mmol), 1-methylpiperazine (0.088 ml ; 0.785 mmol) and sodium iodide (0.02 g ; 0.121 mmol) in acetonitrile (10 ml) was stirred under argon atmosphere overnight. After evaporation to dryness, the residue was purified by flash chromatography eluting with a 5-12 % gradient of methanol/dichloromethane. After evaporation of the appropriate fractions, the solid was triturated in ether/pentane to give *N*-[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide as a solid.

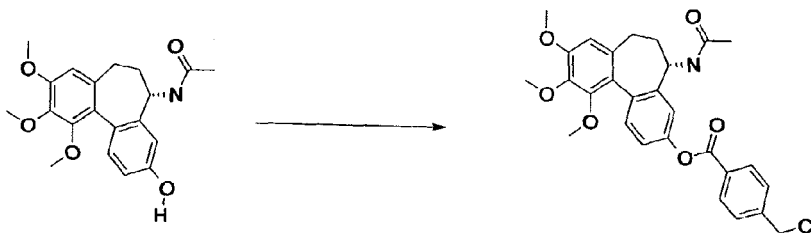
Yield : 75 %

^1H NMR spectrum (DMSO-d_6) : 1.86 (s, 3H) ; 1.83-1.98 (m, 1H) ; 2.00-2.26 (m, 2H) ; 2.31 (br s, 3H) ; 2.4-2.6 (m, 8H) ; 2.53-2.59 (m, 1H) ; 3.54 (s, 3H) ; 3.62 (s, 2H) ; 3.80 (s, 3H) ; 3.85 (s, 3H) ; 4.53-4.64 (m, 1H) ; 6.82 (s, 1H) ; 7.20-7.25 (m, 2H) ; 7.41 (d, 1H) ; 7.56 (d, 2H) ; 8.13 (d, 2H) ; 8.39 (d, 1H).

MS-ESI : 574 $[\text{MH}]^+$

The starting material was prepared as follows

- 72 -



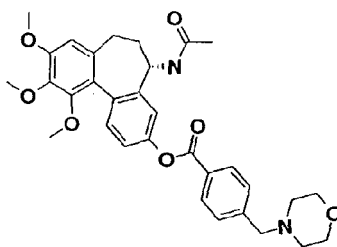
A solution of *N*-acetyl-colchicinol (0.357 g ; 1 mmol), EDCI (0.23 g ; 1.2 mmol), DMAP (0.025 g ; 0.2 mmol) and 4-chloromethylbenzoic acid (0.205 g ; 1.2 mmol) in dichloromethane (8 ml) was stirred under argon atmosphere overnight. After evaporation to dryness, the residue was purified by flash chromatography eluting with dichloromethane/ethanol (98/2) to give (1).

Yield : 72 %

¹H NMR spectrum (DMSO-d₆) : 1.86 (s, 3H) ; 1.91 (m, 1H) ; 1.04-2.14 (m, 1H) ; 2.14-2.67 (m, 1H) ; 2.57 (m, 1H), partially obscured by DMSO peak ; 3.54 (s, 3H) ; 3.80 (s, 3H) ; 3.86 (s, 3H) ; 4.54-4.64 (m, 1H) ; 4.91 (s, 2H) ; 6.83 (s, 1H) ; 7.21-7.28 (m, 2H) ; 7.42 (d, 1H) ; 7.70 (d, 2H) ; 8.14 (d, 2H) ; 8.40 (d, 1H).

Example 17

15



Using an analogous procedure to that described for Example 16, *N*-[(5*S*)-3-(4-chloromethylphenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide was reacted with morpholine to give *N*-[(5*S*)-3-(4-{morpholinomethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide.

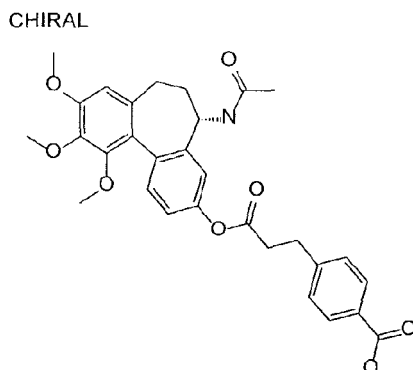
Yield : 86 %

¹H NMR spectrum (DMSO-d₆) : 1.90 (s, 3H) ; 1.88-2.01 (m, 1H) ; 2.06-2.30 (m, 2H) ; 2.43 (br s, 4H) ; 2.54-2.63 (m, 1H) ; 3.30 (m, 2H) ; 3.58 (s, 3H) ; 3.62-3.67 (m, 6H) ; 3.84 (s, 3H) ; 3.89 (s, 3H) ; 4.57-4.67 (m, 1H) ; 3.86 (s, 1H) ; 7.23-7.30 (m, 2H) ; 7.45 (d, 1H) ; 7.61 (d, 1H) ; 8.17 (d, 1H) ; 8.43 (d, 1H).

MS-ESI : 561 [MH]⁺

Elemental analysis	Found	C 66.1	H 6.5	N 4.9
C ₃₂ H ₃₆ N ₂ O ₇ ; 0.3 dichloromethane	Requires	C 66.2	H 6.3	N 4.8%

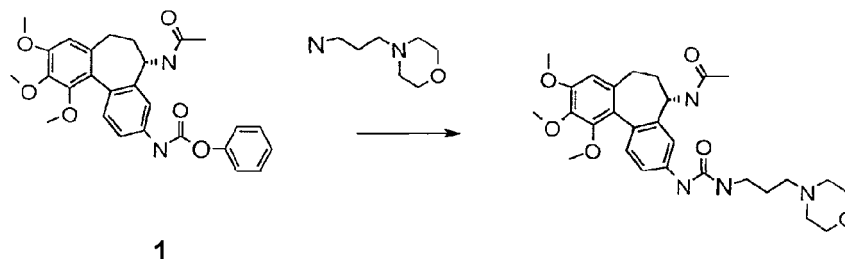
10 **Example 18**



A solution of *N*-acetyl-colchicininol (0.357 g ; 1 mmol), EDCI (0.23 g ; 1.2 mmol), DMAP (0.025 g ; 0.2 mmol) and 3-(4-carboxyphenyl)propionic acid (0.233 g ; 1.2 mmol) was stirred at ambient temperature overnight. After removal of the solvent by evaporation, the residue was purified by preparative HPLC on reverse phase silica eluting with methanol/ammonium carbonate buffer (2 g/l pH7) (50/50) to give **4-(3-[(5S)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl]oxy-3-oxopropyl)benzoic acid**.

20 Yield : 70%

¹H NMR spectrum (DMSO-d₆) : 1.82-1.93 (m, 4H) ; 1.97-2.22 (m, 2H) ; 2.39-2.63 (m, 1H) ; 2.93-2.99 (m, 2H) ; 2.99-3.06 (m, 2H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 3.47-3.56 (m, 1H) ; 6.89 (s, 1H) ; 6.94 (d, 1H) ; 6.99 (dd, 1H) ; 7.30-7.37 (m, 3H) ; 7.85 (d, 2H) ; 8.46 (d, 1H).

MS-ESI : 534 [MH]⁺**Example 19**

5

A solution of *N*-[(5*S*)-3-phenoxy-carbonylamino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (**1**) (0.1 g ; 0.21 mmol) and 4-(3-aminopropyl)morpholine (0.095 g ; 0.66 mmol) in DMSO (1 ml) was stirred at ambient temperature for 1 hour. The mixture was purified by preparative HPLC on reverse phase silica eluting with methanol/ammonium carbonate buffer (2 g/l, pH7) (40/60) to give, after evaporation, *N*-[(5*S*)-9,10,11-trimethoxy-3-[(3-morpholinopropyl)amino]carbonylamino)-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide as a foam.

Yield : 84%

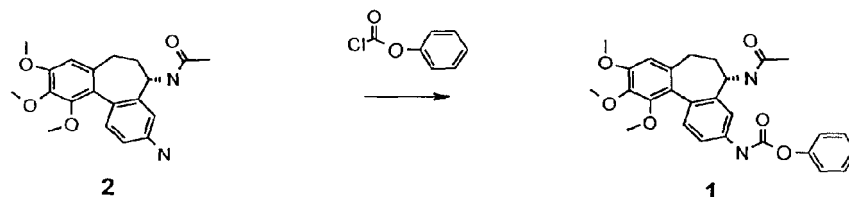
¹H NMR spectrum (DMSO-d₆) : 1.6 (m, 2H) ; 1.88 (s, 3H) ; 1.90 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.3-2.4 (m, 6H) ; 2.45 (m, 1H, signal obscured by DMSO peak) ; 3.15 (m, 2H) ; 3.47 (s, 3H) ; 3.6 (m, 4H) ; 3.78 (s, 3H) ; 3.83 (d, 3H) ; 4.47 (m, 1H) ; 6.13 (t, 1H) ; 6.76 (s, 1H) ; 7.16 (d, 1H) ; 7.29 (d, 1H) ; 7.37 (dd, 1H) ; 8.37 (d, 1H) ; 8.47 (s, 1H).

MS-ESI : 527 [MH]⁺

20	Elemental analysis :	Found	C 63.4	H 7.4	N 10.6
	C ₂₈ H ₃₈ N ₄ O ₆ ; 0.1 H ₂ O	Requires	C 63.6	H 7.3	N 10.6%

The starting material was prepared as follows:

- 75 -

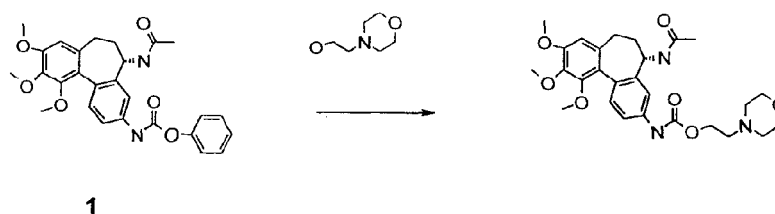


Pyridine (570 μl ; 7 mmol) and phenyl chloroformate (720 μl ; 10.3 mmol) were added to a solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (2) cooled at 0°C (2 g ; 5.6 mmol) in THF (40 ml), under argon atmosphere. The mixture was stirred at 0°C for 5 minutes and then at ambient temperature for 1 hour. The mixture was extracted with ethyl acetate. The organic phase was washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate and brine. After evaporation to dryness the solid was triturated with ether and hexane to give (1) as a solid.

10 Yield : 89%

¹H NMR spectrum (DMSO-*d*₆) : 1.82 (s, 3H) ; 1.85 (m, 1H) ; 2.10 (m, 2H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.47 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.48 (m, 1H) ; 6.77 (s, 1H) ; 7.20-7.50 (m, 7H) ; 7.55 (s, 1H) ; 8.38 (d, 1H) ; 9.31 (s, 1H).

15 **Example 20**



Using an analogous procedure to that described for Example 19, *N*-[(5*S*)-3-phenoxy-carbonylamino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) was reacted with 4-(2-hydroxyethyl)morpholine and the mixture was heated at 60°C for 2 hours to give *N*-[(5*S*)-3-(2-morpholinoethoxycarbonylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide.

Yield : 84%

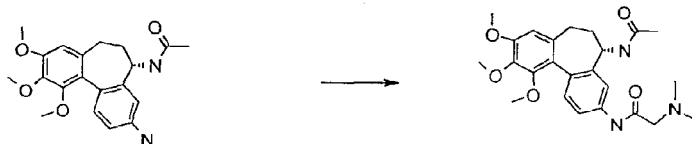
- 76 -

^1H NMR spectrum (DMSO-d_6) : 1.89 (s, 3H) ; 1.90 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.4-2.45 (m, 4H) ; 2.46 (m, 1H, signal obscured by DMSO peak) ; 2.6 (t, 2H) ; 3.42 (s, 3H) ; 3.59 (m, 4H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.22 (m, 2H) ; 4.44 (m, 1H) ; 6.78 (s, 1H) ; 7.22 (d, 1H) ; 7.39 (dd, 1H) ; 7.50 (s, 1H) ; 8.39 (d, 1H) ; 9.73 (s, 1H).

5 MS-ESI : 514 $[\text{MH}]^+$

Elemental analysis :	Found	C 60.4	H 6.6	N 8.0
$\text{C}_{27}\text{H}_{35}\text{N}_3\text{O}_7$; 1.1 H_2O	Required	C 60.8	H 7.0	N 7.9%

Example 21



10

1

A solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) (0.2 g ; 0.56 mmol), *N,N*-dimethylglycine (0.058 g ; 0.56 mmol), EDCI (0.14g ; 0.73 mmol) and DMAP (0.014 g ; 0.11 mmol) in
 15 dichloromethane (8 ml) was stirred at ambient temperature overnight. The mixture was washed with water and the organic phase was evaporated and purified by preparative HPLC on reverse phase silica eluting with methanol/ammonium carbonate buffer (2 g/l, pH7) (40/60) to give, after evaporation, *N*-[(5*S*)-3-(*N,N*-dimethylaminoacetyl)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide as a white foam.

20 Yield : 57%

^1H NMR spectrum (DMSO-d_6) : 1.90 (s, 3H) ; 1.93 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.31 (s, 6H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.09 (d, 1H) ; 3.10 (d, 1H) ; 3.48 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.5 (m, 1H) ; 6.78 (s, 1H) ; 7.25 (d, 1H) ; 7.6 (m, 2H) ; 8.4 (d, 1H) ; 9.73 (s, 1H).

25 MS-ESI : 442 $[\text{MH}]^+$

Elemental analysis :	Found	C 63.0	H 7.0	N 9.2
$\text{C}_{24}\text{H}_{31}\text{N}_3\text{O}_5$; 0.8 H_2O	Required	C 63.2	H 7.2	N 9.2%

Example 22

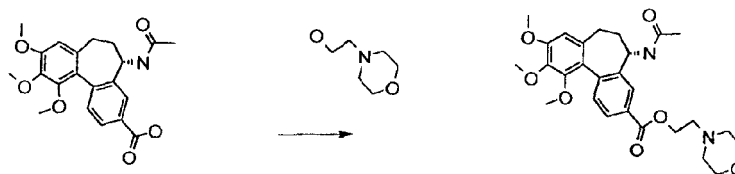
- 5 Using an analogous procedure to that described for Example 21, *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide was reacted with 1-piperidinepropionic acid to give *N*-[(5*S*)-3-(3-piperidinopropanoylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide.

Yield : 61%

- 10 ¹H NMR spectrum (DMSO-d₆) : 1.40 (m, 2H) ; 1.55 (m, 4H) ; 1.89 (s, 3H) ; 1.90 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.4 (br s, 4H) ; 2.45 (m, 2H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 2.62 (m, 2H) ; 3.47 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.45 (m, 1H) ; 6.78 (s, 1H) ; 7.24 (d, 1H) ; 7.5 (s, 1H) ; 7.58 (dd, 1H) ; 8.40 (d, 1H).

MS-ESI : 496 [MH]⁺

- | | | | | | |
|----|---|----------|--------|-------|--------|
| 15 | Elemental analysis : | Found | C 65.7 | H 7.4 | N 8.2 |
| | C ₂₈ H ₃₇ N ₃ O ₅ | Required | C 65.7 | H 7.6 | N 8.2% |

Example 23

1

20

- A solution of *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) (0.385 g ; 1 mmol), EDCI (0.248 g ; 1.3 mmol), DMAP (0.248 g ; 0.2 mmol) and 4-(2-hydroxyethyl)morpholine (127 μl ; 1.05 mmol) was
 25 stirred at ambient temperature overnight. After evaporation to dryness the residue was

purified by preparative HPLC on reverse phase silica eluting with a 40-60% gradient of methanol/ammonium carbonate buffer (2 g/l, pH7) to give, after evaporation, *N*-[(5*S*)-3-(2-morpholinoethoxycarbonyl)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide as a solid.

5 Yield : 47%

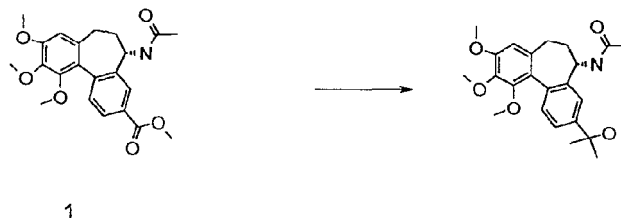
¹H NMR spectrum (DMSO-d₆) : 1.88 (s, 3H) ; 1.88-2.05 (m, 2H) ; 2.2 (m, 1H) ; 2.5 (m, 5H, signal obscured by DMSO peak) ; 2.7 (m, 2H) ; 3.5 (s, 3H) ; 3.6 (m, 4H) ; 3.79 (s, 3H) ; 3.85 (s, 3H) ; 4.4 (m, 2H) ; 4.55 (m, 1H) ; 6.82 (s, 1H) ; 7.47 (d, 1H) ; 7.89 (dd, 1H) ; 7.95 (d, 1H) ; 8.58 (d, 1H).

10 MS-ESI : 499 [MH]⁺

Elemental analysis :	Found	C 63.2	H 6.7	N 5.5
C ₂₇ H ₃₄ N ₂ O ₇ ; 0.6 H ₂ O	Required	C 65.0	H 6.9	N 5.6%

Example 24

15



A solution of methyllithium in ether (1.6 M ; 2.14 ml ; 3.4 mmol) was added at -78°C under argon atmosphere to dry THF (5 ml). After 5 minutes a solution of *N*-[(5*S*)-3-(methoxycarbonyl)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) (0.274 g ; 0.68 mmol) in THF (11 ml) was added. The mixture was stirred at -78°C for 30 minutes, allowed to warm up and further stirred at ambient temperature for 90 minutes. After removal of the solvents by evaporation, the residue was taken up in an aqueous ammonium chloride/ethyl acetate mixture and extracted. The organic phase was evaporated and purified by flash chromatography eluting with ethyl acetate to give *N*-[(5*S*)-3-(1-hydroxy-1-methylethyl)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide as a white foam.

25 Yield : 45%

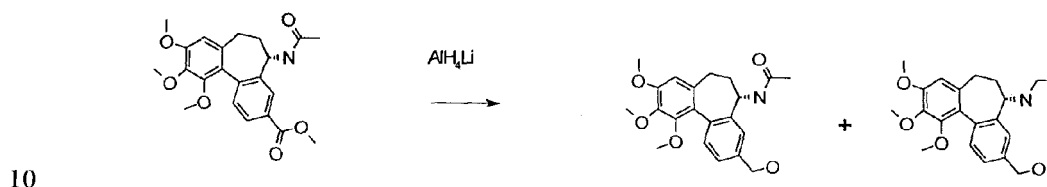
- 79 -

^1H NMR spectrum (DMSO-d_6) : 1.45 (s, 3H) ; 1.48 (s, 3H) ; 1.86 (m, 1H) ; 1.88 (s, 3H) ; 2.03 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.5 (s, 3H) ; 3.77 (s, 3H) ; 3.83 (s, 3H) ; 6.77 (s, 1H) ; 7.23 (d, 1H) ; 7.35 (dd, 1H) ; 7.5 (d, 1H) ; 8.43 (d, 1H).

MS-ESI : 422.1 $[\text{MNa}]^+$

5	Elemental analysis :	Found	C 66.5	H 7.3	N 3.5
	$\text{C}_{23}\text{H}_{29}\text{NO}_5$; 0.8 H_2O	Required	C 66.7	H 7.5	N 3.4%

Examples 25 and 26



A suspension of (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cycloheptene-3-carboxylate (2.28 ml ; 5.7 mmol) and lithium aluminium hydride (0.216 g ; 22.8 mmol) in a mixture of THF (10 ml) and ether (60 ml) was stirred at reflux under argon atmosphere overnight. After addition of water (60 ml), the mixture was stirred for 2 hours. The resulting solid was filtered and the filtrate was evaporated and purified by flash chromatography eluting with a 5-10% gradient of methanol/dichloromethane to give, after evaporation, *N*-[(5*S*)-3-hydroxymethyl-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (yield : 33%) and [(5*S*)-5-(ethylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]methanol (yield : 47%).

20

Example 25

^1H NMR spectrum (DMSO-d_6) : 1.86 (m, 1H) ; 1.87 (s, 3H) ; 2.01 (m, 1H) ; 2.14 (m, 1H) ; 2.48 (m, 1H, signal obscured by DMSO peak) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.83 (s, 3H) ; 4.54 (m, 3H) ; 5.21 (t, 1H) ; 6.78 (s, 1H) ; 7.27 (m, 2H) ; 7.32 (s, 1H) ; 8.42 (d, 1H).

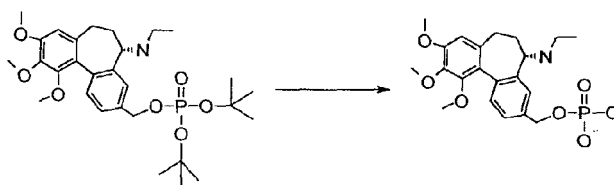
25 MS-ESI : 394.1 $[\text{MH}]^+$

Elemental analysis :	Found	C 66.0	H 6.8	N 3.7
$\text{C}_{21}\text{H}_{25}\text{NO}_5$; 0.5 H_2O	Requires	C 66.3	H 6.9	N 3.7%

Example 26

¹H NMR spectrum (DMSO-d₆) : 1.90 (s, 3H) ; 1.90 (m, 1H) ; 2.01 (m, 1H) ; 2.20 (m, 1H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.53 (s, 3H) ; 3.78 (s, 3H) ; 3.85 (s, 3H) ; 4.53 (m, 1H) ; 6.84 (s, 1H) ; 7.50 (d, 1H) ; 7.72 (d, 1H) ; 7.77 (dd, 1H) ; 8.45 (d, 1H).

5 MS-ESI : 389 [MNa]⁺

Example 27

1

A solution of (5*S*)-5-(ethylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
 10 dibenzo[*a,c*]cyclohepten-3-yl di*tert*butyl phosphate (1) (0.215 g ; 0.391 mmol) in 1M
 hydrogen chloride solution in 1,4-dioxane (2 ml) was stirred at ambient temperature
 overnight. After addition of ether (20 ml) the resulting precipitate was filtered, washed with
 ether and dried to give (5*S*)-5-(ethylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
 dibenzo[*a,c*]cyclohepten-3-yl dihydrogen phosphate.

15 Yield : 88%

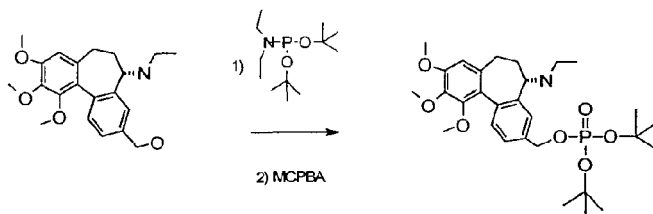
¹H NMR spectrum (DMSO-d₆) : 1.04 (t, 3H) ; 1.78 (m, 1H) ; 2.0 (m, 1H) ; 2.32 (m, 1H) ; 2.5 (m, 3H, signals obscured by DMSO peak) ; 3.48 (s, 3H) ; 3.5 (m, 1H,) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.79 (m, 3H) ; 6.78 (s, 1H) ; 7.27 (s, 2H) ; 7.66 (s, 1H).

MS-ESI : 460 [MH]⁺

20

The starting material was prepared as follows:

- 81 -



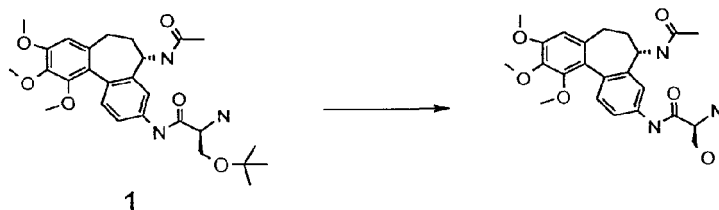
1

1H-Tetrazole (0.182 g ; 2.6 mmol) was added, under argon atmosphere, to a solution of [(5*S*)-5-(ethylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]methanol, (prepared as described in Example 26), (0.3 g ; 0.84 mmol) and di-*tert*-butyl diethylphosphoramidite (0.33 g ; 1.34 mmol) in dry THF (5.5 ml). After 5 minutes, the solution was cooled to -78°C and a solution of *m*-chloroperbenzoic acid (0.375 g ; 1.68 mmol) in dichloromethane (3 ml) was added in portions. The mixture was allowed to warm to ambient temperature and further stirred for 5 minutes. After addition of aqueous ammonium hydrogen carbonate and aqueous sodium sulphite, the organic solvent was removed by evaporation and the residue was taken up in dichloromethane. The organic phase was washed with water, dried and evaporated. The residue was purified by flash chromatography eluting with ethyl acetate/methanol (95/5) to give (1) as a foam.

Yield : 54 %.

¹H NMR spectrum (DMSO-d₆) : 1.03 (t, 3H) ; 1.43 (s, 18H) ; 1.72 (m, 1H) ; 2.0 (m, 1H) ; 2.35 (m, 1H) ; 2.50 (m, 3H, signal obscured by DMSO peak) ; 3.2-3.6 (m, 1H signal obscured by H₂O peak) ; 3.50 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.99 (m, 2H) ; 6.80 (s, 1H) ; 7.35 (m, 2H).

20 **Example 28**



1

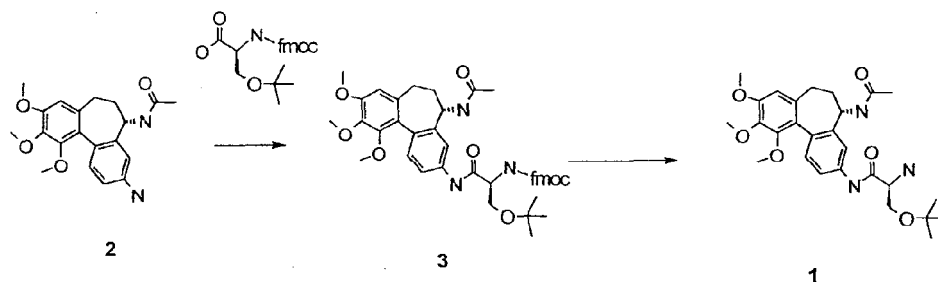
12N Hydrochloric acid (5ml) was added to a solution of (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-amino-3-*tert*butoxypropanamide (1) (0.35 g ; 0.7 mmol) in 1,4-dioxane (5 ml). The mixture was heated at 60°C under argon atmosphere for 1 hour. After dilution with ether, the resulting precipitate was filtered, washed with ether and dried to give (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-amino-3-hydroxypropanamide as a white solid.

Yield : 65%

¹H NMR spectrum (DMSO-d₆) : 1.90 (s, 3H) ; 1.95 (m, 1H) ; 2.05 (m, 1H) ; 2.18 (m, 1H) ; 2.50 (m, 1H, signal obscured by DMSO peak) ; 3.48 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 3.88 (m, 2H) ; 4.05 (m, 1H) ; 4.45 (m, 1H) ; 6.8 (s, 1H) ; 7.29 (d, 1H) ; 7.58 (d, 1H) ; 7.65 (dd, 1H) ; 8.32 (br s, 3H) ; 8.47 (d, 1H).

MS-ESI : 444 [MH]⁺

The starting material was prepared as follows:



O-(7-(Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (0.468 g ; 1.28 mmol) and *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (2) (0.398 g ; 1.12 mmol) were added to a solution of *N*-fmoc-*O-tert*-butyl-L-serine (0.428 g ; 1.12 mmol) in dichloromethane (18ml) and *N,N*-diisopropylethylamine (0.222 ml ; 1.28 mmol). The mixture was stirred overnight under argon atmosphere at ambient temperature. After addition of water, the organic phase was dried over MgSO₄ and evaporated to give (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-(fmoc-amino)-3-(*tert*butoxy)propanamide (3).

Yield : 95%

¹H NMR spectrum (DMSO-d₆) : 1.14 (s, 9H) ; 1.87 (s, 3H) ; 1.80 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.46 (s, 3H) ; 3.50-3.60 (m, 2H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.20-4.35 (m, 4H) ; 4.45 (m, 1H) ; 6.77 (s, 1H) ; 7.25 (d, 1H) ; 7.32 (m, 2H) ; 7.42 (m, 2H) ; 7.54 (m, 2H) ; 7.61 (s, 1H) ; 7.76 (m, 2H) ; 7.89 (m, 2H) ; 8.40 (d, 1H).

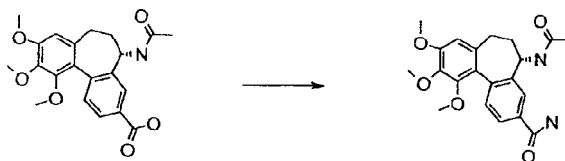
MS-ESI : 744 [MNa]⁺

A solution of (3) (0.75 g ; 1.04 mmol) and piperidine (1 ml) in dichloromethane (1.5 ml) was stirred at ambient temperature for 45 minutes. After evaporation to dryness the residue was purified by flash chromatography eluting with ethyl acetate/methanol (95/5) to give (1) as a foam.

Yield : 70%

¹H NMR spectrum (DMSO-d₆) : 1.14 (s, 9H) ; 1.87 (s, 3H) ; 1.89 (m, 1H) ; 1.90-2.15 (m, 2H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.46 (s, 3H) ; 3.40-3.50 (m, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.49 (m, 1H) ; 6.77 (s, 1H) ; 7.25 (d, 1H) ; 7.58 (m, 2H) ; 8.39 (d, 1H).
MS-ESI : 500.2 [MH]⁺

Example 29



Oxalyl chloride (0.44 g ; 3.4 mmol) and DMF (50 µl) were added to a suspension of *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) under argon atmosphere (1.15 g ; 3 mmol) in dichloromethane (10 ml). The mixture was stirred at ambient temperature for 2 hours, evaporated to dryness and redissolved in dichloromethane (20 ml). The solution was cooled at -78° C and ammonia gas was allowed to bubble through the solution for 5 minutes. The mixture was allowed to warm up and further stirred at ambient temperature for 15 minutes. After evaporation to dryness, the residue was taken up in aqueous sodium hydrogen carbonate/ethyl acetate. The organic phase was separated, evaporated and purified by flash chromatography eluting with ethyl

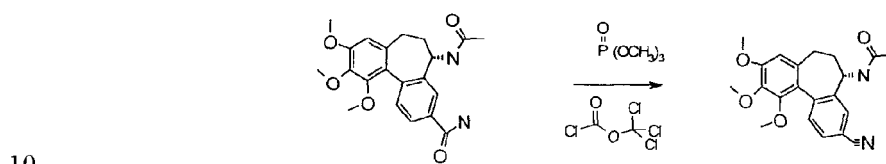
acetate/methanol (95/5) to give *N*-[(5*S*)-3-carbamoyl-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide.

Yield : 20 %

¹H NMR spectrum (DMSO-d₆) : 1.89 (s, 3H) ; 1.89 (m, 1H) ; 1.96 (m, 1H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.49 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.54 (m, 1H) ; 6.81 (s, 1H) ; 7.36 (d, 1H) ; 7.78 (dd, 1H) ; 7.87 (d, 1H) ; 7.96 (s, 1H) ; 8.47 (d, 1H).

MS-ESI : 407 [MNa]⁺

Example 30



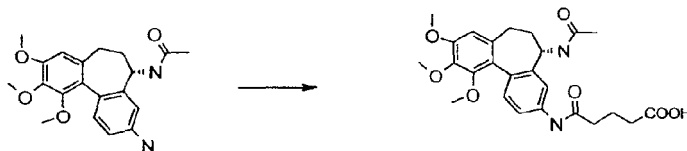
Trichloromethyl chloroformate (0.094 ml ; 0.77 mmol) was added in portions at 0°C to a solution of *N*-[(5*S*)-3-carbamoyl-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (0.2 g ; 0.484 mmol), (prepared as described for Example 29) and trimethyl phosphate (0.306 ml ; 2.6 mmol). The mixture was allowed to warm up and then heated at 60°C for 5 minutes. The reaction mixture was poured onto ice and stirred. The resulting precipitate was filtered, dried and purified by flash chromatography, eluting with dichloromethane/ethyl acetate (1/1) to give *N*-[(5*S*)-3-cyano-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide.

Yield : 61 %.

20 ¹H NMR spectrum (DMSO-d₆) : 0.97 (t, 3H) ; 1.60 (m, 1H) ; 1.95 (m, 2H) ; 2.30 (m, 2H) ; 2.45 (m, 1H, signal partially obscured by DMSO peak) ; 3.46 (s, 3H) ; 3.76 (s, 3H) ; 3.82 (s, 3H) ; 4.54 (m, 2H) ; 5.16 (t, 1H) ; 6.75 (s, 1H) ; 7.15-7.30 (m, 2H) ; 7.53 (s, 1H).

MS-ESI : 358 [MH]⁺

25 Example 31



A solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) (0.3 g ; 84 mmol) and glutaric anhydride (0.199 g ; 84 mmol) in dichloromethane (20 ml) was stirred at ambient temperature for 90 minutes.

- 5 After removal of the solvent by evaporation, the residue was purified by flash chromatography, eluting with dichloromethane /methanol (80/20) to give, after trituration in ether, *N*-[(5*S*)-3-(4-carboxybutanoylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide as a white solid.

Yield : 63 %

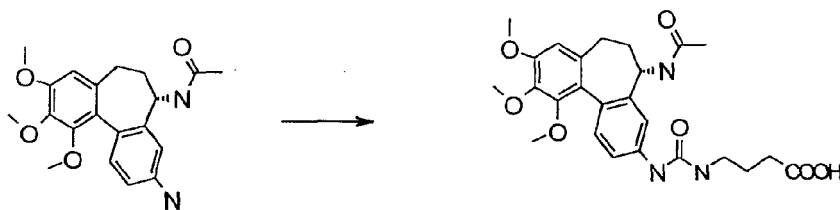
- 10 ¹H NMR spectrum (DMSO_d₆) : 1.8-2 (m, 3H) ; 1.88 (s, 3H) ; 2-2.25 (m, 2H) ; 2.26 (t, 2H) ; 2.36 (t, 2H) ; 2.5 (s, 1H, signal partially observed by DMSO peak) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.48 (m, 1H) ; 6.76 (s, 1H) ; 7.22 (d, 1H) ; 7.53 (d, 1H) ; 7.56 (s, 1H) ; 8.4 (d, 1H) ; 9.97 (s, 1H).

MS-ESI / 471 [MH]⁺

- | | | | | |
|---|----------|--------|-------|--------|
| 15 Elemental analysis | Found | C 59.2 | H 6.2 | N 5.4 |
| C ₂₅ H ₃₀ N ₂ O ₇ | Requires | C 63.8 | H 6.4 | N 6.0% |

Example 32

20



- A suspension of 4-aminobutyric acid (0.111 g ; 1.08 mmol) and *N,O*-bis(trimethylsilyl)acetamide (1.8 ml ; 7.3 mmol) in dichloromethane (10 ml) was stirred at ambient temperature for 2 hours. The mixture was evaporated to dryness and redissolved in dichloromethane (10 ml) under argon atmosphere. A solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (0.35 g ; 0.98 mmol), phenyl chloroformate (135 μ l ; 1.08 mmol) and triethylamine (151 μ l ; 1.08 mmol) in

dichloromethane (10 ml) was stirred for 1 hour under argon atmosphere and added to the above solution. The mixture was stirred overnight, evaporated and purified by preparative HPLC on reverse phase silica eluting with a 0-30 % gradient of methanol/ammonium carbonate buffer pH7 to give, after evaporation, 4-[[[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]aminocarbonyl]amino]butanoic acid as a solid

Yield : 50 %

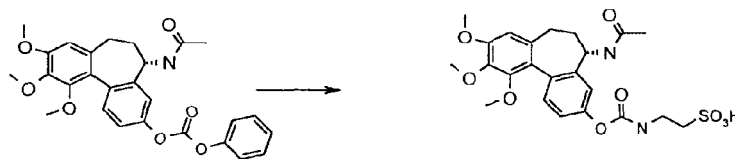
¹H NMR spectrum (DMSO-d₆, CF₃CO₂D) : 1.68 (m, 2H) ; 1.88 (s, 3H) ; 1.85-2 (m, 1H) ; 2-2.2 (m, 2H) ; 2.27 (m, 2H) ; 2.5 (m, 1H, signal partially observed by DMSO peak) ; 3.12 (m, 2H) ; 3.47 (s, 3H) ; 3.78 (s, 3H) ; 3.83 (s, 3H) ; 4.48 (m, 1H) ; 6.75 (s, 1H) ; 7.17 (d, 1H) ; 7.3 (s, 1H) ; 7.39 (d, 1H).

MS-ESI : 486 [MH]⁺

Elemental analysis	Found	C 58.3	H 6.5	N 8.7
C ₂₅ H ₃₁ N ₃ O ₇	Requires	C 61.8	H 6.4	N 8.7%

15

Example 33



20 A solution of *N*-[(5*S*)-3-phenoxy-carbonyloxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) (0.35 g ; 0.73 mmol), 2-aminomethanesulphonic acid (0.156 g ; 1.25 mmol) and triethylamine (174 μl ; 1.25 mmol) in DMSO (2.5 ml) was heated at 70°C for 2 days. The mixture was taken up in water and purified by preparative HPLC eluting with a 0-30 % gradient of methanol/ammonium carbonate buffer (2 g/l pH7).

25 The appropriate fractions were evaporated and the resulting solid triturated in ether and dried

to give 2-[[[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]oxycarbonyl]amino]ethane-1-sulphonic acid.

Yield : 22 %

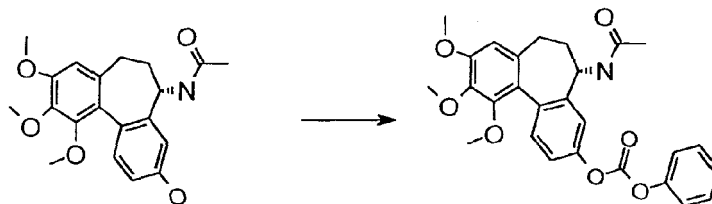
¹H NMR spectrum (DMSO-d₆ ; CF₃CO₂D) : 1.89 (s, 3H) ; 1.8-1.95 (m, 1H) ; 2-2.5 (m, 2H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 2.71 (t, 2H) ; 3.4 (m, 2H) ; 3.54 (s, 3H) ; 3.8 (s, 3H) ; 3.85 (s, 3H) ; 4.58 (m, 1H) ; 6.8 (s, 1H) ; 7.08 (d, 1H) ; 7.1 (s, 1H) ; 7.32 (d, 1H).

MS-ESI : 509 [MH]⁺

Elemental analysis: Found C 48.1 H 6.0 N 7.3 S 5.2

10 C₂₃H₂₈N₂O₉S ; 1 NH₃·2.5 H₂O Requires C 48.4 H 6.4 N 7.4 S 5.6%

The starting material was prepared as follows:



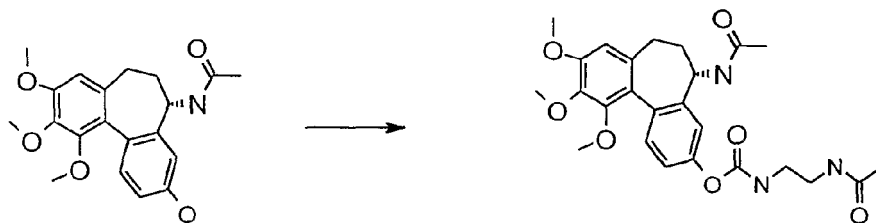
15

A solution of *N*-acetyl colchicinol (0.35 g ; 0.98 mmol), phenyl chloroformate (145 μl ; 1.08 mmol) and triethylamine (150 μl ; 1.08 mmol) in dichloromethane (20 ml) was stirred at ambient temperature for 1 hour. The mixture was washed with water and the organic phase evaporated. The residue was purified by flash chromatography, eluting with ethyl

20 acetate/petroleum ether (80/20) to give *N*-[(5*S*)-3-phenoxy-carbonyloxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide as a foam.

Yield : 79 %

¹H NMR spectrum (DMSO-d₆) : 1.89 (s, 3H) ; 1.8-1.95 (m, 1H) ; 2-2.3 (m, 2H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.52 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.58 (m, 25 1H) ; 6.8 (s, 1H) ; 7.2 - 7.6 (m, 8H) ; 8.41 (d, 1H).

Example 34

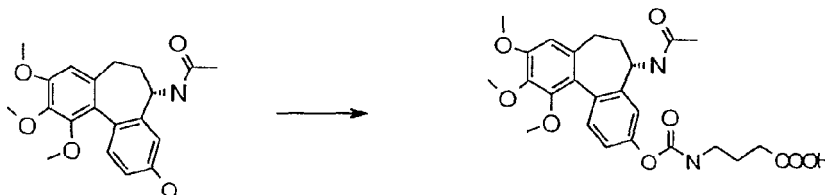
- 5 A solution of *N*-acetyl-cochicinol (0.25 g ; 0.7 mmol), 4-nitrophenyl chloroformate (0.169 g ; 0.84 mmol) and triethylamine (117 μ l ; 0.84 mmol) in dichloromethane (10 ml) was stirred under argon atmosphere for 1 hour. *N*-Acetylenehtylenediamine (0.086 g ; 0.84 mmol) was then added and the mixture was stirred further for 3 hours. After evaporation to dryness, the residue was purified by flash chromatography, eluting with
- 10 methanol/acetonitrile/dichloromethane (4/48/48) to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl *N*-[2-(acetylamino)ethyl]carbamate**.

Yield : 49 %

- ¹H NMR spectrum (DMSO-d₆) : 1.84 (s, 3H) ; 1.88 (s, 3H) ; 1.85-1.95 (m, 1H) ; 2-2.25 (m, 2H) ; 2.5 (m, 1H, signal partially observed by H₂O peak) ; 3.53 (s, 3H) ; 3.79 (s, 3H) ; 3.85 (s, 3H) ; 4.55 (m, 1H) ; 6.81 (s, 1H) ; 7.06 (dd, 1H) ; 7.07 (d, 1H) ; 7.32 (d, 1H) ; 7.79 (m, 1H) ; 7.8 (m, 1H) ; 8.41 (d, 1H).
- 15

MS-ESI : 486.1 [MH]⁺

Elemental analysis	Found	C 60.3 H 6.6 N 8.3
20 C ₂₅ H ₃₁ N ₃ O ₇ 0.6 H ₂ O	Requires	C 60.5 H 6.5 N 8.5%

Example 35

A suspension of 4-aminobutyric acid (0.087 g ; 0.84 mmol) and *N,O*-bis(trimethylsilyl)acetamide (0.865 ml ; 3.5 mmol) in dichloromethane (10 ml) was stirred under argon atmosphere for 3 hours and evaporated to dryness. The residue was then
 5 redissolved in dichloromethane (10 ml). 4-Nitrophenyl chloroformate (0.17 g ; 0.84 mmol) and triethylamine (0.117 ml ; 0.84 mmol) were added to a solution of *N*-acetyl-colchicinol (0.25 g ; 0.7 mmol) in dichloromethane (10 ml). The solution was stirred for 1 hour and added to the above solution. The resulting mixture was stirred further for 3 hours. After evaporation to dryness the residue was purified by preparative HPLC on reverse phase silica
 10 eluting with methanol/ammonium carbonate buffer (2 g/l pH7) (30/70) to give, after evaporation of the appropriate fractions, **4-[(*(5S)*-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl)oxycarbonyl)amino]butanoic acid**.

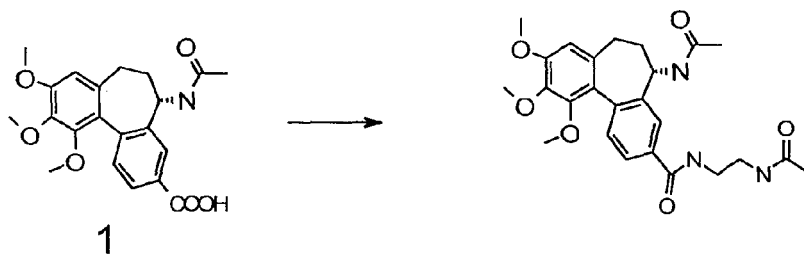
Yield : 44 %

¹H NMR spectrum (DMSO-d₆, CF₃CO₂D) : 1.73 (m, 2H) ; 1.88 (s, 3H) ; 1.8-1.95 (m, 1H) ; 2-
 15 2.25 (m, 2H) ; 2.3 (m, 2H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.11 (m, 2H) ; 3.53 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.45 (m, 1H) ; 6.8 (s, 1H) ; 7.05 (dd, 1H) ; 7.07 (d, 1H) ; 7.31 (d, 1H) ; 7.87 (m, 1H) ; 8.42 (d, 1H).

MS-ESI : 487.1 [MH]⁺

Elemental analysis	Found	C 60.2	H 6.3	N 6.0
20 C ₂₅ H ₃₀ N ₂ O ₈ 0.5 H ₂ O	Requires	C 60.6	H 6.3	N 5.7%

Example 36



25 A mixture of *N*-[(*5S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) (0.3 g ; 0.78 mmol), DCCI (0.193 g ; 0.93 mmol),

- 90 -

DMAP (0.019 g ; 0.15 mmol) and *N*-acetythylenediamine (0.096 g ; 0.985 mmol) in dichloromethane was stirred at ambient temperature overnight. After evaporation of the solvent, the residue was purified by flash chromatography and eluted with methanol/dichloromethane (10/90) to give *N*-[(5*S*)-3-(2-acetylaminoethylcarbamoyl)-

5 **9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide.**

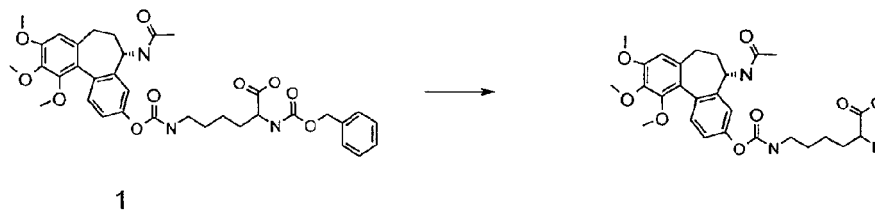
Yield : 71 %

¹H NMR spectrum (DMSO-d₆) : 1.82 (s, 3H) ; 1.89 (s, 3H) ; 1.85-2.05 (m, 2H) ; 2.17 (m, 1H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.22 (m, 2H) ; 3.32 (m, 2H, signal partially observed by H₂O peak) ; 3.49 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 10 6.81 (s, 1H) ; 7.38 (d, 1H) ; 7.75 (dd, 1H) ; 7.84 (d, 1H) ; 8 (m, 1H) ; 8.52 (m, 2H).

MS-ESI : 470.2 [MH]⁺

Elemental analysis:	Found	C 60.3	H 6.6	N 8.2
C ₂₅ H ₃₁ N ₃ O ₆ 0.4 dichloromethane	Requires	C 60.6	H 6.4	N 8.4%

15 **Example 37**



A solution of 6-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
20 dibenzo[*a,c*]cyclohepten-3-yl]oxy}carbonyl)amino]-2-(benzyloxycarbonylamino)-hexanoic
acid (1) (0.4 g ; 0.6 mmol) in ethanol (80 ml) was hydrogenated in the presence of 10%
palladium on carbon (0.08 g). After filtration of the catalyst and evaporation to dryness, the
residue was purified by preparative HPLC eluting with a 0-40% gradient of
methanol/ammonium carbonate buffer (2 g/l pH7) to give, after evaporation and trituration in
25 ether, 6-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
dibenzo[*a,c*]cyclohepten-3-yl]oxy}carbonyl)amino]-2-aminohexanoic acid as a solid.
Yield : 75 %

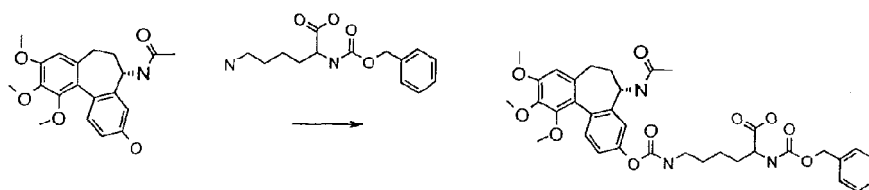
- 91 -

¹H NMR spectrum (DMSO-d₆) : 1.35-1.55 -m, 4H) ; 1.62 (m, 1H) ; 1.72 (m, 1H) ; 1.9 (s, 3H) ; 1.9 (m, 1H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3-3.2 (m, 3H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.83 (s, 3H) ; 4.55 (m, 1H) ; 6.79 (s, 1H) ; 7.02 (dd, 1H) ; 7.12 (d, 1H) ; 7.29 (d, 1H) ; 7.74 (m, 1H) ; 8.7 (d, 1H).

5 MS-ESI : 530.1 [MH]⁺

Elemental analysis:	Found	C 60.2	H 7.0	N 7.8
C ₂₇ H ₃₅ N ₃ O ₉ · 0.5 H ₂ O	Requires	C 60.2	H 6.7	N 7.8%

The starting material was prepared as follows:



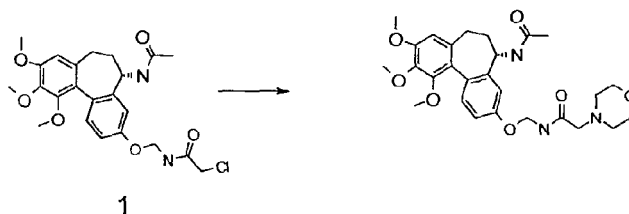
1

10

A suspension of *N*-(carboxybenzyloxy)-L-lysine (0.141 g ; 0.5 mmol) and *N,O*-bis(trimethylsilyl)acetamide (0.519 ml ; 2 mmol) in dichloromethane (10 ml) was stirred at ambient temperature under argon atmosphere for 3 hours. The mixture was evaporated to dryness and the residue redissolved in dichloromethane (10 ml). A solution of *N*-acetyl-
 15 colchicinol (0.15 g ; 0.42 mmol), and 4-nitrophenyl chloroformate (0.102g; 0.5mmol) was stirred at ambient temperature for 1 hour and then added to the above solution. The resulting mixture was stirred overnight, evaporated to dryness and purified by preparative HPLC eluting with a 0-55% gradient of methanol/ammonium carbonate buffer (2 g/l pH7) to give (1).

20 Yield : 63 %

¹H NMR spectrum (DMSO-d₆) : 1.35 (m, 2H) ; 1.49 (m, 2H) ; 1.62 (m, 1H) ; 1.72 (m, 1H) ; 1.89 (s, 3H) ; 1.9 (m, 1H) ; 2.07 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.07 (m, 2H) ; 3.52 (s, 3H) ; 3.8 (s, 3H) ; 3.85 (s, 3H) ; 4.57 (m, 1H) ; 5.05 (m, 2H) ; 6.81 (s, 1H) ; 7 (m, 1H) ; 7.05 (dd, 1H) ; 7.1 (d, 1H) ; 7.32 (d, 1H) ; 7.3-7.4 (m, 5H)
 25 ; 7.75 (m, 1H) ; 8.58 (d, 1H).

Example 38

5

A solution of *N*-([(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]oxymethyl)-2-chloroacetamide (1) (0.25 g ; 0.55 mmol) in morpholine (2 ml)) was stirred at ambient temperature for 2 hours. After addition of dichloromethane and removal of the insoluble material by filtration, the filtrate was

10 evaporated to dryness and the residue was purified by preparative HPLC eluting with a 0-45% gradient of ethanol/ammonium carbonate buffer (2 g/l pH7) to give, after evaporation and trituration in ether, *N*-([(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]oxymethyl)-2-morpholinoacetamide.

Yield : 60 %

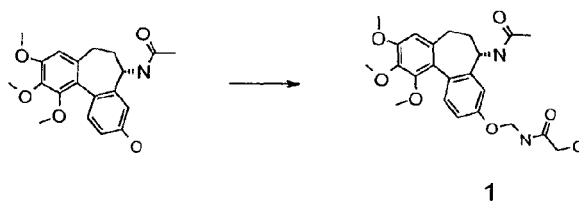
15 ¹H NMR spectrum (DMSO-d₆) : 1.85 (m, 1H) ; 1.88 (s, 3H) ; 1.95-2.2 (m, 2H) ; 2.4 (m, 4H) ; 2.5 (m, 1H, signal partially observed by DMSO peak) ; 3 (s, 2H) ; 3.47 (s, 3H) ; 3.57 (m, 4H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.5 (m, 1H) ; 5.18 (m, 2H) ; 6.76 (s, 1H) ; 6.91 (d, 1H) ; 6.98 (dd, 1H) ; 7.22 (d, 1H) ; 8.32 (m, 1H) ; 8.85 (m, 1H).

MS-ESI : 514.1 [MH]⁺

20	Elemental analysis	Found	C 61.2	H 6.9	N 7.9
	C ₂₇ H ₃₅ N ₃ O ₇	Requires	C 61.4	H 7.0	N 8.0%

The starting material was prepared as follows:

- 93 -

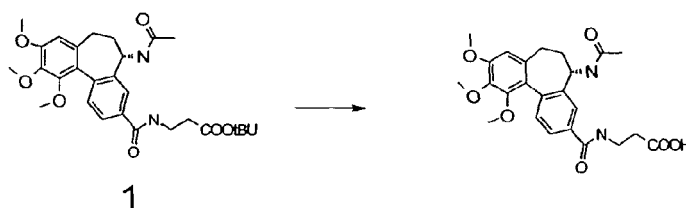


2-Chloro-*N*-(hydroxymethyl)-acetamide (0.342 g ; 2.7 mmol), triphenylphosphine (1.1 g ; 4.19 mmol) and DEAD (0.6 ml ; 4.19 mmol) were added to a solution of *N*-acetyl-
 5 colchicinol (0.3 g ; 0.84 mmol) in dichloromethane (20 ml) under argon atmosphere. The mixture was stirred at ambient temperature for 2 hours, evaporated and purified by flash chromatography eluting with ethyl acetate/dichloromethane (50/50) and dichloromethane/methanol (98/2) to give (1).

Yield : 76 %

10 MS-ESI : 485.1 [MH]⁺

Example 39



15 *N*-[(5*S*)-3-(2-*tert*Butoxycarbonyl)ethylcarbamoyl]-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) (0.26 g ; 0.5 mmol) in solution in dichloromethane (10 ml) was treated with TFA (10 ml) at ambient temperature for 1 hour. After evaporation to dryness, the residue was purified by preparative HPLC eluting with methanol/ammonium carbonate buffer (2 g/l pH7) (35/65) to give, after evaporation and
 20 trituration in ether, 3-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-ylcarbonylamino]propanoic acid as a white solid.

Yield : 56%

¹H NMR spectrum (DMSO-d₆) : 1.91 (s, 3H) ; 1.85-2.1 (m, 2H) ; 2.2 (m, 1H) ; 2.5 (m, 1H, signal partially observed by DMSO peak) ; 3.2-3.6 (m, 4H, signal partially obscured by H₂O

- 94 -

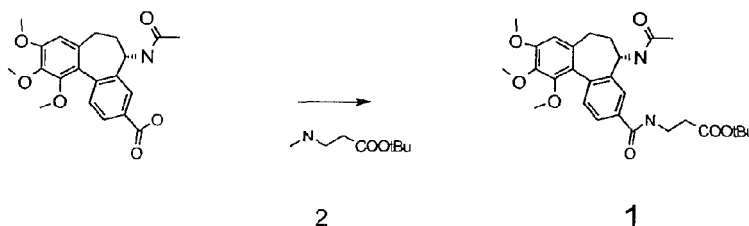
peak) ; 3.5 (s, 3H) ; 3.8 (s, 3H) ; 3.86 (s, 3H) ; 4.6 (m, 1H) ; 6.82 (s, 1H) ; 7.39 (d, 1H) ; 7.74 (dd, 1H) ; 7.85 (d, 1H) ; 8.54 (d, 1H) ; 8.62 (m, 1H).

MS-ESI : 457.1 [MH]⁺

Elemental analysis: Found C 60.9 H 6.7 N 6.7

5 C₂₄H₂₈N₂O₇ Requires C 63.2 H 6.2 N 6.1%

The starting material was prepared as follows:



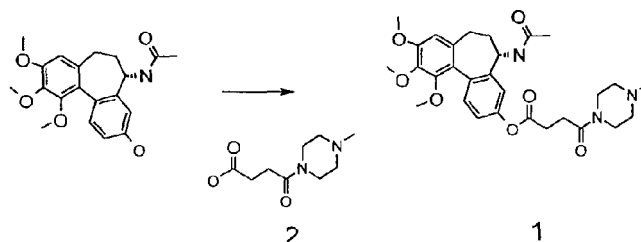
A mixture of *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (1) (0.3 g ; 0.78 mmol), EDCI (0.179 g ; 0.93 mmol), DMAP (0.019 g ; 0.15 mmol), triethylamine (0.13 ml ; 0.985 mmol) and *tert*butyl 3-methylaminopropanoate (0.17 g ; 0.985 mmol) in dichloromethane was stirred at ambient temperature overnight. After removal of the solvent by evaporation, the residue was purified by flash chromatography and eluted with ethyl acetate to give (1).

15 Yield : 84%

¹H NMR spectrum (DMSO-d₆) : 1.4 (s, 9H) ; 1.89 (s, 3H) ; 1.9 (m, 1H) ; 2 (m, 1H) ; 2.17 (m, 1H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.25-3.55 (m, 4H) ; 3.49 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 6.81 (s, 1H) ; 7.37 (d, 1H) ; 7.7 (dd, 1H) ; 7.81 (d, 1H) ; 8.5 (m, 2H).

20

Example 40



A suspension of 3-(4-methylpiperazin-1-ylcarbonyl)propanoic acid (2) (0.219 g ; 1.1 mmol), DCCI (0.226 ml ; 1.1 mmol) and DMAP (0.052 ml ; 0.42 mmol) in dichloromethane (20 ml) was stirred under argon atmosphere for 1 hour. *N*-Acetyl-colchicinol (0.3 g ; 0.84 mmol) was then added and the mixture was stirred overnight. After removal of the insoluble material by filtration, the filtrate was evaporated and purified by preparative HPLC eluting with a 0-50% gradient of methanol/ammonium carbonate buffer (2 g/l pH7) to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[4-methylpiperazin-1-ylcarbonyl]propanoate**.

Yield : 48 %

¹H NMR spectrum (DMSO-d₆) : 1.80-1.96 (m, 1H) ; 1.88 (s, 3H) ; 2.07 (m, 1H) ; 2.18 (m, 1H) ; 2.20 (s, 3H) ; 2.26 (m, 2H) ; 2.33 (m, 2H) ; 2.59 (m, 1H, signal partially obscured by DMSO peak) ; 2.73 (m, 2H) ; 2.79 (m, 2H) ; 3.48 (m, 4H) ; 3.53 (s, 3H) ; 3.80 (s, 3H) ; 3.86 (s, 3H) ; 4.55 (m, 1H) ; 6.82 (s, 1H) ; 7.03-7.12 (m, 2H) ; 7.36 (d, 1H) ; 8.43 (d, 1H).

MS-ESI : 540 [MH]⁺

15	Elemental analysis	Found	C 63.9	H 7.1	N 7.5
	C ₂₉ H ₃₇ N ₃ O ₇ ; 0.3 H ₂ O	Requires	C 63.9	H 7.0	N 7.7%

The starting material was prepared as follows:

A suspension of *N*-methylpiperazine (1.1 ml ; 10 mmol) and succinic anhydride (1.2 g ; 12 mmol) in dichloromethane (20 ml) was stirred under argon atmosphere for 24 hours.

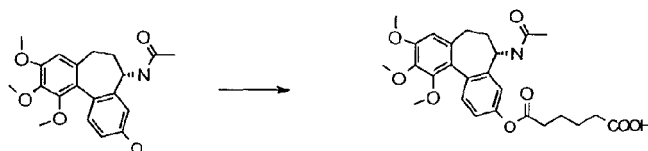
After evaporation to dryness, the residue was triturated in ether/pentane to give (2) as a solid.

Yield : 91 %

¹H NMR Spectrum (DMSO-d₆) : 2.37 (s, 3H) ; 2.53 (m, 2H) ; 2.58 (m, 2H) ; 2.64 (m, 4H) ; 3.59 (m, 2H) ; 3.69 (m, 2H) ; 5.70 (br s, 1H).

25

Example 41



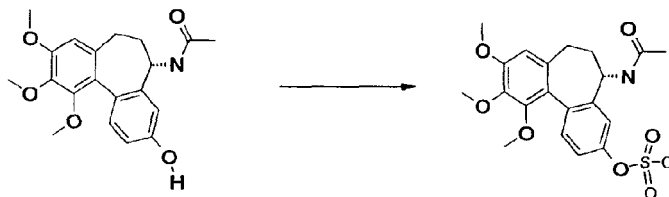
N-Acetyl-colchicinol (0.3 g ; 0.84 mmol) was added under argon atmosphere to a solution of adipic acid (0.147 g ; 1 mmol, *O*-(7-(azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (0.383 g ; 1 mmol) and diisopropylethylamine (0.352 ml ; 2 mmol) in acetonitrile (20 ml). The reaction mixture was stirred at ambient
 5 temperature overnight and evaporated to dryness. The residue was taken up in water (4 ml), the pH was adjusted to 6.5 with 0.1M hydrochloric acid. The solution was purified by preparative HPLC eluting with a 0-40% gradient of methanol/ammonium carbonate buffer (2 g/l pH7) to give 5-[(*5S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-**dibenzo[*a,c*]cyclohepten-3-yl**]oxycarbonyl]pentanoic acid.

10 Yield : 31 %

¹H NMR spectrum (DMSO-d₆) : 1.54-1.75 (m, 4H) ; 1.85-1.90 (m, 1H) ; 1.87 (s, 3H) ; 1.98-2.28 (m, 4H) ; 2.57 (m, 1H, signal partially obscured by DMSO peak) ; 2.61 (t, 2H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 6.80 (s, 1H) ; 7.04-7.11 (m, 2H) ; 7.34 (d, 1H) ; 8.43 (d, 1H).

15 MS-ESI : 508 [MNa]⁺

Example 42



20 Chlorosulphonic acid (1 ml) was added at 0°C in portions to a solution of pyridine (10 ml). After 15 minutes at 0°C, a solution of *N*-acetyl-colchicinol (1 g, 2.8 mmol) in pyridine (10 ml) was added. The solution was stirred overnight at ambient temperature. Water (30 ml) was added and the mixture was adjusted to pH8 by addition of sodium hydrogen carbonate. The aqueous layer was extracted with ether (3 x 20 ml) and purified on HP20SS resin, eluted
 25 with a 0-40 % gradient of methanol/water. The volatiles were removed by evaporation to give (*5S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-**dibenzo[*a,c*]cyclohepten-3-yl hydrogen sulphate** as a white solid.

Yield = 71 %

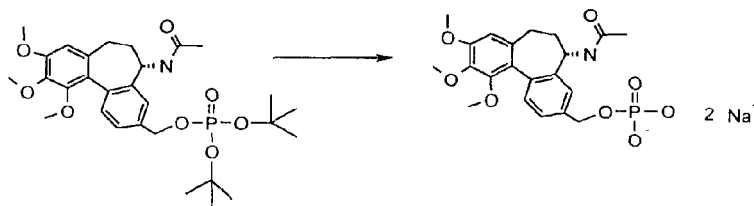
- 97 -

¹H NMR spectrum (DMSO-d₆) : 1.9 (s, 3H), 2-2.2 (m, 2H), 2.5 (m, 1H, signal obscured by DMSO peak), 3.5 (s, 3H), 3.77 (s, 3H), 3.83 (s, 3H), 4.5 (m, 1H), 6.77 (s, 1H), 7.1 (s, 1H), 7.2 (2s, 2H), 8.4 (d, 1H).

MS-ESI : 482 [M Na]⁺

5	Elemental analysis:	Found	C 48.1	H 4.9	N 2.8	S 6.2
	C ₂₀ H ₂₂ O ₈ NSNa, 2 H ₂ O	Requires	C 48.5	H 5.3	N 2.8	S 6.5%

Example 43



10

Using an analogous procedure to that described for Example 27 [(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]methyl di*tert*butyl phosphate was treated with 1M hydrogen chloride in 1,4-dioxane to give [(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]methyl dihydrogen phosphate.

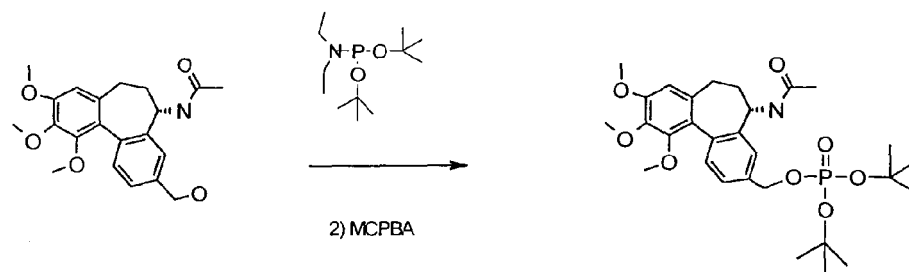
Yield : 95 %

The sodium salt was prepared by addition of 2N sodium hydroxide to a suspension of [(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]methyl dihydrogen phosphate in water until the mixture was at pH7. After freeze-drying, the sodium salt was obtained as a white solid.

¹H NMR Spectrum (D₂O) : 1.94 (m, 1H) ; 1.98 (s, 3H) ; 2.15 (m, 1H) ; 2.25 (m, 1H) ; 2.50 (m, 1H) ; 3.50 (s, 3H) ; 3.80 (s, 3H) ; 3.84 (s, 3H) ; 4.48 (m, 1H) ; 4.80 (m, 2H) ; 6.80 (s, 1H) ; 7.40 (m, 3H).

25 MS - ESI : 496 [MH]⁺

The starting material was prepared as follows:



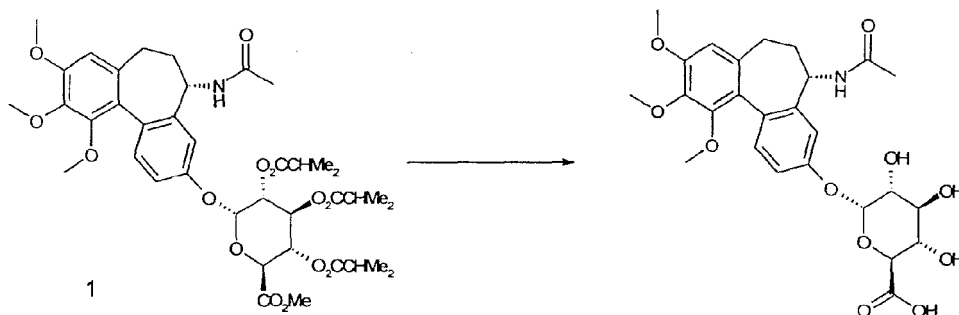
Using an analogous procedure to that described for the starting material in Example 27, *N*-[(5*S*)-3-hydroxymethyl-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide, (prepared as described in Example 25), was reacted with di-*tert*-butyl diethylphosphoramidite to give [(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]methyl di-*tert*-butyl phosphate.

Yield : 59 %

¹H NMR spectrum (DMSO-d₆) : 1.432 (s, 9H) ; 1.435 (s, 9H) ; 1.88 (s, 3H) ; 1.90 (m, 1H) ;
 2.02 (m, 1H) ; 2.18 (m, 1H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.50 (s, 3H) ;
 3.79 (s, 3H) ; 3.85 (s, 3H) ; 4.56 (m, 1H) ; 4.97 (d, 2H) ; 6.81 (s, 1H) ; 7.35 (m, 2H) ; 7.38 (s, 1H) ; 8.46 (d, 1H).

Example 44

15



A solution of methyl (2*S*,3*R*,4*S*,5*R*,6*R*)-6-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]oxy-3,4,5-tris(isobutyryloxy)tetrahydro-2*H*-
 20 pyran-2-carboxylate (1) (515 mg ; 0.68 mol) in methanol (10 ml) and water (1 ml) was treated with lithium hydroxide monohydrate (214 mg ; 5.1 mmol). The reaction mixture was stirred

at ambient temperature and additional solution of lithium hydroxide monohydrate (86 mg ; 2 mmol) in H₂O (1 ml) was added after 12 hours and then again after a further 10 hours to complete the reaction. After a total of 30 hours at ambient temperature, the methanol was removed and the remaining solution was adjusted to pH6 with 2N hydrochloric acid. The resulting heterogeneous solution was deposited on a column of HP20 SS resin (35 ml) for purification, eluting with a 0 to 75 % aqueous solution of methanol. After removal of the solvents by evaporation, the solid was purified further by preparative HPLC on reverse phase silica eluting with a 0-50 % gradient of methanol/water to give, after removal of the methanol by evaporation and freeze drying, (2*S*,3*S*,4*S*,5*R*,6*R*)-6-[[*(5S)*-5-(acetyl-amino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]oxy}-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylic acid as a white solid (260 mg).

Yield : 68 %

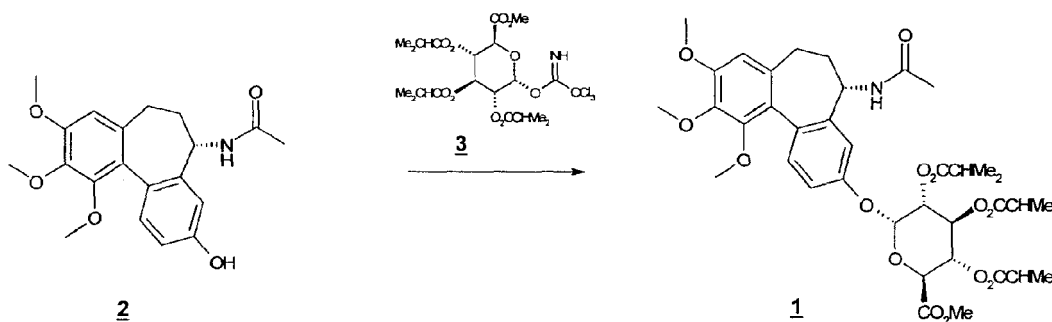
¹H NMR spectrum (DMSO-d₆, CF₃CO₂D) : 1.88 (m, 1H) ; 1.89 (s, 3H) ; 2.08 (m, 1H) ; 2.15 (m, 1H) ; 2.52 (m, 1H, signal obscured partially by DMSO peak) ; 3.25-3.36 (m, 3H) ; 3.44 (t, 1H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 3.91 (d, 1H) ; 4.50 (m, 1H) ; 5.03 (d, 1H) ; 6.77 (s, 1H) ; 6.98 (s, 1H) ; 7.00 (d, 1H) ; 7.26 (d, 1H).

MS - ESI : 534 [MH]⁺

Elemental analysis	Found	C 55.7	H 6.1	N 2.5
C ₂₆ H ₃₁ NO ₁₁ ; 1.5 H ₂ O	Requires	C 56.0	H 5.9	N 2.6%

20

The starting material was prepared as follows :



25 Freshly distilled boron trifluoride-diethyl ether (0.22 ml ; 1.7 mmol) was added at 0°C under argon atmosphere to a stirred solution of *N*-acetyl-colchicinol (2) (303 mg ; 0.85 mmol)

- 100 -

and methyl (trichloroacetimidoyl 2, 3, 4-tri-O-isobutyryl- α -D-glucopyranosid) uronate (3) (955 mg ; 1.7 mmol), (THL 36, 8601, 1995), in dichloromethane (8 ml). The mixture was stirred at 0°C for 15 minutes and then at ambient temperature for 2 hours. The reaction mixture was diluted with dichloromethane, washed with aqueous saturated sodium hydrogen carbonate, water, then dried (MgSO₄) and evaporated. The residue was purified by flash chromatography eluting with a 0 to 35 % gradient of dichloromethane/ether to give, after evaporation, (1) as a light yellow-green foam.

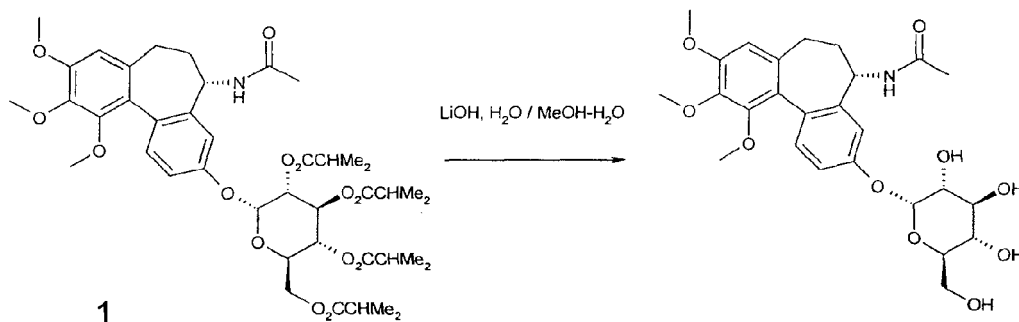
Yield : 82 %

¹H NMR spectrum (DMSO-d₆ + CD₃CO₂D) : 1.01-1.06 (m, 18H) ; 1.87 (m, 1H) ; 1.89 (s, 3H) ; 2.13 (m, 1H) ; 2.26 (m, 1H) ; 2.50 (m, 4H, signal obscured partially by DMSO peak) ; 3.50 (s, 3H) ; 3.65 (s, 3H) ; 3.78 (s, 3H) ; 3.83 (s, 3H) ; 4.58 (m, 1H) ; 4.74 (d, 1H) ; 5.11 (t, 1H) ; 5.17 (d, 1H) ; 5.60 (t, 1H) ; 5.73 (d, 1H) ; 6.77 (s, 1H) ; 6.94 (s, 1H) ; 6.95 (d, 1H) ; 7.28 (d, 1H) ; 8.37 (d, 1H).

MS - ESI : 758 [MH]⁺

15

Example 45



(2*R*,3*R*,4*S*,5*R*,6*R*)-2-[(5*S*)-5-(Acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[α , c]cyclohepten-3-yl]oxy-3,5-bis(isobutyryloxy)-6-[(isobutyryloxy)methyl]tetrahydro-2*H*-pyran-4-yl 2-methylpropanoate (1) (304 mg ; 0.38 mmol) and H₂O (0.25 ml) were added to a 0.48M solution of lithium hydroxide monohydrate in methanol (6 ml). The mixture was stirred at ambient temperature for 6 hours. After removal of the methanol by evaporation, the remaining aqueous solution was adjusted to pH6.2 with 2N hydrochloric acid. The resulting heterogeneous solution was deposited on a

column of HP20 SS resin (35 ml) for purification, eluting with a 0 - 60 % gradient of methanol/water. After concentration and freeze drying *N*-((5*S*)-9,10,11-trimethoxy-3-[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl]oxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl)acetamide was obtained as a white solid (180 mg).

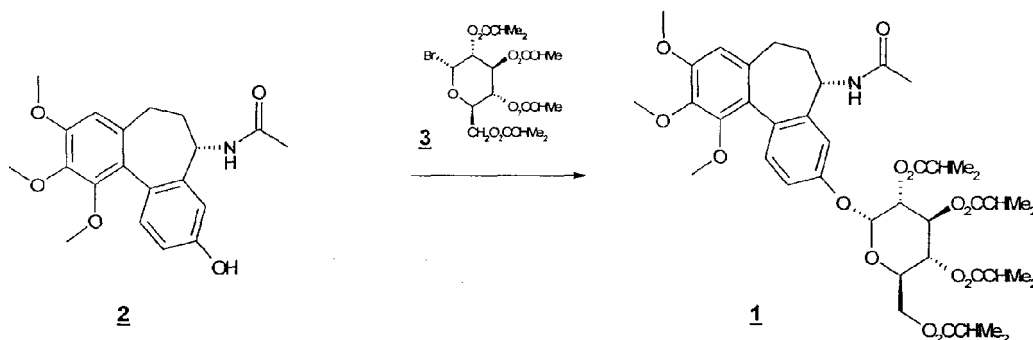
Yield : 84 %

¹H NMR spectrum (DMSO-d₆, CF₃CO₂D) : 1.88 (m, 1H) ; 1.90 (s, 3H) ; 2.10 (m, 1H) ; 2.18 (m, 1H) ; 2.52 (m, 1H, signal obscured partially by DMSO peak) ; 3.21-3.37 (m, 4H) ; 3.51 (s, 3H) ; 3.48-3.58 (m, 1H) ; 3.74-3.81 (m, 1H) ; 3.80 (s, 3H) ; 3.84 (s, 3H) ; 4.50 (m, 1H) ; 4.92 (d, 1H) ; 6.78 (s, 1H) ; 6.98 (d, 1H) ; 7.00 (dd, 1H) ; 7.26 (d, 1H) ; 8.36 (d, 1H).

MS - ESI : 542 [MNa]⁺

Elemental analysis	Found	C 55.3	H 6.6	N 2.6
C ₂₆ H ₃₃ NO ₁₀ 2.6 H ₂ O	Required	C 55.1	H 6.8	N 2.5%

The starting material was prepared as follows:



N-Benzyltributylammomium bromide (523 mg ; 1 mmol) and *N*-acetyl-colchicinol (2) (357 mg ; 1 mmol) in a 1.25N aqueous solution of sodium hydroxide were added at 0°C to a solution of the (2*R*,3*R*,4*S*,5*S*,6*R*)-2-bromo-3,5-bis(isobutyryloxy)-6-[(isobutyryloxy)methyl]tetrahydro-2*H*-pyran-4-yl 2-methylpropanoate (3) (523 mg ; 1 mmol), (J. Chem. Soc. Perkins Trans. 1 1995 p 577), in trichloromethane (2 ml). After 1 hour the reaction mixture was stirred at ambient temperature. Additional reagent (3) (250 mg ; 0.48 mmol and 1.33 mg ; 0.33 mmol) and 1.25N sodium hydroxide (0.2 ml and 0.1 ml) were added

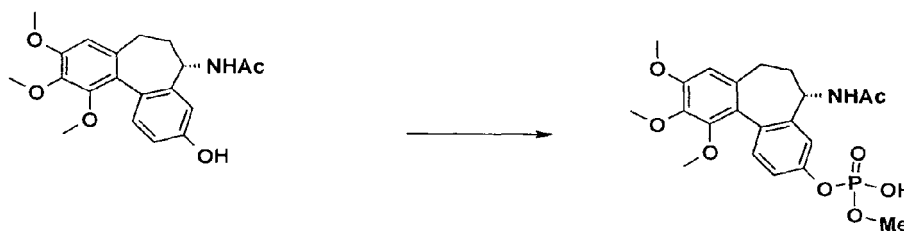
to the reaction mixture after 6 hours at ambient temperature and then again after a further 14 hours at ambient temperature. After a total of 24 hours, the reaction mixture was diluted with dichloromethane, washed successively with water, brine and then dried (MgSO₄). After removal of the solvent, the residue was purified by flash chromatography eluting with
 5 dichloromethane/ether (8/2 to 6/4) to give (1) (320 mg) as a foam.

Yield : 40 %

¹H NMR spectrum (DMSO-d₆) : 1.00-1.11 (m, 24H) ; 1.88 (m, 1H) ; 1.89 (s, 3H) ; 2.08 (m, 1H) ; 2.21 (m, 1H) ; 2.46-2.66 (m, 5H, signal obscured partially by DMSO peak) ; 3.48 (s, 3H) ; 3.77 (s, 3H) ; 3.83 (s, 3H) ; 4.16-4.24 (m, 2H) ; 4.32 (m, 1H) ; 4.47 (m, 1H) ; 5.08 (m, 10 2H) ; 5.54 (t, 1H) ; 5.67 (d, 1H) ; 6.77 (s, 1H) ; 6.92 (d, 1H) ; 6.95 (dd, 1H) ; 7.25 (d, 1H) ; 8.38 (d, 1H).

MS-ESI : 800 [MH]⁺

Example 46



1

15

A solution of *N*-acetyl-colchicinol (1) (0.45 g ; 1.26 mmol) in THF (40 ml) under argon was cooled to 0°C and treated with a 1.0M solution of lithiumHMDS in THF (1.39 ml ; 1.39 mmol). The mixture was stirred at 0°C for 1 hour and then added in portions over about
 20 15 minutes to a solution of methyl dichlorophosphate (625 µl ; 4.16 mmol) in THF (150 ml). The mixture was stirred at ambient temperature for 15 minutes. After addition of water (200 ml) the THF was removed by evaporation. After removal of the insoluble material by filtration, the filtrate was purified on HP20 SS resin eluting with a gradient of 0-60% methanol/water. The appropriate fractions were freeze-dried to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl methyl hydrogen phosphate** as a white solid (391 mg).
 25

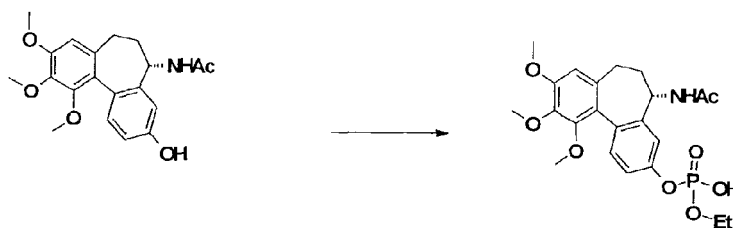
Yield : 69%

¹H NMR spectrum (DMSO d₆ ; CF₃CO₂D) : 1.89 (s, 3H) ; 1.9 (m, 1H) ; 2.05 (m, 1H) ; 2.18 (m, 1H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.53 (s, 3H) ; 3.73 (d, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.51 (m, 1H) ; 6.79 (s, 1H) ; 7.14 (d, 1H) ; 7.15 (s, 1H) ; 7.32 (d, 1H) ; 8.46 (d, 1H).

MS-ESI : 451 [MH]⁺

Elemental analysis :	Found	C 54.1	H 5.9	N 3.1
C ₂₁ H ₂₆ NO ₈ P; 0.7 H ₂ O	Requires	C 54.2	H 6.0	N 3.0%

10 Example 47



1

A solution of *N*-acetyl-colchicinol (1) (0.36 g ; 1.0 mmol) in THF (40 ml) under argon was cooled to 0°C and treated with a 1.0M solution of lithiumHMDS in THF (1.1 ml ; 1.1 mmol). The mixture was stirred at 0°C for 1 hour and then added in portions over about 2 hours to a solution of ethyl dichlorophosphate (400 µl ; 3.3 mmol) in THF (150 ml). The mixture was stirred at ambient temperature for 15 minutes. After addition of water (200 ml) the THF was removed by evaporation. After removal of the insoluble material by filtration, the filtrate was purified on HP20 SS resin eluting with a gradient of 0-60% methanol/water. The appropriate fractions were freeze-dried to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl ethyl hydrogen phosphate** as a white solid (259 mg).

Yield : 56%

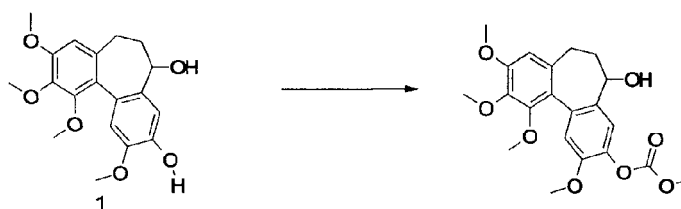
¹H NMR spectrum (DMSO d₆ ; CF₃CO₂D) : 1.25 (dt, 3H) ; 1.89 (s, 3H) ; 1.9 (m, 1H) ; 2.05 (m, 1H) ; 2.19 (m, 1H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.53 (s, 3H) ;

- 104 -

3.79 (s, 3H) ; 3.85 (s, 3H) ; 4.09 (m, 2H) ; 4.52 (m, 1H) ; 6.80 (s, 1H) ; 7.13 (d, 1H) ; 7.15 (s, 1H) ; 7.32 (d, 1H) ; 8.45 (d, 1H).

MS-ESI : 466 [MH]⁺

Elemental analysis :	Found	C 54.6	H 6.0	N 3.0
5 C ₂₂ H ₂₈ NO ₈ P; 1.0 H ₂ O	Requires	C 54.7	H 6.3	N 2.9%

Example 48

10

Triethylamine (140 μ l ; 1.0 mmol) and methyl chloroformate (80 μ l ; 1.0 mmol) were added to a solution of 5-hydroxy-2,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-ol (1) (0.18 g ; 0.5 mmol) in THF (10 ml). The mixture was stirred at ambient temperature overnight. After removal of the insoluble material by filtration the residue was purified by flash chromatography eluting with increasingly polar mixtures of ethyl acetate/hexanes (5 to 60% ethyl acetate) to give **5-hydroxy-2,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl methyl carbonate** as a hard oil/white solid (163 mg).

20 Yield : 81%

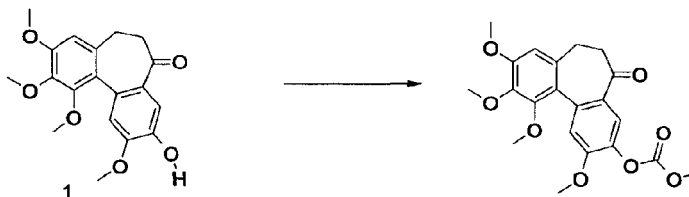
¹H NMR spectrum (CDCl₃) : 1.89 (m, 1H) ; 2.39 (m, 2H) ; 2.54 (m, 1H) ; 3.61 (s, 3H) ; 3.87 (s, 3H) ; 3.91 (s, 3H) ; 3.92 (s, 3H) ; 3.93 (s, 3H) ; 4.56 (m, 1H) ; 6.59 (s, 1H) ; 7.15 (s, 1H) ; 7.45 (s, 1H).

MS-ESI : 427 [MNa]⁺

25

Example 49

- 105 -



Triethylamine (35 μ l ; 0.225 mmol) and methyl chloroformate (20 μ l ; 0.225 mmol) were added to a solution of 3-hydroxy-2,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[*a,c*]cyclohepten-5-one (1) (0.052 g ; 0.15 mmol) in THF (5 ml). The mixture was stirred at ambient temperature for 5 hours. After removal of the insoluble material by filtration the residue was purified by flash chromatography eluting with increasingly polar mixtures of ethyl acetate/hexanes (0 to 100% ethyl acetate) to give **methyl 2,9,10,11-tetramethoxy-5-oxo-6,7-dihydro-5H-dibenzo[*a,c*]cyclohepten-3-yl carbonate** as a white solid (57 mg).

Yield : 95%

^1H NMR spectrum (CDCl_3) : 2.69 (m, 1H) ; 2.85 (m, 1H) ; 2.97 (m, 1H) ; 3.16 (m, 1H) ; 3.53 (s, 3H) ; 3.93 (s, 3H) ; 3.94 (s, 3H) ; 3.94 (s, 3H) ; 3.95 (s, 3H) ; 6.64 (s, 1H) ; 7.24 (s, 1H) ; 7.47 (s, 1H).

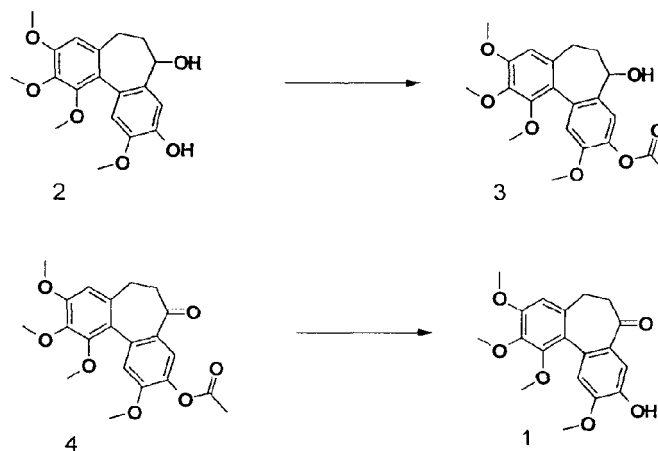
MS-ESI : 403 $[\text{MH}]^+$

Elemental analysis :	Found	C 62.0	H 5.5
$\text{C}_{21}\text{H}_{22}\text{O}_8$; 0.2 H_2O	Requires	C 62.1	H 5.6%

The starting material was prepared as follows:

20

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Triethylamine (1.05 ml ; 7.5 mmol) and acetyl chloride (540 μ l ; 7.5 mmol) were added to a solution of 5-hydroxy-2,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[*a,c*]cyclohepten-3-ol (2) (1.05 g ; 3.0 mmol) in THF (50 ml). The mixture was stirred at ambient temperature overnight. After removal of the insoluble material by filtration the residue was purified by flash chromatography eluting with increasingly polar mixtures of ethyl acetate/hexanes (0 to 100% ethyl acetate) to give methyl 5-hydroxy-2,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[*a,c*]cyclohepten-3-yl carboxylate (3) as a white solid (880 mg).

Yield : 76%

^1H NMR spectrum (CDCl_3) : 1.89 (m, 1H) ; 2.34 (s, 3H) ; 2.38 (m, 2H) ; 2.53 (m, 1H) ; 3.61 (s, 3H) ; 3.84 (s, 3H) ; 3.90 (s, 3H) ; 3.91 (s, 3H) ; 4.55 (m, 1H) ; 6.59 (s, 1H) ; 7.14 (s, 1H) ; 7.35 (s, 1H).

MS-ESI : 411 [MNa] $^+$

Elemental analysis :	Found	C 65.0	H 6.3
$\text{C}_{21}\text{H}_{24}\text{O}_7$	Requires	C 64.9	H 6.2%

A solution of (3) (0.776 g ; 2.0 mmol) in dichloromethane (30 ml) was added to a solution of Collins Reagent (3.1 g ; 12.0 mmol) in dichloromethane (30 ml). The mixture was stirred at ambient temperature for 30 minutes. After removal of the insoluble material by filtration the filtrate was washed with 2N hydrochloric acid, then brine and dried over MgSO_4 . The residue was purified by flash chromatography eluting with increasingly polar mixtures of

ethyl acetate/hexanes (0 to 60% ethyl acetate) to give methyl 5-oxo-2,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl carboxylate (4) as a white solid (706 mg).

Yield : 91%

¹H NMR spectrum (CDCl₃) : 2.34 (s, 3H) ; 2.64 (m, 1H) ; 2.82 (m, 1H) ; 2.93 (m, 1H) ; 3.14 (m, 1H) ; 3.50 (s, 3H) ; 3.88 (s, 3H) ; 3.91 (s, 3H) ; 3.92 (s, 3H) ; 6.61 (s, 1H) ; 7.20 (s, 1H) ; 7.36 (s, 1H).

MS-ESI : 387 [MH]⁺

Elemental analysis :	Found	C 65.6	H 6.0
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C ₂₁ H ₂₂ O ₇	Requires	C 65.3	H 5.7%
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10 Water (10 ml) and saturated aqueous sodium hydrogen carbonate (10 ml) were added to a solution of (4) (0.58 g ; 1.5 mmol) in methanol (50 ml). The mixture was stirred at ambient temperature overnight. After dilution with ethyl acetate the organic phase was washed with 2N hydrochloric acid, then brine and dried over MgSO₄. The residue was triturated with ether and hexanes to give (1) as a white solid (441 mg).

15 Yield : 85%

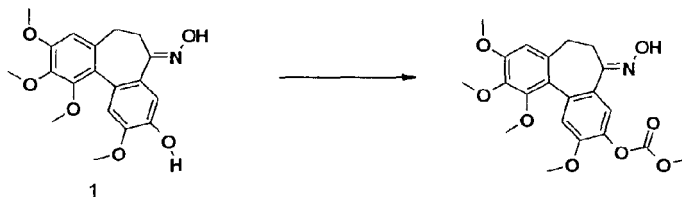
¹H NMR spectrum (CDCl₃) : 2.61 (m, 1H) ; 2.80 (m, 1H) ; 2.91 (m, 1H) ; 3.06 (m, 1H) ; 3.45 (s, 3H) ; 3.88 (s, 3H) ; 3.88 (s, 3H) ; 3.91 (s, 3H) ; 5.73 (s br, 1H) ; 6.58 (s, 1H) ; 7.09 (s, 1H) ; 7.17 (s, 1H).

MS-ESI : 345 [MH]⁺

20 Elemental analysis :	Found	C 65.52	H 6.10
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C ₁₉ H ₂₀ O ₆ ; 0.2 H ₂ O	Requires	C 65.58	H 5.91
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Example 50



Triethylamine (18 μ l ; 0.12 mmol) and methyl chloroformate (10 μ l ; 0.12 mmol) were added to a solution of 5-(hydroxyimino)-2,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-ol (1) (0.035 g ; 0.1 mmol) in THF (3 ml). The mixture was stirred at ambient temperature overnight. After removal of the insoluble material by filtration the residue was purified by flash chromatography eluting with increasingly polar mixtures of ethyl acetate/hexanes (0 to 100% ethyl acetate) to give 5-(hydroxyimino)-2,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl methyl carbonate (E isomer) as a white solid (22 mg), followed by the second (Z) isomer (7 mg).

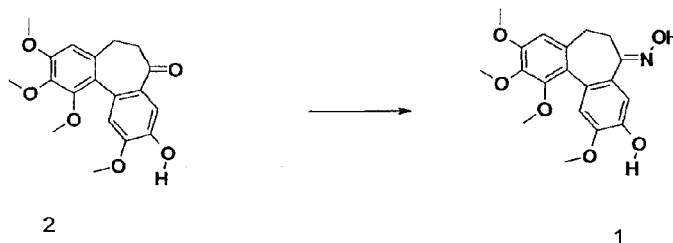
Yield : 54%

¹H NMR spectrum (CDCl₃) : 2.59 (m, 1H) ; 2.82 (m, 2H) ; 3.20 (m, 1H) ; 3.51 (s, 3H) ; 3.88 (s, 3H) ; 3.90 (s, 3H) ; 3.91 (s, 3H) ; 3.93 (s, 3H) ; 6.59 (s, 1H) ; 7.21 (s, 1H) ; 7.26 (s, 1H) ; 8.61 (br s, 1H).

MS-ESI : 418 [MH]⁺

Elemental analysis :	Found	C 58.1	H 5.7	N 3.0
15 C ₂₁ H ₂₃ NO ₈ ; 0.8 H ₂ O	Requires	C 58.4	H 5.7	N 3.2%

The starting material was prepared as follows :



20

Hydroxylamine hydrochloride (70 mg ; 1.0 mmol) was added to a solution of 3-hydroxy-2,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-one (2) (0.172 g ; 0.5 mmol) in pyridine (3.0 ml). The mixture was stirred at ambient temperature overnight. After dilution with 2N hydrochloric acid and extraction with ethyl acetate, the organic phase was washed with 2N hydrochloric acid, then brine and dried over MgSO₄. The residue was triturated with ether and hexanes to give (1) (a 3:1 mixture of E:Z isomers) as a white solid (170 mg).

Yield : 95%

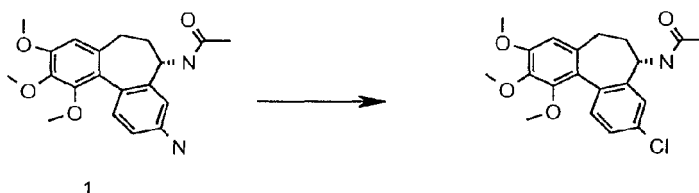
¹H NMR spectrum (CDCl₃), major isomer : 2.56 (m, 1H) ; 2.66-2.9 (m, 2H) ; 3.18 (m, 1H) ; 3.45 (s, 3H) ; 3.86 (s, 3H) ; 3.88 (s, 3H) ; 3.89 (s, 3H) ; 6.55 (s, 1H) ; 7.08 (s, 1H) ; 7.24 (s, 1H).

5 MS-ESI : 360 [MH]⁺

Elemental analysis :	Found	C 62.6	H 6.3	N 3.6
C ₁₉ H ₂₁ NO ₆ ; 0.3 H ₂ O	Requires	C 62.6	H 6.0	N 3.8%

Example 51

10



A solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (0.712 g ; 2 mmol) in ethanol (3.75 ml) and 36% hydrochloric acid (1.57 ml) was slowly added into a mixture of ice (6 ml) and 36% hydrochloric acid (1.57 ml). At 0°C a solution of sodium nitrite (0.14 g ; 2 mmol) in water (0.25 ml) was added. The mixture was stirred at 0°C for 1 hour and then transferred into a separate flask containing a solution of copper(I) chloride (0.218 g ; 2.2 mmol) in water (0.35 ml) and 36% hydrochloric acid (0.4 ml). The resulting mixture was stirred at 30°C for 30 minutes and extracted with toluene/ethyl acetate (50/50). The organic phase was washed with water, dilute sodium hydroxide, and saturated sodium chloride solution, then dried and the volatiles were removed by evaporation. The residue was purified by flash chromatography eluting with ethyl acetate to give *N*-[(5*S*)-3-chloro-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide.

25 Yield : 46%.

¹H NMR Spectrum (DMSO-d₆) : 1.89 (s, 3H) ; 1.90 (m, 1H) ; 2.02 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H) ; 3.50 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.52 (m, 1H) ; 6.80 (s, 1H) ; 7.35 (m, 3H) ; 8.43 (d, 1H).

MS-ESI : 398 [MNa]⁺**Example 52**

5

A solution of (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-(*N*-*tert*butoxycarbonylamino)-5-[(2-nitroethanimidoyl)amino]pentanamide (1) (0.15 g, 0.28 mmol) in dichloromethane (2 ml) was treated at 0°C with TFA (2 ml). The mixture was stirred at ambient temperature for 2 hours and evaporated. The residue was taken up in methanol/dichloromethane and evaporated to give an oil which was triturated in ether to give (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-amino-5-[(2-nitroethanimidoyl)amino]pentanamide as a solid.

15 Yield : 95 %

¹H NMR spectrum (DMSO-d₆) : 1.60 (m, 2H) ; 1.83 (m, 2H) ; 1.90 (s, 3H) ; 1.92 (m, 1H) ; 2.06 (m, 1H) ; 2.20 (m, 1H) ; 2.5 (m, 1H ; signal obscured by DMSO Peak) ; 3.22 (m, 2H) ; 3.50 (s, 3H) ; 3.79 (s, 3H) ; 3.85 (s, 3H) ; 3.95 (m, 1H) ; 4.48 (m, 1H) ; 6.80 (s, 1H) ; 7.32 (d, 1H) ; 7.45 (d, 1H) ; 7.75 (dd, 1H) ; 8.45 (d, 1H).

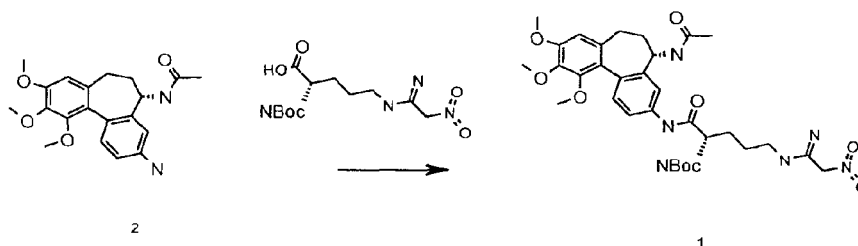
20 MS - ESI : 558 [MH]⁺

Elemental analysis	Found	C 48.1	H 5.7	N 13.2
C ₂₆ H ₃₅ N ₇ O ₇ ; 1.4 TFA; 0.5 methanol	Requires	C 48.0	H 5.3	N 13.4%

The starting material was prepared as follows:

25

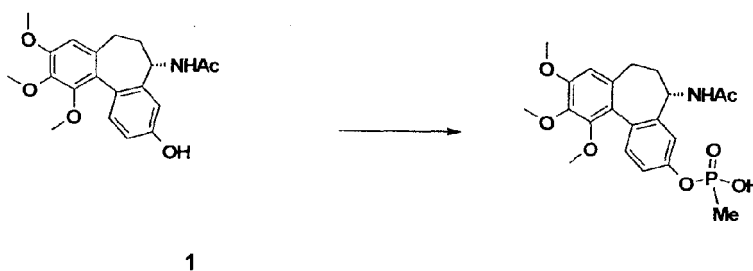
- 111 -



A solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide (2) (0.45 g ; 1.26 mmol), *N*α-*tert*-butoxycarbonyl-ω-nitro-L-arginine (50.402 g ; 1.26 mmol), EDCI (0.312 g ; 1.63 mmol) and DMAP (0.03 g ; 0.25 mmol) in dichloromethane (18 ml) was stirred at ambient temperature overnight. After addition of water (2 ml) and extraction, the organic phase was evaporated to give an oil which was purified by flash chromatography eluting with ethyl acetate/methanol (95/5) to give (1).
Yield : 28 %

¹H NMR (DMSO-d₆) : 1.38 (m, 2H) ; 1.40 (s, 9H) ; 1.60 (m, 2H) ; 1.90 (s, 3H) ; 1.91 (m, 1H) ; 2.15 (m, 2H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.20 (m, 2H) ; 3.48 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.13 (m, 1H) ; 4.50 (m, 1H) ; 6.80 (s, 1H) ; 7.10 (d, 1H) ; 7.27 (d, 2H) ; 7.55 (s, 1H) ; 7.62 (d, 1H) ; 8.40 (d, 1H).
MS - ESI : 658 [MH]⁺

Example 53



A solution of *N*-acetyl-colchicinol (0.36 g ; 1.0 mmol) in THF (40 ml) under argon was cooled to 0°C and treated with a 1.0M solution of lithiumHMDS in THF (1.1 ml ; 1.1 mmol). The mixture was stirred at 0°C for 1 hour and then added in portions over about 2

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hours to a solution of methylphosphonic dichloride (0.53 mg ; 4.0 mmol) in THF (150 ml). The mixture was stirred at ambient temperature for 15 minutes. After addition of water (200 ml) the THF was removed by evaporation. After removal of the insoluble material by filtration, the filtrate was purified on HP20 SS resin eluting with a gradient of 0-60% methanol/water. The methanol was removed by evaporation and the mixture was adjusted to pH7.14 with sodium hydroxide (0.1 M). The appropriate fractions were freeze-dried to give **(5S)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl hydrogen methylphosphonate** as a beige solid (180 mg).

Yield : 41%

¹H NMR spectrum (DMSO d₆ ; CF₃CO₂D) : 1.53 (d, 3H) ; 1.88 (s, 3H) ; 1.9 (m, 1H) ; 2.06 (m, 1H) ; 2.16 (m, 1H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.52 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.51 (m, 1H) ; 6.79 (s, 1H) ; 7.13 (s, 1H) ; 7.14 (d, 1H) ; 7.30 (d, 1H) ; 8.45 (d, 1H).

MS-ESI : 458 [MNa]⁺

Example 54

The following illustrate representative pharmaceutical dosage forms containing the compound of formula I, or a pharmaceutically acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

(a)	<u>Tablet I</u>	<u>mg/tablet</u>
	Compound X	100
	Lactose Ph.Eur.....	182.75
25	Croscarmellose sodium	12.0
	Maize starch paste (5% w/v paste)	2.25
	Magnesium stearate	3.0
(b)	<u>Tablet II</u>	<u>mg/tablet</u>
30	Compound X	50
	Lactose Ph.Eur.....	223.75
	Croscarmellose sodium	6.0

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		Maize starch.....	15.0
		Polyvinylpyrrolidone (5% w/v paste).....	2.25
		Magnesium stearate	3.0
	(c)	<u>Tablet III</u>	<u>mg/tablet</u>
5		Compound X	1.0
		Lactose Ph.Eur.....	93.25
		Croscarmellose sodium	4.0
		Maize starch paste (5% w/v paste)	0.75
		Magnesium stearate	1.0
10			
	(d)	<u>Capsule</u>	<u>mg/capsule</u>
		Compound X	10
		Lactose Ph.Eur.....	488.5
		Magnesium stearate	1.5
15			
	(e)	<u>Injection I</u>	<u>(50 mg/ml)</u>
		Compound X	5.0% w/v
		1M Sodium hydroxide solution.....	15.0% v/v
		0.1M Hydrochloric acid	
20		(to adjust pH to 7.6)	
		Polyethylene glycol 400	4.5% w/v
		Water for injection to 100%	
	(f)	<u>Injection II</u>	<u>10 mg/ml)</u>
25		Compound X	1.0% w/v
		Sodium phosphate BP.....	3.6% w/v
		0.1M Sodium hydroxide solution.....	15.0% v/v
		Water for injection to 100%	
30	(g)	<u>Injection III</u>	<u>(1mg/ml,buffered to pH6)</u>
		Compound X	0.1% w/v
		Sodium phosphate BP.....	2.26% w/v

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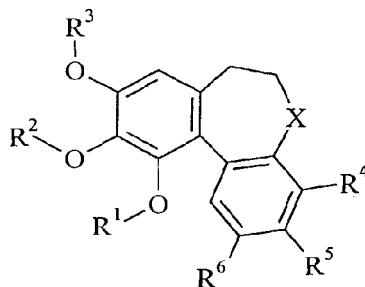
Citric acid0.38% w/v
Polyethylene glycol 4003.5% w/v
Water for injection to 100%

Note

- 5 The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

CLAIMS

1. The use of a compound of the formula I:



(I)

wherein

X is

-C(O)-, -C(S)-, -C=NOH, or -CH(R⁷)- wherein R⁷ is hydrogen, hydroxy, C₁₋₇alkoxy, -OR⁸ or -NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR¹¹-, -SO₂- or -SO₂NR¹²- (wherein R¹¹ and R¹², which may be the same or different, each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is selected from one of the following nine groups:

1) hydrogen, C₁₋₇alkyl, C₃₋₇cycloalkyl, C₁₋₄alkylY⁸C₁₋₄alkyl wherein Y⁸ is as defined herein, or phenyl,

(which alkyl, cycloalkyl, alkylY⁸alkyl or phenyl group may bear one or more substituents selected from:

halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, carboxy, carbamoyl, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, phenyl, nitro, sulphate, phosphate,

Z¹ (wherein Z¹ represents a 5-6 membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and Z² (wherein Z² is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms,

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selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)),

C₁₋₄alkylZ¹ (wherein Z¹ is as defined herein), and

a group -Y²R¹³ (wherein Y² is -NR¹⁴C(O)- or -O-C(O)- (wherein R¹⁴ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹³ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R¹⁵ wherein R¹⁵ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR¹⁶R¹⁷ and -NR¹⁸COR¹⁹ (wherein R¹⁶, R¹⁷, R¹⁸ and R¹⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))));

2) R¹⁵ wherein R¹⁵ is as defined herein;

3) C₂₋₇alkenylR¹⁵ (wherein R¹⁵ is as defined herein);

4) C₃₋₇alkynylR¹⁵ (wherein R¹⁵ is as defined herein);

5) Z¹ (wherein Z¹ is as defined herein);

6) C₁₋₇alkylZ¹ (wherein Z¹ is as defined herein);

7) C₁₋₇alkylY⁸Z¹ (wherein Z¹ is as defined herein and Y⁸ is -C(O)-, -NR⁵⁹C(O)-, -NR⁵⁹C(O)C₁₋₄alkyl-, -C(O)NR⁶⁰- or -C(O)NR⁶⁰C₁₋₄alkyl-, (wherein R⁵⁹ and R⁶⁰, which may be the same or different, each represents hydrogen, C₁₋₃alkyl, C₁₋₃hydroxyalkyl or C₁₋₃alkoxyC₂₋₃alkyl));

8) (C₁₋₇alkyl)_cY⁹Z³ (wherein c is 0 or 1, Z³ is an amino acid group and Y⁹ is a direct bond, -C(O)- or -NR⁶¹- (wherein R⁶¹ is hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)); and

9) C₁₋₇alkylR¹⁵ (wherein R¹⁵ is as defined herein);

and R⁹ is hydrogen, C₁₋₇alkyl or C₃₋₇cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C₁₋₄alkoxy and phenyl);

R¹, R² and R³ are each independently

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hydrogen, PO_3H_2 , sulphate, C_{3-7} cycloalkyl, C_{2-7} alkenyl, C_{2-7} alkynyl, C_{1-7} alkanoyl, a group $\text{R}^{20}\text{C}_{1-7}$ alkyl (wherein R^{20} is phenyl which may bear one or more substituents selected from C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} aminoalkyl and C_{1-4} hydroxyalkoxy), C_{1-7} alkyl or C_{1-7} alkylsulphonyl (which alkyl or alkylsulphonyl group may bear one or more substituents selected from:

5 halogeno, amino, C_{1-4} alkylamino, $\text{di}(\text{C}_{1-4}$ alkyl)amino, hydroxy, C_{1-4} alkoxy, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-\text{Y}^2\text{R}^{21}$ (wherein Y^2 is $-\text{NR}^{22}\text{C}(\text{O})-$ or $-\text{O}-\text{C}(\text{O})-$ (wherein R^{22} represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{21} is C_{1-7} alkyl, C_{3-7} cycloalkyl or a group R^{23} wherein R^{23} is a phenyl group or a 5-10-membered

10 aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, $-\text{CONR}^{24}\text{R}^{25}$ and $-\text{NR}^{26}\text{COR}^{27}$ (wherein R^{24} , R^{25} , R^{26} and

15 R^{27} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl));

with the proviso that at least two of R^1 , R^2 and R^3 are C_{1-7} alkyl;

R^4 , R^5 and R^6 are each independently selected from:

- 20 hydrogen, $-\text{OPO}_3\text{H}_2$, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C_{1-7} alkoxy, C_{1-7} alkanoyl, C_{1-7} thioalkoxy, C_{1-7} alkyl, (which alkyl group may bear one or more substituents selected from:
- halogeno, amino, C_{1-4} alkylamino, $\text{di}(\text{C}_{1-4}$ alkyl)amino, hydroxy, C_{1-4} alkoxy, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy,
- 25 phenyl, sulphate, phosphate and a group $-\text{Y}^3\text{R}^{28}$ (wherein Y^3 is $-\text{NR}^{29}\text{C}(\text{O})-$ or $-\text{O}-\text{C}(\text{O})-$ (wherein R^{29} represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{28} is C_{1-7} alkyl, C_{3-7} cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear
- 30 one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, $-\text{CONR}^{31}\text{R}^{32}$ and $-\text{NR}^{31}\text{COR}^{32}$ (wherein R^{31} , R^{32} , R^{33} and

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R^{34} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl)), and
 a group $-Y^4R^{35}$
 (wherein Y^4 is $-C(O)-$, $-OC(O)-$, $-O-$, $-SO-$, $-SO_2-$, $-OSO_2-$, $-NR^{36}-$, $-C_{1-4}$ alkyl $NR^{36}-$, $-C_{1-4}$ alkyl $C(O)-$, $-NR^{37}C(O)-$, $-OC(O)O-$, $-C(O)NR^{38}-$ or $-NR^{39}C(O)O-$ (wherein R^{36} , R^{37} , R^{38} and R^{39} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and
 R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, hydroxy, amino, C_{1-7} alkyl, C_{1-7} alkoxy, C_{1-7} alkanoyl, C_{1-7} alkylamino, di(C_{1-7} alkyl)amino, amino C_{1-7} alkylamino, C_{1-7} alkylamino C_{1-7} alkylamino, C_{1-7} alkanoylamino C_{1-7} alkyl, di(C_{1-7} alkyl)amino C_{1-7} alkylamino, C_{1-7} alkylphosphate, C_{1-7} alkylphosphonate, C_{1-7} alkylcarbamoyl C_{1-7} alkyl,
 (which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino, alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:
 halogeno, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-4} hydroxyalkyl, C_{1-4} alkoxy, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphonyl, C_{1-4} alkoxycarbonylamino, C_{1-4} alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^5R^{40}$ (wherein Y^5 is $-NR^{41}C(O)-$, $-C(O)NR^{42}-$, $-C(O)-O-$ or $-O-C(O)-$ (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{40} is C_{1-7} alkyl, C_{3-7} cycloalkyl, carboxy C_{1-7} alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy, cyano, $-CONR^{44}R^{45}$ and $-NR^{46}COR^{47}$ (wherein R^{44} , R^{45} , R^{46} and R^{47} , which may be the same or different, each represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl))),
 R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected

independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, di(C₁₋₄hydroxyalkyl)aminoC₁₋₄alkyl, di(C₁₋₄aminoalkyl)aminoC₁₋₄alkyl, C₁₋₄hydroxyalkoxy, carboxy, C₁₋₄carboxyalkyl, phenyl, cyano, -CONR⁴⁹R⁵⁰, -NR⁵¹COR⁵² (wherein R⁴⁹, R⁵⁰, R⁵¹ and R⁵², which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and C₁₋₄alkylR⁵³ (wherein R⁵³ is as defined herein), C₁₋₇alkylR⁴⁸ (wherein R⁴⁸ is as defined herein),

R⁵³ (wherein R⁵³ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄carboxyalkyl, C₁₋₄aminoalkyl, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and R⁵⁴ (wherein R⁵⁴ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonylC₁₋₄alkyl)), or

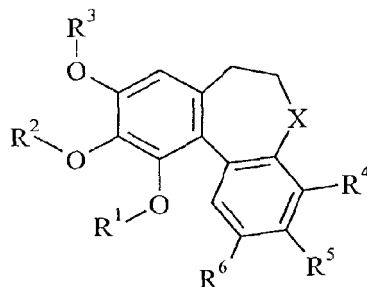
(CH₂)_aY⁶(CH₂)_bR⁵³ (wherein R⁵³ is as defined herein, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y⁶ represents a direct bond, -O-, -C(O)-, -NR⁵⁵-, -NR⁵⁶C(O)- or -C(O)NR⁵⁷- (wherein R⁵⁵, R⁵⁶, and R⁵⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl), and wherein one or more of the (CH₂)_a or (CH₂)_b groups may bear one or more substituents selected from hydroxy, amino and halogeno));

with the proviso that R⁵ is not hydroxy, alkoxy, substituted alkoxy (wherein R⁵ is Y⁴R³⁵ and Y⁴ is -O- and R³⁵ is C₁₋₇alkyl bearing one or more substituents selected from the list given herein), -OPO₃H₂, -O-C₁₋₇alkanoyl or benzyloxy;

or a salt thereof, a pharmaceutically acceptable salt thereof, a solvate or hydrate thereof, or a

prodrug thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.

2. A compound of the formula IIa:



(IIa)

5 wherein

X is

-C(O)-, -C(S)-, -C=NOH, or -CH(R⁷)- wherein R⁷ is hydrogen, hydroxy, C₁₋₇alkoxy, -OR⁸ or -NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR¹¹-, -SO₂- or -SO₂NR¹²- (wherein R¹¹ and R¹², which may be the same or
10 different, each independently represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is selected from one of the following nine groups:

1) hydrogen, C₁₋₇alkyl, C₃₋₇cycloalkyl, C₁₋₄alkylY⁸C₁₋₄alkyl wherein Y⁸ is as defined herein, or phenyl,

(which alkyl, cycloalkyl, alkylY⁸alkyl or phenyl group may bear one or more substituents

15 selected from:

halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, carboxy, carbamoyl, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, phenyl, nitro, sulphate, phosphate,

Z¹ (wherein Z¹ represents a 5-6 membered saturated heterocyclic group (linked via
20 carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and Z² (wherein Z² is a 5-6-membered saturated

25 heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

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oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl, C₁₋₇alkanoyl, cyanoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonyl(C₁₋₄alkyl)),

C₁₋₄alkylZ¹ (wherein Z¹ is as defined herein), and

- 5 a group -Y²R¹³ (wherein Y² is -NR¹⁴C(O)- or -O-C(O)- (wherein R¹⁴ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹³ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R¹⁵ wherein R¹⁵ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more
- 10 substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR¹⁶R¹⁷ and -NR¹⁸COR¹⁹ (wherein R¹⁶, R¹⁷, R¹⁸ and R¹⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- 15 2) R¹⁵ wherein R¹⁵ is as defined herein;
- 3) C₂₋₇alkenylR¹⁵ (wherein R¹⁵ is as defined herein);
- 4) C₃₋₇alkynylR¹⁵ (wherein R¹⁵ is as defined herein));
- 5) Z¹ (wherein Z¹ is as defined herein);
- 6) C₁₋₇alkylZ¹ (wherein Z¹ is as defined herein);
- 20 7) C₁₋₇alkylY⁸Z¹ (wherein Z¹ is as defined herein and Y⁸ is -C(O)-, -NR⁵⁹C(O)-, -NR⁵⁹C(O)C₁₋₄alkyl-, -C(O)NR⁶⁰- or -C(O)NR⁶⁰C₁₋₄alkyl-, (wherein R⁵⁹ and R⁶⁰, which may be the same or different, each represents hydrogen, C₁₋₃alkyl, C₁₋₃hydroxyalkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- 8) (C₁₋₇alkyl)_cY⁹Z³ (wherein c is 0 or 1, Z³ is an amino acid group and Y⁹ is a direct bond, -C(O)- or -NR⁶¹- (wherein R⁶¹ is hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl)); and
- 25 9) C₁₋₇alkylR¹⁵ (wherein R¹⁵ is as defined herein);
- and R⁹ is hydrogen, C₁₋₇alkyl or C₃₋₇cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C₁₋₄alkoxy and phenyl);
- R¹, R² and R³ are each independently
- hydrogen, PO₃H₂, sulphate, C₃₋₇cycloalkyl, C₂₋₇alkenyl, C₂₋₇alkynyl, C₁₋₇alkanoyl, a group
- 30 R²⁰C₁₋₇alkyl (wherein R²⁰ is phenyl which may bear one or more substituents selected from C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄aminoalkyl and C₁₋₄hydroxyalkoxy), C₁₋₇alkyl or C₁₋₇alkylsulphonyl
- (which alkyl or alkylsulphonyl group may bear one or more substituents selected from:

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- halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y²R²¹ (wherein Y² is -NR²²C(O)- or -O-C(O)- (wherein R²² represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²¹ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R²³ wherein R²³ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR²⁴R²⁵ and -NR²⁶COR²⁷ (wherein R²⁴, R²⁵, R²⁶ and R²⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- with the proviso that at least two of R¹, R² and R³ are C₁₋₇alkyl;
- R⁴ is
- hydrogen, cyano, halogeno, nitro, amino, hydroxy, C₁₋₇alkoxy, C₁₋₇thioalkoxy, C₁₋₇alkanoyl or C₁₋₇alkyl,
- (which alkyl group may bear one or more substituents selected from:
- halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y³R²⁸ (wherein Y³ is -NR²⁹C(O)- or -O-C(O)- (wherein R²⁹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²⁸ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R³⁰ wherein R³⁰ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR³¹R³² and -NR³¹COR³² (wherein R³¹, R³², R³³ and R³⁴, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl));
- R⁵ and R⁶ are each independently selected from
- hydrogen, -OPO₃H₂, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C₁₋₇alkoxy, C₁₋₇alkanoyl, C₁₋₇thioalkoxy, C₁₋₇alkyl,

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- (which alkyl group may bear one or more substituents selected from:
halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy,
phenyl, sulphate, phosphate and a group -Y³R²⁸ (wherein Y³ is -NR²⁹C(O)- or -O-C(O)-
5 (wherein R²⁹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R²⁸ is C₁₋₇alkyl, C₃₋₇cycloalkyl or a group R³⁰ wherein R³⁰ is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR³¹R³² and -NR³¹COR³² (wherein R³¹, R³², R³³ and R³⁴, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))), and
10 a group -Y⁴R³⁵
15 (wherein Y⁴ is -C(O)-, -OC(O)-, -O-, -SO-, -SO₂-, -OSO₂-, -NR³⁶-, -C₁₋₄alkylNR³⁶-, -C₁₋₄alkylC(O)-, -NR³⁷C(O)-, -OC(O)O-, -C(O)NR³⁸- or -NR³⁹C(O)O- (wherein R³⁶, R³⁷, R³⁸ and R³⁹, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and
R³⁵ is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate,
20 hydroxy, amino, C₁₋₇alkyl, C₁₋₇alkoxy, C₁₋₇alkanoyl, C₁₋₇alkylamino, di(C₁₋₇alkyl)amino, aminoC₁₋₇alkylamino, C₁₋₇alkylaminoC₁₋₇alkylamino, C₁₋₇alkanoylaminoC₁₋₇alkyl, di(C₁₋₇alkyl)aminoC₁₋₇alkylamino, C₁₋₇alkylphosphate, C₁₋₇alkylphosphonate, C₁₋₇alkylcarbamoylC₁₋₇alkyl,
25 (which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino, alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:
halogeno, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, hydroxy, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, C₁₋₄alkoxycarbonylamino, C₁₋₄alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y⁵R⁴⁰ (wherein Y⁵
30 is -NR⁴¹C(O)-, -C(O)NR⁴²-, -C(O)-O- or -O-C(O)- (wherein R⁴¹ and R⁴² which may be the same or different each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and

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- R⁴⁰ is C₁₋₇alkyl, C₃₋₇cycloalkyl, carboxyC₁₋₇alkyl or a group R⁴³ wherein R⁴³ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, C₁₋₄hydroxyalkoxy, carboxy, cyano, -CONR⁴⁴R⁴⁵ and -NR⁴⁶COR⁴⁷ (wherein R⁴⁴, R⁴⁵, R⁴⁶ and R⁴⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl))),
- 10 R⁴⁸ (wherein R⁴⁸ is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from
- hydroxy, nitro, halogeno, amino, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, di(C₁₋₄hydroxyalkyl)aminoC₁₋₄alkyl, di(C₁₋₄aminoalkyl)aminoC₁₋₄alkyl, C₁₋₄hydroxyalkoxy, carboxy, C₁₋₄carboxyalkyl, phenyl, cyano, -CONR⁴⁹R⁵⁰, -NR⁵¹COR⁵² (wherein R⁴⁹, R⁵⁰, R⁵¹ and R⁵², which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and C₁₋₄alkylR⁵³ (wherein R⁵³ is as defined herein),
- 20 C₁₋₇alkylR⁴⁸ (wherein R⁴⁸ is as defined herein),
- R⁵³ (wherein R⁵³ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from
- 25 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄carboxyalkyl, C₁₋₄aminoalkyl, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and R⁵⁴ (wherein R⁵⁴ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from
- 30 oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonyl(C₁₋₄alkyl)), or
- (CH₂)_aY⁶(CH₂)_bR⁵³ (wherein R⁵³ is as defined herein, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y⁶ represents a direct bond, -O-, -C(O)-, -NR⁵⁵-, -NR⁵⁶C(O)- or -

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C(O)NR⁵⁷- (wherein R⁵⁵, R⁵⁶, and R⁵⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl), and wherein one or more of the (CH₂)_a or (CH₂)_b groups may bear one or more substituents selected from hydroxy, amino and halogeno));

- 5 with the proviso that R⁵ is not hydroxy, alkoxy, substituted alkoxy (wherein R⁵ is Y⁴R³⁵ and Y⁴ is -O- and R³⁵ is C₁₋₇alkyl bearing one or more substituents selected from the list given herein), -OPO₃H₂, -O-C₁₋₇alkanoyl or benzyloxy;
with the further proviso that at least one of R⁵ or R⁶ is a group -Y⁴R³⁵ (wherein Y⁴ and R³⁵ are as defined herein) but with the further provisos
- 10 that when R⁵ is -Y⁴R³⁵ and R⁶ is hydrogen, hydroxy, methoxy or methoxycarbonyl, -Y⁴R³⁵ is not selected from cases wherein:

Y⁴ is -C(O)-, -OC(O)-, -O-, -SO-, -OSO₂-, -NR³⁶-, -NR³⁷C(O)- or -C(O)NR³⁸- (wherein R³⁶, R³⁷ and R³⁸, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R³⁵ is

- 15 a glycine, valine or lysine group, a dipeptide of glycine and valine groups, C₁₋₇alkyl, C₁₋₇alkoxy, C₁₋₇alkanoyl,

(which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno, hydroxy, and a group -Y⁵R⁴⁰ (wherein Y⁵ is -O-C(O)- and R⁴⁰ is C₁₋₇alkyl)),
or

- 20 R⁴⁸ (wherein R⁴⁸ is a tetrazolyl group (which may or may not be substituted as herein defined), a phenyl group or a benzyl group which phenyl or benzyl group may bear one or more substituents selected from C₁₋₄alkyl); and

that when R⁶ is -Y⁴R³⁵ and R⁵ is hydrogen, hydroxy, methoxy or methoxycarbonyl, -Y⁴R³⁵ is not selected from cases wherein:

- 25 Y⁴ is -C(O)-, -O- or -OSO₂- and R³⁵ is C₁₋₇alkyl, C₁₋₇alkoxy

(which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno),

- 30 R⁴⁸ (wherein R⁴⁸ is a benzyl group which benzyl group may bear one or more substituents selected from C₁₋₄alkyl), or

R⁵³ (wherein R⁵³ is piperidinyl);

or a salt thereof.

3. The use of a compound of the formula IIa as defined in claim 2, or a salt thereof, a pharmaceutically acceptable salt thereof, a solvate or hydrate thereof, or a prodrug thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.
4. A compound according to claim 2 wherein X is -CH(R⁷)- wherein R⁷ is -OR⁸ or -NR⁸R⁹ (wherein R⁸ is a group -Y¹R¹⁰ (wherein Y¹ is -C(O)-, -C(O)O- or -C(O)NR¹¹- (wherein R¹¹ represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and R¹⁰ is as defined in claim 2) and R⁹ is as defined in claim 2).
5. A compound according to claim 2 or claim 4 wherein R¹, R² and R³ are each methyl.
6. A compound according to any one of claims 2, 4 or 5 wherein R⁴ is hydrogen.
7. A compound according to any one of claims 2, 4, 5 or 6 wherein R⁶ is hydrogen, halogeno, amino, carboxy, hydroxy, C₁₋₇alkoxy or a group Y⁴R³⁵ (wherein Y⁴ is -C(O)-, -O- or -OSO₂- and R³⁵ is C₁₋₇alkyl, C₁₋₇alkoxy (which alkyl or alkoxy may bear one or more substituents selected from halogeno), R⁴⁸ (wherein R⁴⁸ is a benzyl group) or R⁵³ (wherein R⁵³ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms selected independently from O, S and N)).
8. A compound according to any one of claims 2, 4, 5, 6 or 7 wherein R⁶ is hydrogen, C(O)OCH₃ or methoxy.
9. A compound according to any one of claims 2, 4, 5, 6, 7 or 8 wherein R⁵ is hydrogen, halogeno, amino, carboxy, carbamoyl, C₁₋₇alkanoyl, C₁₋₇thioalkoxy, or a group -Y⁴R³⁵ (wherein Y⁴ is -C(O)-, -OC(O)-, -O-, -SO-, -OSO₂-, -NR³⁶-, -NR³⁷C(O)- or -C(O)NR³⁸- (wherein R³⁶, R³⁷ and R³⁸, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and

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R³⁵ is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, C₁₋₇alkyl, C₁₋₇alkoxy, C₁₋₇alkanoyl, C₁₋₇alkanoylaminoC₁₋₇alkyl,

(which alkyl, alkoxy, alkanoyl, alkanoylaminoalkyl may bear one or more substituents selected from:

5 halogeno, amino, hydroxy, carboxy, and a group -Y⁵R⁴⁰ (wherein Y⁵ is -C(O)-O- or -O-C(O)- and R⁴⁰ is C₁₋₇alkyl or a group R⁴³ wherein R⁴³ is a benzyl group),

R⁴⁸ (wherein R⁴⁸ is a phenyl group, a benzyl group or a 5-10-membered aromatic

heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected

independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may

10 bear one or more substituents selected from

hydroxy, fluoro, amino, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄aminoalkyl, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, di(C₁₋₄hydroxyalkyl)aminoC₁₋₄alkyl, di(C₁₋₄aminoalkyl)aminoC₁₋₄alkyl, C₁₋₄hydroxyalkoxy, carboxy, C₁₋₄carboxyalkyl, cyano, -CONR⁴⁹R⁵⁰, -NR⁵¹COR⁵² (wherein R⁴⁹, R⁵⁰, R⁵¹ and R⁵², which may be the

15 same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl) and C₁₋₄alkylR⁵³ (wherein R⁵³ is as defined herein),

C₁₋₇alkylR⁴⁸ (wherein R⁴⁸ is as defined herein),

R⁵³ (wherein R⁵³ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which

20 heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, fluoro, chloro, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄carboxyalkyl, C₁₋₄aminoalkyl, di(C₁₋₄alkyl)aminoC₁₋₄alkyl, C₁₋₄alkoxyC₁₋₄alkyl, C₁₋₄alkylsulphonylC₁₋₄alkyl and R⁵⁴ (wherein R⁵⁴ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N,

25 which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₁₋₄alkoxy, C₁₋₄alkoxyC₁₋₄alkyl and C₁₋₄alkylsulphonyl(C₁₋₄alkyl)), or

(CH₂)_aY⁶(CH₂)_bR⁵³ (wherein R⁵³ is as defined herein, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y⁶ represents a direct bond, -O-, -C(O)-, -NR⁵⁵-, -NR⁵⁶C(O)- or -

30 C(O)NR⁵⁷- (wherein R⁵⁵, R⁵⁶, and R⁵⁷, which may be the same or different, each represents hydrogen, C₁₋₃alkyl or C₁₋₃alkoxyC₂₋₃alkyl), and wherein one or more of the (CH₂)_a or

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(CH₂)_b groups may bear one or more substituents selected from hydroxy, amino and halogeno));

with the proviso that R⁵ is not alkoxy, substituted alkoxy (wherein R⁵ is Y⁴R³⁵ and Y⁴ is -O- and R³⁵ is C₁₋₇alkyl bearing one or more substituents selected from the list given herein), -O-

5 C₁₋₇alkanoyl or benzyloxy.

10. A compound according to claim 2 selected from:

(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-
 {[(2*R*)-2,6-diaminohexanoyl]amino}propanoate,

10 (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[(2-
 aminoacetyl)amino]propanoate,

N-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-
 yl]oxymethyl)-2-morpholinoacetamide,

(2*S*,3*S*,4*S*,5*R*,6*R*)-6-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
 15 dibenzo[*a,c*]cyclohepten-3-yl]oxy}-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylic acid,
N-{[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-
 dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide,
N-{[(5*S*)-3-(4-{morpholinomethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-
 dibenzo[*a,c*]cyclohepten-5-yl]acetamide,

20 (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl 3-[4-
 methylpiperazin-1-ylcarbonyl]propanoate,
 5-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-
 yl}oxycarbonyl]pentanoic acid,

4-(3-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-
 25 yl]oxy-3-oxopropyl}benzoic acid and

(2*S*)-*N*-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-
 yl]-2-amino-3-hydroxypropanamide,
 and salts thereof.

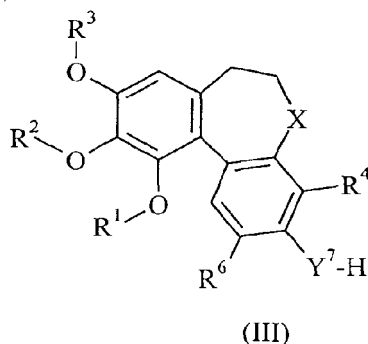
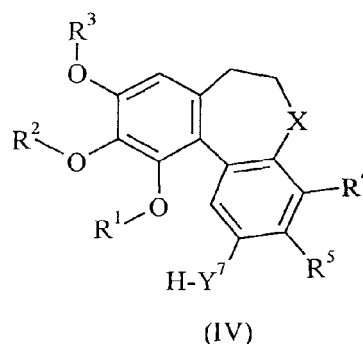
30 11. A compound according to claim 2 selected from

N-{[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-
 dihydro-5*H*-dibenzo[*a,c*]cyclohepten-5-yl]acetamide and

(2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-amino-3-hydroxypropanamide,
and salts thereof.

- 5 12. A compound according to claim 2 selected from
(2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl]-2-amino-5-[(2-nitroethanimidoyl)amino]pentanamide
and salts thereof.

- 10 13. A process for the manufacture of a compound of formula IIa as defined in claim 2 which comprises:
(a) for the preparation of compounds of formula IIa and salts thereof in which R⁵ or R⁶ is a group Y⁴R³⁵ (wherein R³⁵ is as defined in claim 2 and Y⁴ is a group -OC(O)- or -NHC(O)-), the reaction of a compound of formula III or IV:



(wherein X, R¹, R², R³, R⁴, R⁵, R⁶ are as defined in claim 2 and Y⁷ is -O- or -NH-), by acylation or coupling reactions;

- 20 (b) for the preparation of compounds of formula IIa and salts thereof in which R⁵ or R⁶ is a group Y⁴R³⁵ (wherein R³⁵ is C₁₋₇alkoxy which may be substituted as defined in claim 2 and Y⁴ is a group -OC(O)- or -NHC(O)-), the reaction of a compound of formula III and IV, by acylation reactions;
- (c) for the preparation of compounds of formula IIa and salts thereof in which R⁵ or R⁶ is a
25 group Y⁴R³⁵ (wherein R³⁵ is aminoC₁₋₇alkylamino, C₁₋₇alkylaminoC₁₋₇alkylamino, di(C₁₋₇alkyl)aminoC₁₋₇alkylamino and may be substituted as defined in claim 2, or is R⁵³ (wherein

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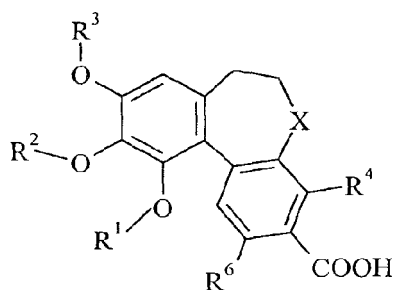
R^{53} is as defined in claim 2) and Y^4 is a group $-OC(O)-$ or $-NHC(O)-$, can be prepared by the reaction of a compound of formula III or IV, acylation reactions;

(d) for the preparation of compounds of formula IIa and salts thereof in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is a sugar moiety and Y^4 is a group $-O-$ or $-NH-$), the reaction of a compound of formula III or IV, glycosylation reactions;

(e) for the preparation of compounds of formula IIa and salts thereof in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is sulphate and Y^4 is a group $-O-$ or $-NH-$), the reaction of a compound of formula III or IV, by sulphonylation reactions;

(f) for the preparation of compounds of formula IIa and salts thereof in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is C_{1-7} alkylphosphate and may be substituted as defined in claim 2 and Y^4 is a group $-O-$ or $-NH-$), the reaction of a compound of formula III or IV, by phosphorylation reactions;

(g) for the preparation of compounds of formula IIa and salts thereof in which R^5 is amino the reaction of a carboxylic acid of formula V:



(V)

(wherein X , R^1 , R^2 , R^3 , R^4 and R^6 are as defined in claim 2) via Curtius rearrangement and hydrolysis; and

(h) for the preparation of compounds of formula IIa and salts thereof in which R^5 or R^6 is chloro the reaction of a compound of formula III or IV by the Sandmeyer reaction; and when a pharmaceutically acceptable salt of a compound of formula IIa is required, reaction of the compound obtained with an acid or base whereby to obtain the desired pharmaceutically acceptable salt.

14. A pharmaceutical composition which comprises as active ingredient a compound of formula IIa as defined in claim 2 or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable excipient or carrier.
- 5 15. A method for producing a vascular damaging effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula IIa or a pharmaceutically acceptable salt thereof as defined in claim 2.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/GB 99/04436

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C13/547 A61K31/165 A61K31/22 A61K31/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 99 02166 A (ANGIOGENE PHARM LTD ;DOUGHERTY GRAEME (GB)) 21 January 1999 (1999-01-21) the whole document	1-15
X	FR 4 685 M (ROUSSEL-UCLAF) 23 January 1967 (1967-01-23) page 1, left-hand column	2, 4-9
X	US 5 760 092 A (TIMASHEFF SERGE M ET AL) 2 June 1998 (1998-06-02) the whole document	1-15
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

29 March 2000

Date of mailing of the international search report

12/04/2000

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/04436

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 88, no. 17, 1978 Columbus, Ohio, US; abstract no. 121468j, KISELEV ET AL.: "Benzoid rearrangement of colchicine in the presence of ethylene glycol" page 572; XP002134241 abstract & ZH. ORG. KHIM., vol. 13, no. 11, - 1977 pages 2337-2342,	2,4-9
X	O BOYE ET AL: "Synthesis of carbon-14 labeled electrophilic ligands of the colchicine binding site of tubulin: chloroacetates of demethylthiocolchicines and of N-acetylcolchinal, isothiocyanates of 9-deoxy-N-acetylcolchinal" JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS, GB, SUSSEX, vol. 33, no. 4, 1 January 1993 (1993-01-01), pages 293-299, XP002081866 ISSN: 0362-4803 Page 296, Scheme 3	2,4-9
X	R BRECHT ET AL: "Dihydrocolchicine 8,12-endoperoxide. a novel starting material for convenient syntheses of the allocolchicinoids N-acetylcolchinal O-methyl ether and androbiphenylene" LIEBIGS ANNALEN/RECUEIL: ORGANIC AND BIOORGANIC CHEMISTRY - A EUROPEAN JOURNAL, US, VCH PUBLISHERS, FLORIDA, no. 11, 1 January 1997 (1997-01-01), pages 2275-2279, XP002081867 ISSN: 0947-3440 Compounds 6a, 6b page 2276, right-hand column	2,4-9
X	GIL-JONG KANG ET AL: "n-Acetylcolchinal O-methyl ether and thiocolchicine, potent analogs of colchicine modified in the C-ring" JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 265, no. 18, 25 June 1990 (1990-06-25), pages 10255-10259, XP002081868 ISSN: 0021-9258 page 10259, left-hand column; figure 1	2,4-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/04436

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9902166	A	21-01-1999	AU 8231198 A	08-02-1999
FR 4685	M		NONE	
US 5760092	A	02-06-1998	NONE	



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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
19/043,303	01/31/2025		2600	50535-710.305	19	1

CONFIRMATION NO. 1040

FILING RECEIPT



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21971

WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

Date Mailed: 03/11/2025

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Alexis HOWERTON, San Francisco, CA;
Hal GERBER, San Francisco, CA;

Applicant(s)

Spruce Biosciences, Inc., South San Francisco, CA;

Power of Attorney: None

Domestic Applications for which benefit is claimed

This application is a CON of 18/941,832 11/08/2024
which is a CON of 17/667,285 02/08/2022 ABN
which is a CON of 17/081,694 10/27/2020 PAT 11,304,950
which is a CON of PCT/US2019/029486 04/26/2019
which claims benefit of 62/822,815 03/23/2019
and claims benefit of 62/663,951 04/27/2018

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.) - **None.**
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 03/10/2025

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 19/043,303**

Projected Publication Date: 06/19/2025

Non-Publication Request: No

Early Publication Request: No

Title

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at <http://www.uspto.gov/web/offices/pac/doc/general/index.html>.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD						Application or Docket Number 19/043,303					
Substitute for Form PTO-875											
APPLICATION AS FILED - PART I											
		(Column 1)	(Column 2)		SMALL ENTITY		OR	OTHER THAN SMALL ENTITY			
FOR		NUMBER FILED		NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)		
BASIC FEE <small>(37 CFR 1.16(a) (b), or (c))</small>		N/A		N/A	N/A			N/A	350		
SEARCH FEE <small>(37 CFR 1.16(k), (l), or (m))</small>		N/A		N/A	N/A			N/A	770		
EXAMINATION FEE <small>(37 CFR 1.16(o) (p), or (q))</small>		N/A		N/A	N/A			N/A	880		
TOTAL CLAIMS <small>(37 CFR 1.16(i))</small>		19	minus 20 =	*			OR	x	= 0		
INDEPENDENT CLAIMS <small>(37 CFR 1.16(h))</small>		1	minus 3 =	*				x	= 0		
APPLICATION SIZE FEE <small>(37 CFR 1.16(s))</small>	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).								0		
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))									0		
					TOTAL			TOTAL	2000		
* If the difference in column 1 is less than zero, enter "0" in column 2.											
APPLICATION AS AMENDED - PART II											
		(Column 1)	(Column 2)		(Column 3)		SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
AMENDMENT A		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
	Total <small>(37 CFR 1.16(i))</small>	*	Minus	**	=	x	=	OR	x	=	
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus	***	=	x	=	OR	x	=	
	Application Size Fee (37 CFR 1.16(s))							OR			
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR			
					TOTAL ADD'L FEE			OR	TOTAL ADD'L FEE		
AMENDMENT B											
AMENDMENT B		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
	Total <small>(37 CFR 1.16(i))</small>	*	Minus	**	=	x	=	OR	x	=	
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus	***	=	x	=	OR	x	=	
	Application Size Fee (37 CFR 1.16(s))							OR			
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR			
					TOTAL ADD'L FEE			OR	TOTAL ADD'L FEE		
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.											



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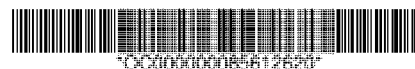
APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
19/043,303	01/31/2025	Alexis HOWERTON	50535-710.305

CONFIRMATION NO. 1040

21971

WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

INFORMAL NOTICE



Date Mailed: 03/11/2025

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
Alexis HOWERTON
Hal GERBER

An inventor's oath or declaration in compliance with 37 CFR 1.63 or 1.64 executed by or with respect to each inventor must be submitted no later than the date on which the issue fee is paid in response to a notice requiring such fee. See 37 CFR 1.53(f).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at (571) 272-4000 or (571) 272-4200 or 1-888-786-0101.

/eekubay/

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
19/043,303	01/31/2025	Alexis HOWERTON	50535-710.305	1040
21971 7590 04/01/2025 WILSON SONSINI GOODRICH & ROSATI 650 PAGE MILL ROAD PALO ALTO, CA 94304-1050			EXAMINER CENTRAL, DOCKET	
			ART UNIT OPAP	PAPER NUMBER
			NOTIFICATION DATE 04/01/2025	DELIVERY MODE ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

<i>Decision Granting Request for Prioritized Examination (Track I)</i>	Application No. 19/043,303	Applicant(s) HOWERTON et al.	
	Examiner TERRI S JACKSON	Art Unit OPET	AIA (FITF) Status Yes
<p>1. THE REQUEST FILED <u>31 January 2025</u> IS GRANTED .</p> <p>The above-identified application has met the requirements for prioritized examination</p> <p>A. ☒ for an original nonprovisional application (Track I).</p> <p>B. ☐ for an application undergoing continued examination (RCE).</p> <p>2. The above-identified application will undergo prioritized examination. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:</p> <p>A. filing a <u>petition for extension of time</u> to extend the time period for filing a reply;</p> <p>B. filing an <u>amendment to amend the application to contain more than four independent claims, more than thirty total claims</u>, or a multiple dependent claim;</p> <p>C. filing a <u>request for continued examination</u> ;</p> <p>D. filing a notice of appeal;</p> <p>E. filing a request for suspension of action;</p> <p>F. mailing of a notice of allowance;</p> <p>G. mailing of a final Office action;</p> <p>H. completion of examination as defined in 37 CFR 41.102; or</p> <p>I. abandonment of the application.</p> <p>Telephone inquiries with regard to this decision should be directed to TERRI JACKSON at (571)272-2991. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.</p>			
/TERRI S JACKSON/ Paralegal Specialist, OPET			



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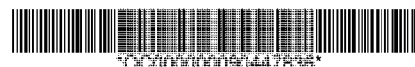
APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
19/043,303	01/31/2025	Alexis HOWERTON	50535-710.305

CONFIRMATION NO. 1040

21971

WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PUBLICATION NOTICE



Date Mailed: 06/20/2025

Title:METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Publication No.US-2025-0195527-A1

Publication Date:06/19/2025

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be viewed using the USPTO's publicly available Searchable Databases via the Patent Public Search tool at www.uspto.gov. The direct link to access the Patent Public Search tool is currently <https://ppubs.uspto.gov/pubwebapp/static/pages/ppubsbasic.html>.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through Patent Center, the USPTO's electronic patent application filing and management system. The direct link to access this status information is currently <https://patentcenter.uspto.gov/>. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of Patent Center.

Further assistance in electronically accessing the publication, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

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Doc Code: ECOMM.AUTH/ECOMM.WTDW

Doc Description: Internet Communications Authorization/Internet Communications Authorization Withdrawal

PTO/SB/439 (11-15)

**AUTHORIZATION FOR INTERNET
COMMUNICATIONS IN A PATENT
APPLICATION OR REQUEST TO
WITHDRAW AUTHORIZATION FOR
INTERNET COMMUNICATIONS**

Application No.	19/043,303
Filing Date	01/31/2025
First Named Inventor	Alexis HOWERTON
Art Unit	1627
Examiner Name	Sarah PIHONAK
Practitioner Docket No.	50535-710.305

To: Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I. To authorize permission for Internet Communications.

☒ Recognizing that Internet communications are not secure, I hereby authorize the USPTO to communicate with the undersigned and practitioners in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application via video conferencing, instant messaging, or electronic mail. I understand that a copy of these communications will be made of record in the application file. (MPEP 502.03)

II. To withdraw authorization for Internet Communications.

☐ The authorization given on _____, to the USPTO to communicate with the undersigned and any practitioner in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application via Internet communications such as video conferencing, instant messaging, or electronic mail is hereby withdrawn. I understand that the withdrawal is effective when approved rather than when received.

I am the

- ☐ applicant.
- ☐ attorney or agent of record. Registration number _____.
- ☒ attorney or agent acting under 37 CFR 1.34. Registration number 79,641.

/Victoria Cheng/

August 8, 2025

Signature

Date

Victoria CHENG

(832) 605-2488

Typed or printed name

Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Juristic entities must be represented by a patent practitioner (see 37 CFR 1.31, which is applicable to any paper filed on or after September 16, 2012, that is presented on behalf of a juristic entity, regardless of application filing date). Submit multiple forms if more than one signature is required, see below*.

☒ * Total of 1 forms are submitted.

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Routine uses of the information in this record may include disclosure to: 1) law enforcement, in the event that the system of records indicates a violation or potential violation of law; 2) a federal, state, local, or international agency, in response to its request; 3) a contractor of the USPTO having need for the information in order to perform a contract; 4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record; 5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record; 6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations; 7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals; 8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)); 9) the Office of Personnel Management (OPM) for personnel research purposes; and 10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION # 19/043,303	RECEIPT DATE / TIME 08/08/2025 03:06:31 PM Z ET	ATTORNEY DOCKET # 50535-710.305
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Title of Invention

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	1040	FILED BY	Colleen Mensching
PATENT CENTER #	71766517	FILING DATE	01/31/2025
CUSTOMER #	21971	FIRST NAMED INVENTOR	Alexis HOWERTON
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Victoria Cheng

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
Spruce 50535-710.305 - Internet Comms Auth.pdf	2	Internet Communications Authorization	217 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
Spruce 50535-710.305 - Internet Comms Auth.pdf	CFB2952341841C8ED0F0F3F4B9C2C50E1B0CA381DD2A918B 8708E5377317ED0590750BBD0712DD3D8FA1CB06A6E7976DF 1CFCB9029FAE66DA5449DB041F48220

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	HOWERTON; Alexis et al.	Group Art Unit:	1627
Serial Number:	19/043,264	Examiner:	PIHONAK, SARAH
Filing or 371 (c) Date:	2025-01-31	CONFIRMATION NO:	6961
Title:	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS		

FILED ELECTRONICALLY ON: August 13, 2025

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

A. ☐ 37 CFR § 1.97 (b). This Information Disclosure Statement should be considered by the Office because:

☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;

-- OR --

☐ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

☐ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

B. ☒ 37 CFR § 1.97(c). Although this Information Disclosure Statement is being filed after the period specified in 37 CFR § 1.97(b), above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

☐ a statement as specified in §1.97 (e) provided concurrently herewith;

-- OR --

☒ a fee of [\$280.00/112.00/56.00] as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. ☐ 37 CFR § 1.97 (d). Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in § 1.97 (e);

-- AND --

ii. a fee of [\$280.00/112.00/56.00] as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. ☐ 37 CFR §1.97 (e). Statement.

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);

-- AND/OR --

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);

-- AND/OR --

☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

- E. ☐ *Statement Under 37 C.F.R. §1.704(d)*. Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay. The Patent Term Adjustment Statement Under 37 CFR 1.704(d) on Form PTO/SB/133 is filed herewith.
- F. ☒ *37 CFR §1.98 (a) (2)*. The content of the Information Disclosure Statement is as follows:
- ☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.
- OR --
- ☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.
- AND/OR --
- ☒ Copies of Foreign Patent Documents and/or Non-Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).
- AND/OR --
- ☐ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).
- G. ☐ *37 CFR §1.98(a)(3)*. The Information Disclosure Statement includes non-English patents and/or references.
- ☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.
- ☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.
- OR --
- ☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____
- ☐ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.
- H. ☐ *37 CFR §1.98(d)*. Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:
- ☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.
- Application in which the information was submitted: _____
- Information Disclosure Statement(s) filed on: _____
- AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

- I. ☒ *Fee Authorization.* The Commissioner is hereby authorized to charge the above-referenced fees of \$280.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No. 50535-710.306).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: August 13, 2025

By: /Kaia R. Parenti/
Kaia R. Parenti, Reg. No. 81050

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	1	of	9	Attorney Docket Number	50535-710.306

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
		None			

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T ⁶
		Country Code ³ Number ⁴ Kind Code ⁵ (if known)				
	001	EP-3784233-B1	06-05-2024	SPRUCE BIOSCIENCES INC [US]		☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	
*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant			
¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.			

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

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				Examiner Name	PIHONAK, SARAH
Sheet	2	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	001	AUCHUS, Richard J. et al. Phase 3 trial of crinecerfont in adult congenital adrenal hyperplasia. New England Journal of Medicine 391(6):504-514 (2024).	☐
	002	AUCHUS, Richard J. et al. Supplemental Information: Phase 3 trial of crinecerfont in adult congenital adrenal hyperplasia. New England Journal of Medicine 391(6):504-514 (2024).	☐
	003	AUCHUS, Richard J. Management Considerations for the Adult With Congenital Adrenal Hyperplasia. Molecular and Cellular Endocrinology 408:190-197 (2015).	☐
	004	AVILA, N. A. et al. Testicular adrenal rest tissue in congenital adrenal hyperplasia: serial sonographic and clinical findings. AJR. American journal of roentgenology 172(5):1235-1238 (1999).	☐
	005	AYCAN, Zehra et al. Prevalence and long-term follow-up outcomes of testicular adrenal rest tumours in children and adolescent males with congenital adrenal hyperplasia. Clinical endocrinology 78(5):667-672 (2013).	☐
	006	CLAAHSEN-VAN DER GRINTEN, H. L. et al. Testicular adrenal rest tumours in congenital adrenal hyperplasia. International Journal of pediatric endocrinology 2009:624823, 1-8 (2009).	☐
	007	CLAAHSEN-VAN DER GRINTEN, Hedi L. et al. Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. European journal of endocrinology 157(3):339-344 (2007).	☐
	008	CLAAHSEN-VAN DER GRINTEN, Hedi L. et al. Repeated successful induction of fertility after replacing hydrocortisone with dexamethasone in a patient with congenital adrenal hyperplasia and testicular adrenal rest tumors. Fertility and Sterility 88(3):705.e5-705.e8 (2007).	☐

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				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	3	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	009	ClinicalTrials.gov Identifier: NCT04457336. A Ph2b to Evaluate Clinical Efficacy and Safety of Tildacerfont in Adult CAH, Record created June 25, 2020. pp. 1-17. [retrieved on 2025-08-12] Available at URL: https://clinicaltrials.gov/study/NCT04457336 .	☐
	010	DELFINO, Michele et al. Testicular adrenal rest tumors in patients with congenital adrenal hyperplasia: prevalence and sonographic, hormonal, and seminal characteristics. Journal of ultrasound in medicine 31(3):383-388 (2012).	☐
	011	ENGELS, Manon et al. Testicular adrenal rest tumors: current insights on prevalence, characteristics, origin, and treatment. Endocrine Reviews 40(4):973-987 (2019).	☐
	012	EP19793181.9 Brief Communication - Opposition Proceedings dated August 5, 2025.	☐
	013	EP19793181.9 Reply from the opponent to submission of proprietor dated July 18, 2025.	☐
	014	EP19793181.9 Reply of the Patent Proprietor to the notice(s) of opposition dated June 18, 2025.	☐
	015	FLECK, Beth A. et al. Binding Kinetics Redefine the Antagonist Pharmacology of the Corticotropin-releasing Factor Type 1 Receptor. The Journal of Pharmacology and Experimental Therapeutics 341(2):518-531 (2012).	☐
	016	JOSEPH, Dana N., and Shannon Whirlledge. Stress and the HPA axis: balancing homeostasis and fertility. International journal of molecular sciences 18(10):2224, 1-15 (2017).	☐
	017	MA, Li et al. Sonographic features of the testicular adrenal rests tumors in patients with congenital adrenal hyperplasia: a single-center experience and literature review. Orphanet Journal of Rare Diseases 14(1):242, 1-8 (2019).	☐

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				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	4	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	018	MAZZILLI, Rossella et al. The High Prevalence of Testicular Adrenal Rest Tumors in Adult Men With Congenital Adrenal Hyperplasia Is Correlated With ACTH Levels. Front Endocrinol (Lausanne) 10:335 (2019).	☐
	019	Neurocrine Biosciences, Inc. (Claimant) v. Spruce Biosciences, Inc. (Defendant). Defence dated March 13, 2025 (Claim No. HP-2025-000005) (pp. 1-3).	☐
	020	Neurocrine Biosciences, Inc. (Claimant) v. Spruce Biosciences, Inc. (Defendant). Grounds of Invalidity dated January 31, 2025 (Claim No. HP-2025-000005) (pp. 1-10).	☐
	021	Neurocrine Biosciences, Inc. (Claimant) v. Spruce Biosciences, Inc. (Defendant). Particulars of Claim dated January 31, 2025 (Claim No. HP-2025-000005) (pp. 1-10).	☐
	022	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Patent Owner's Response in U.S. Patent No. 12,115,166 dated July 8, 2025 (pp 1-6) (PGR-2025-00032).	☐
	023	Neurocrine Biosciences, Inc. (Plaintiff) v. Spruce Biosciences, Inc. (Defendant). Memorandum dated June 9, 2025 (Case No. 1:25-cv-00059-JDW (pp. 1-10).	☐
	024	NISSEN, Trygve et al. The clinical case report: a review of its merits and limitations. BMC Research Notes 7:264, 1-7 (2014).	☐
	025	REISCH, Nicole et al. High prevalence of reduced fecundity in men with congenital adrenal hyperplasia. The Journal of Clinical Endocrinology and Metabolism 94(5):1665-1670 (2009).	☐
	026	Spruce Biosciences announces topline results from CAHMELIA-203 in adult classic CAH and CAHPTAIN-205 in pediatric classic CAH. Spruce Biosciences :1-7 (2024).	☐

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				First Named Inventor	HOWERTON; Alexis
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				Examiner Name	PIHONAK, SARAH
Sheet	5	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
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	027	Spruce Biosciences announces topline results from CAHMELIA-204 in adult CAH and CAHPTAIN-205 in adult and pediatric CAH. Spruce Biosciences :1-4 (2024).	☐
	028	STIKKELBROECK, Nike M. M. L. et al. High prevalence of testicular adrenal rest tumors, impaired spermatogenesis, and Leydig cell failure in adolescent and adult males with congenital adrenal hyperplasia. The Journal of Clinical Endocrinology and Metabolism 86(12):5721-5728 (2001).	☐
	029	The Journal of Clinical Endocrinology and Metabolism Author Guidelines. Oxford Academic (2025); [retrieved on 2025-06-09]. Available at URL: https://academic.oup.com/jcem/pages/author_guidelines .	☐
	030	TURCU, Adina F. and Richard J. Auchus. Adrenal steroidogenesis and congenital adrenal hyperplasia. Endocrinology and Metabolism Clinics 44(2):275-296 (2015).	☐
	031	TURCU, Adina F., and Richard J. Auchus. Novel treatment strategies in congenital adrenal hyperplasia. Current Opinion in Endocrinology, Diabetes and Obesity 23(3):225-232 (2016).	☐
	032	TURCU, Adina F. et al. Supplemental Information: Single-dose study of a corticotropin-releasing factor receptor-1 antagonist in women with 21-hydroxylase deficiency. The Journal of Clinical Endocrinology and Metabolism 101(3):1174-1180 (2016).	☐
	033	U.S. Provisional Application No. 62/822,815 filed on March 23, 2019.	☐
	034	WALDRON, James. Spruce saws off only drug after 2nd hyperplasia fail, leaving biotech's direction in doubt. Fierce Biotech :1-2 (2024).	☐
	035	WANG, Zhu et al. Diagnosis of testicular adrenal rest tumors on ultrasound: a retrospective study of 15 cases report. Medicine 94(36):e1471, 1-6 (2015).	☐

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				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	6	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	036	World Health Organization Drug Information, 32(2):369-370 (2018).	☐
	037	YAMADA, Yasuki et al. New class of corticotropin-releasing factor (CRF) antagonists: small peptides having high binding affinity for CRF receptor. Journal of medicinal chemistry 47(5):1075-1078 (2004).	☐
	038	YASIR, Muhammad et al. Corticosteroid Adverse Effects. StatPearls, July 3, 2023; [retrieved on 2025-06-17]. Available at URL: https://www.ncbi.nlm.nih.gov/books/NBK531462/ pp. 1-14.	☐
	039	YU, Min Kyung et al. Clinical manifestations of testicular adrenal rest tumor in males with congenital adrenal hyperplasia. Annals of pediatric endocrinology and metabolism 20(3):155-161 (2015).	☐

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				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	7	of	9	Attorney Docket Number	50535-710.306

TIMING STATEMENT

Please see 37 CFR 1.97(e) to make the appropriate selection(s):

- ☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

- ☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

- ☐ See attached timing statement under 37 CFR 1.97(e).

- ☒ A timing statement under 37 CFR 1.97(e) is not submitted herewith.

TIMING FEE

- ☒ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

COPIES

- ☐ An identification of an earlier application pursuant to 37 CFR 1.98(d)(1) is attached.

SIZE FEE ASSERTION

Please see 37 CFR 1.17(v) and attached instructions for completing this form to make the appropriate selection of an assertion under 37 CFR 1.98. For the information disclosure statement (IDS) submitted herewith, the applicant or patent owner certifies the following with respect to the cumulative number of applicant-provided or patent-owner provided items of information submitted to date including those in the accompanying IDS (select only one):

- ☐ No IDS size fee is required under 37 CFR 1.17 (v) at this time.
- ☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(1).
- ☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(2).
- ☒ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(3).

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 11.18. Please see CFR 1.4(d) for the form of the signature.

Signature:	/Kaia R. Parenti/	Date (YYYY-MM-DD)	2025-08-13
Name/Print	Kaia R. Parenti	Registration Number	81050

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

<div>Substitute for form 1449/PTO</div> <div>INFORMATION DISCLOSURE STATEMENT BY APPLICANT</div> <div>(Use as many sheets as necessary)</div>				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	8	of	9	Attorney Docket Number	50535-710.306

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	HOWERTON; Alexis et al.	Group Art Unit:	1627
Serial Number:	19/043,264	Examiner:	PIHONAK, SARAH
Filing or 371 (c) Date:	2025-01-31	CONFIRMATION NO:	6961
Title:	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS		

FILED ELECTRONICALLY ON: August 13, 2025

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

- A. ☐ 37 CFR § 1.97 (b). This Information Disclosure Statement should be considered by the Office because:
- ☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);
 - OR --
 - ☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;
 - OR --
 - ☐ (3) It is being filed before the mailing of a first Office action on the merits;
 - OR --
 - ☐ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.
- B. ☒ 37 CFR § 1.97(c). Although this Information Disclosure Statement is being filed after the period specified in 37 CFR § 1.97(b), above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:
- ☐ a statement as specified in § 1.97 (e) provided concurrently herewith;
 - OR --
 - ☒ a fee of [\$280.00/112.00/56.00] as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.
- C. ☐ 37 CFR § 1.97 (d). Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:
- i. a statement as specified in § 1.97 (c);
 - AND --
 - ii. a fee of [\$280.00/112.00/56.00] as set forth in § 1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.
- D. ☐ 37 CFR § 1.97 (e). Statement.
- ☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);
 - AND/OR --
 - ☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);
 - AND/OR --
 - ☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

- E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay. The Patent Term Adjustment Statement Under 37 CFR 1.704(d) on Form PTO/SB/133 is filed herewith.
- F. ☒ *37 CFR §1.98 (a) (2).* The content of the Information Disclosure Statement is as follows:
- ☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.
- OR --
- ☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.
- AND/OR --
- ☒ Copies of Foreign Patent Documents and/or Non-Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).
- AND/OR --
- ☐ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).
- G. ☐ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.
- ☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.
- ☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.
- OR --
- ☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____
- ☐ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.
- H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:
- ☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.
- Application in which the information was submitted: _____
- Information Disclosure Statement(s) filed on: _____
- AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

- I. ☒ *Fee Authorization.* The Commissioner is hereby authorized to charge the above-referenced fees of \$280.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No. 50535-710.306).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: August 13, 2025

By: /Kaia R. Parenti/
Kaia R. Parenti, Rcg. No. 81050

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	1	of	9	Attorney Docket Number	50535-710.306

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
		None			

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T ⁶
		Country Code ³ Number ⁴ Kind Code ⁵ (if known)				
	001	EP-3784233-B1	06-05-2024	SPRUCE BIOSCIENCES INC [US]		☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	
*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant			
¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.			

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				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	2	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	001	AUCHUS, Richard J. et al. Phase 3 trial of crinecerfont in adult congenital adrenal hyperplasia. New England Journal of Medicine 391(6):504-514 (2024).	☐
	002	AUCHUS, Richard J. et al. Supplemental Information: Phase 3 trial of crinecerfont in adult congenital adrenal hyperplasia. New England Journal of Medicine 391(6):504-514 (2024).	☐
	003	AUCHUS, Richard J. Management Considerations for the Adult With Congenital Adrenal Hyperplasia. Molecular and Cellular Endocrinology 408:190-197 (2015).	☐
	004	AVILA, N. A. et al. Testicular adrenal rest tissue in congenital adrenal hyperplasia: serial sonographic and clinical findings. AJR. American journal of roentgenology 172(5):1235-1238 (1999).	☐
	005	AYCAN, Zehra et al. Prevalence and long-term follow-up outcomes of testicular adrenal rest tumours in children and adolescent males with congenital adrenal hyperplasia. Clinical endocrinology 78(5):667-672 (2013).	☐
	006	CLAAHSEN-VAN DER GRINTEN, H. L. et al. Testicular adrenal rest tumours in congenital adrenal hyperplasia. International Journal of pediatric endocrinology 2009:624823, 1-8 (2009).	☐
	007	CLAAHSEN-VAN DER GRINTEN, Hedi L. et al. Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. European journal of endocrinology 157(3):339-344 (2007).	☐
	008	CLAAHSEN-VAN DER GRINTEN, Hedi L. et al. Repeated successful induction of fertility after replacing hydrocortisone with dexamethasone in a patient with congenital adrenal hyperplasia and testicular adrenal rest tumors. Fertility and Sterility 88(3):705.e5-705.e8 (2007).	☐

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				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	3	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
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	009	ClinicalTrials.gov Identifier: NCT04457336. A Ph2b to Evaluate Clinical Efficacy and Safety of Tildacerfont in Adult CAH, Record created June 25, 2020. pp. 1-17. [retrieved on 2025-08-12] Available at URL: https://clinicaltrials.gov/study/NCT04457336 .	☐
	010	DELFINO, Michele et al. Testicular adrenal rest tumors in patients with congenital adrenal hyperplasia: prevalence and sonographic, hormonal, and seminal characteristics. Journal of ultrasound in medicine 31(3):383-388 (2012).	☐
	011	ENGELS, Manon et al. Testicular adrenal rest tumors: current insights on prevalence, characteristics, origin, and treatment. Endocrine Reviews 40(4):973-987 (2019).	☐
	012	EP19793181.9 Brief Communication - Opposition Proceedings dated August 5, 2025.	☐
	013	EP19793181.9 Reply from the opponent to submission of proprietor dated July 18, 2025.	☐
	014	EP19793181.9 Reply of the Patent Proprietor to the notice(s) of opposition dated June 18, 2025.	☐
	015	FLECK, Beth A. et al. Binding Kinetics Redefine the Antagonist Pharmacology of the Corticotropin-releasing Factor Type 1 Receptor. The Journal of Pharmacology and Experimental Therapeutics 341(2):518-531 (2012).	☐
	016	JOSEPH, Dana N., and Shannon Whirlledge. Stress and the HPA axis: balancing homeostasis and fertility. International journal of molecular sciences 18(10):2224, 1-15 (2017).	☐
	017	MA, Li et al. Sonographic features of the testicular adrenal rests tumors in patients with congenital adrenal hyperplasia: a single-center experience and literature review. Orphanet Journal of Rare Diseases 14(1):242, 1-8 (2019).	☐

EXAMINER SIGNATURE			
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Sheet	4	of	9	Attorney Docket Number	50535-710.306

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	018	MAZZILLI, Rossella et al. The High Prevalence of Testicular Adrenal Rest Tumors in Adult Men With Congenital Adrenal Hyperplasia Is Correlated With ACTH Levels. Front Endocrinol (Lausanne) 10:335 (2019).	☐
	019	Neurocrine Biosciences, Inc. (Claimant) v. Spruce Biosciences, Inc. (Defendant). Defence dated March 13, 2025 (Claim No. HP-2025-000005) (pp. 1-3).	☐
	020	Neurocrine Biosciences, Inc. (Claimant) v. Spruce Biosciences, Inc. (Defendant). Grounds of Invalidity dated January 31, 2025 (Claim No. HP-2025-000005) (pp. 1-10).	☐
	021	Neurocrine Biosciences, Inc. (Claimant) v. Spruce Biosciences, Inc. (Defendant). Particulars of Claim dated January 31, 2025 (Claim No. HP-2025-000005) (pp. 1-10).	☐
	022	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Patent Owner's Response in U.S. Patent No. 12,115,166 dated July 8, 2025 (pp 1-6) (PGR-2025-00032).	☐
	023	Neurocrine Biosciences, Inc. (Plaintiff) v. Spruce Biosciences, Inc. (Defendant). Memorandum dated June 9, 2025 (Case No. 1:25-cv-00059-JDW (pp. 1-10).	☐
	024	NISSEN, Trygve et al. The clinical case report: a review of its merits and limitations. BMC Research Notes 7:264, 1-7 (2014).	☐
	025	REISCH, Nicole et al. High prevalence of reduced fecundity in men with congenital adrenal hyperplasia. The Journal of Clinical Endocrinology and Metabolism 94(5):1665-1670 (2009).	☐
	026	Spruce Biosciences announces topline results from CAHMELIA-203 in adult classic CAH and CAHPTAIN-205 in pediatric classic CAH. Spruce Biosciences :1-7 (2024).	☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	
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				Art Unit	1627
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Sheet	5	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
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	027	Spruce Biosciences announces topline results from CAHMELIA-204 in adult CAH and CAHPTAIN-205 in adult and pediatric CAH. Spruce Biosciences :1-4 (2024).	☐
	028	STIKKELBROECK, Nike M. M. L. et al. High prevalence of testicular adrenal rest tumors, impaired spermatogenesis, and Leydig cell failure in adolescent and adult males with congenital adrenal hyperplasia. The Journal of Clinical Endocrinology and Metabolism 86(12):5721-5728 (2001).	☐
	029	The Journal of Clinical Endocrinology and Metabolism Author Guidelines. Oxford Academic (2025); [retrieved on 2025-06-09]. Available at URL: https://academic.oup.com/jcem/pages/author_guidelines .	☐
	030	TURCU, Adina F. and Richard J. Auchus. Adrenal steroidogenesis and congenital adrenal hyperplasia. Endocrinology and Metabolism Clinics 44(2):275-296 (2015).	☐
	031	TURCU, Adina F., and Richard J. Auchus. Novel treatment strategies in congenital adrenal hyperplasia. Current Opinion in Endocrinology, Diabetes and Obesity 23(3):225-232 (2016).	☐
	032	TURCU, Adina F. et al. Supplemental Information: Single-dose study of a corticotropin-releasing factor receptor-1 antagonist in women with 21-hydroxylase deficiency. The Journal of Clinical Endocrinology and Metabolism 101(3):1174-1180 (2016).	☐
	033	U.S. Provisional Application No. 62/822,815 filed on March 23, 2019.	☐
	034	WALDRON, James. Spruce saws off only drug after 2nd hyperplasia fail, leaving biotech's direction in doubt. Fierce Biotech :1-2 (2024).	☐
	035	WANG, Zhu et al. Diagnosis of testicular adrenal rest tumors on ultrasound: a retrospective study of 15 cases report. Medicine 94(36):e1471, 1-6 (2015).	☐

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				Art Unit	1627
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Sheet	6	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
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	036	World Health Organization Drug Information, 32(2):369-370 (2018).	☐
	037	YAMADA, Yasuki et al. New class of corticotropin-releasing factor (CRF) antagonists: small peptides having high binding affinity for CRF receptor. Journal of medicinal chemistry 47(5):1075-1078 (2004).	☐
	038	YASIR, Muhammad et al. Corticosteroid Adverse Effects. StatPearls, July 3, 2023; [retrieved on 2025-06-17]. Available at URL: https://www.ncbi.nlm.nih.gov/books/NBK531462/ pp. 1-14.	☐
	039	YU, Min Kyung et al. Clinical manifestations of testicular adrenal rest tumor in males with congenital adrenal hyperplasia. Annals of pediatric endocrinology and metabolism 20(3):155-161 (2015).	☐

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				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	7	of	9	Attorney Docket Number	50535-710.306

TIMING STATEMENT			
Please see 37 CFR 1.97(e) to make the appropriate selection(s):			
☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).			
OR			
☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).			
☐ See attached timing statement under 37 CFR 1.97(e).			
☒ A timing statement under 37 CFR 1.97(e) is not submitted herewith.			
TIMING FEE			
☒ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.			
COPIES			
☐ An identification of an earlier application pursuant to 37 CFR 1.98(d)(1) is attached.			
SIZE FEE ASSERTION			
Please see 37 CFR 1.17(v) and attached instructions for completing this form to make the appropriate selection of an assertion under 37 CFR 1.98. For the information disclosure statement (IDS) submitted herewith, the applicant or patent owner certifies the following with respect to the cumulative number of applicant-provided or patent-owner provided items of information submitted to date including those in the accompanying IDS (select only one):			
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SIGNATURE			
A signature of the applicant or representative is required in accordance with CFR 1.33, 11.18. Please see CFR 1.4(d) for the form of the signature.			
Signature:	/Kaia R. Parenti/	Date (YYYY-MM-DD)	2025-08-13
Name/Print	Kaia R. Parenti	Registration Number	81050

<div>Substitute for form 1449/PTO</div> <div>INFORMATION DISCLOSURE STATEMENT BY APPLICANT</div> <div>(Use as many sheets as necessary)</div>				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	8	of	9	Attorney Docket Number	50535-710.306

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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ELECTRONIC PAYMENT RECEIPT

APPLICATION # 19/043,303	RECEIPT DATE / TIME 08/13/2025 06:18:52 PM Z ET	ATTORNEY DOCKET # 50535-710.305
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Title of Invention

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	1040	FILED BY	Jennifer Vail
PATENT CENTER #	71844644	AUTHORIZED BY	Kaia Parenti
CUSTOMER #	21971	FILING DATE	01/31/2025
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Alexis HOWERTON

Payment Information

PAYMENT METHOD DA / 232415	PAYMENT TRANSACTION ID E20258CI19367619	PAYMENT AUTHORIZED BY Jennifer Vail
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1834	FILING AN INFORMATION DISCLOSURE STATEMENT THAT CAUSES THE CUMULATIVE NUMBER OF APPLICANT- PROVIDED ITEMS OF INFORMATION TO EXCEED 200	600.00	1	600.00
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under 35 USC 111(a)

PATENT # -

CONFIRMATION # 1040

FILED BY Jennifer Vail

PATENT CENTER # 71844644

FILING DATE 01/31/2025

CUSTOMER # 21971

FIRST NAMED INVENTOR Alexis HOWERTON

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Kaia Parenti

Documents

TOTAL DOCUMENTS: 39

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
NPL_TURCU_Supplemental_Single_1174_1180_2016.pdf	9	Non Patent Literature	1210 KB
NPL_US62822815_Provisional_2019_03_23.pdf	25	Non Patent Literature	1257 KB
NPL_TURCU_Single_dose_1174_1180_2016.pdf	7	Non Patent Literature	3996 KB
NPL_US16639540_OA_16NOV2021.pdf	4	Non Patent Literature	160 KB
NPL_US16388620_OA_15July2019.pdf	8	Non Patent Literature	262 KB
NPL_US16388620_OA_25Nov2019.pdf	9	Non Patent Literature	316 KB

NPL_US13063226_OA_24Jan2013.pdf	12	Non Patent Literature	416 KB
NPL_US16639540_OA_28March2024_.pdf	11	Non Patent Literature	652 KB
NPL_US16639540-OA_11Jul2022.pdf	9	Non Patent Literature	508 KB
NPL_US16639541_OA_4APRIL2022.pdf	15	Non Patent Literature	532 KB
NPL_US17063592_OA_7DEC2021.pdf	13	Non Patent Literature	461 KB
NPL_US17078054_OA_14DEC2020.pdf	13	Non Patent Literature	469 KB
NPL_US17359411_OA_6JAN2022.pdf	17	Non Patent Literature	582 KB
NPL_US17586228_OA_30MAR2020.pdf	17	Non Patent Literature	588 KB
NPL_US17359414_OA_16NOV2021.pdf	14	Non Patent Literature	470 KB
NPL_US17586228_OA10jun2022.pdf	16	Non Patent Literature	533 KB
NPL_US16639540_OA_25MAY2023.pdf	6	Non Patent Literature	4847 KB
NPL_US18078649_OA_09Oct2024.pdf	9	Non Patent Literature	710 KB
NPL_US18078649_OA_21MARCH2024.pdf	15	Non Patent Literature	1356 KB
NPL_US16639540_OA_28SEP2023.pdf	7	Non Patent Literature	5762 KB
NPL_US19043264_OA_25April2025.pdf	32	Non Patent Literature	1123 KB
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NPL_US19043275_OA_30May2025.pdf	30	Non Patent Literature	764 KB
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NPL_World_Drug_Information_369_370_2018.pdf	2	Non Patent Literature	76 KB
NPL_US17720074_OA_26AUG2022.pdf	9	Non Patent Literature	6808 KB
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NPL_US18078649_OA_08SEP2023.pdf	11	Non Patent Literature	9496 KB
NPL_ZORRILLA_Therapeutic_potential_799_828_2004.pdf	31	Non Patent Literature	4538 KB
Spruce 50535-710_306_IDS.pdf	13	-	351 KB
Spruce 50535-710_306_IDS-TRAN.LET.pdf	(1-4) 4	Transmittal Letter	155 KB
Spruce 50535-710_306_IDS-IDS.pdf	(5-13) 9	Information Disclosure Statement (IDS) Form (SB08)	250 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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Bibliographic data

Title: CORTICOTROPIN RELEASING FACTOR ANTAGONIST FOR PREVENTING SUDDEN DEATH
Pub/Pat no: JP2000302693A
Pub/Issue Date: 2000-10-31
Inventor(s): FOSSA ANTHONY ANDREA|FOSSA ANTHONY ANDREA
Applicant(s): PFIZER PROD INC
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Application number: JP20000100606 2000-04-03
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Abstract of JP2000302693A

PROBLEM TO BE SOLVED: To obtain a medicine having low side effect, effective for preventing the sudden death of animal and useful for the treatment of cardiac diseases, hypertension, osteoporosis, anxiety, diabetes, or the like, by including a therapeutically effective amount of a corticotropin releasing factor antagonist. SOLUTION: The objective medicine contains a therapeutically effective amount of a corticotropin releasing factor antagonist such as a compound (salt) expressed by formula I or II [the dotted line is optionally existing double bond; A is N or CR7 (R7 is H, a 1-4C alkyl, or the like); B is NR1R2, CR1R2R11 (R1 is H or a 1-6C alkyl; R2 is a 1-6C alkyl, an aryl, or the like; R11 is H, hydroxy, or the like), or the like; D is N, CR10 (R10 is H, a 1-6C alkyl, or the like), or the like; E is N, CR4 (R4 is H, a 1-6C alkyl, or the like), or the like; Z is NH, O, S, or the like; R3 is H, a halogen, a 1-6C alkyl, or the like; R5 is phenyl, naphthyl, or the like], e.g. 4-(1-ethyl-propoxy)-2,5-dimethyl-6-(2,4,6-trimethyl-benzyl)-pyrimidine. The daily administration rate of the compound is preferably 1.0-20 mg/kg body-weight.

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(54) 【発明の名称】 突然死予防のためのコルチコトロピン放出因子アンタゴニスト

(57) 【要約】

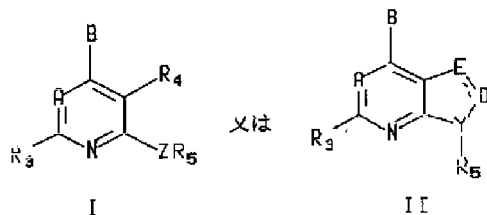
コルチコトロピン放出因子アンタゴニストの治療上有効量を動物に投与する。本発明の方法は、心臓疾患、高血圧、頻脈、うっ血性心不全、脳卒中、骨粗鬆症、早産、心理的萎縮、ストレス性発熱、潰瘍、下痢、術後腸閉塞、精神的不安及びストレスに関連する腸の過敏、糖尿病、神経性疾患、脳障害、ギランバレー症候群、小児突然死症候群、先天性過換気症候群又は尿毒症性神経障害の患者に特に有用である。

【特許請求の範囲】

【請求項1】 動物における突然死の予防のための、治療上有効量のコルチコトロピン放出因子アンタゴニストを含む、薬剤。

【請求項2】 コルチコトロピン放出因子アンタゴニストが、式I又はII：

【化1】



〔式中、点線は所望により存在する二重結合を表し、Aは $-\text{CR}_7$ 又はNであり、Bは $-\text{NR}_1\text{R}_2$ 、 $-\text{CR}_1\text{R}_2\text{R}_{11}$ 、 $-\text{C}(=\text{CR}_1\text{R}_{12})\text{R}_2$ 、 $-\text{NHCR}_{11}\text{R}_1\text{R}_2$ 、 $-\text{OCR}_{11}\text{R}_1\text{R}_2$ 、 $-\text{SCR}_{11}\text{R}_1\text{R}_2$ 、 $-\text{CR}_{11}\text{R}_2\text{OR}_1$ 、 $-\text{CR}_{11}\text{R}_2\text{SR}_1$ 、 $-\text{C}(\text{S})\text{R}_2$ 、 $-\text{NHR}_1\text{R}_2$ 、 $-\text{CR}_2\text{R}_1\text{NHR}_1$ 又は $-\text{C}(\text{O})\text{R}_2$ であり；DはEとDとが二重結合で結合し、及びEが $-\text{CR}_4$ であるとき、N又は $-\text{CR}_{10}$ であり；EとDとが二重結合で結合し、及びEがNであるとき、 $-\text{CR}_{10}$ であり；又はEとDとが単結合で結合しているとき、 $-\text{CR}_8\text{R}_9$ 、 $-\text{CHR}_{10}$ 、 $-\text{C}=\text{O}$ 、 $-\text{C}=\text{S}$ 、 $-\text{C}=\text{NH}$ 又は $-\text{C}=\text{NCH}_3$ であり；EはEとDとが二重結合で結合しているとき、 $-\text{CR}_4$ 又はNであり；又はEとDとが単結合で結合しているとき、 $-\text{CR}_4\text{R}_6$ 又は $-\text{NR}_6$ であり；YはN又は $-\text{CH}$ であり；ZはNH、O、S、 $-\text{N}(\text{C}_1\sim\text{C}_2\text{アルキル})$ 又は $-\text{CR}_{12}\text{R}_{13}$ であり、このとき R_{12} 及び R_{13} は各々独立に水素、トリフルオロメチル若しくはメチルであるか、又は R_{12} 及び R_{13} の一方がシアノ、他方が水素若しくはメチルであり； R_1 は水素又は $\text{C}_1\sim\text{C}_6$ アルキルであり、このとき前記 $\text{C}_1\sim\text{C}_6$ アルキルはヒドロキシ、シアノ、ニトロ、フルオロ、クロロ、ブロモ、ヨード、 CF_3 、 $\text{C}_1\sim\text{C}_4$ アルコキシ、 $-\text{O}-\text{CO}-$ ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{O}-\text{CO}-\text{NH}$ ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{O}-\text{CO}-\text{N}$ ($\text{C}_1\sim\text{C}_4$ アルキル) ($\text{C}_1\sim\text{C}_2$ アルキル)、 NH ($\text{C}_1\sim\text{C}_4$ アルキル)、 N ($\text{C}_1\sim\text{C}_2$ アルキル) ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{S}$ ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{N}$ ($\text{C}_1\sim\text{C}_4$ アルキル) CO ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{NHCO}$ ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{CO}_2$ ($\text{C}_1\sim\text{C}_2$ アルキル)、 $-\text{CONH}$ ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{CON}$ ($\text{C}_1\sim\text{C}_4$ アルキル) ($\text{C}_1\sim\text{C}_2$ アルキル)、($\text{C}_1\sim\text{C}_4$ アルキル)スルフィニル、($\text{C}_1\sim\text{C}_4$ アルキル)スルホニル及び($\text{C}_1\sim\text{C}_4$ アルキル)スルファニルから独立して選択される2個以下の置換基で置換されていてもよく、このとき前述の R_1 基中の前記 $\text{C}_1\sim\text{C}_6$ アルキル、 $\text{C}_1\sim\text{C}_4$ アルコキシ及び $\text{C}_1\sim\text{C}_6$ アルキル部分は、1個の二重結合又は三重結合を含んでもよく； R_2 は $\text{C}_1\sim\text{C}_6$ アルキル、ヘテロアリール、アリール、ヘテロアリール ($\text{C}_1\sim\text{C}_4$ アルキル)又はアリール ($\text{C}_1\sim\text{C}_4$ アルキル)であり、このとき前記アリール及び前記 ($\text{C}_1\sim\text{C}_4$ アルキル) C_1

$\sim\text{C}_4$ アルキルのアリール部分はフェニル及びナフチルから選択され、及び前記ヘテロアリール及び前記 ($\text{C}_1\sim\text{C}_4$ アルキル) $\text{C}_1\sim\text{C}_4$ アルキルのヘテロアリール部分はチエニル、ベンゾチエニル、ピリジル、チアゾリル、キノリル、ピラジニル、ピリミジル、イミダゾリル、フラニル、ベンゾフラニル、ベンゾチアゾリル、イソチアゾリル、ベンズイソチアゾリル、ベンズイソキサゾリル、ベンズイミダゾリル、インドリル及びベンズキサゾリルから選択されるか；又は R_2 は $\text{C}_3\sim\text{C}_8$ シクロアルキル又は ($\text{C}_3\sim\text{C}_8$ シクロアルキル) $\text{C}_1\sim\text{C}_6$ アルキルであり、このとき少なくとも4個の環員を有する前記シクロアルキル及び少なくとも4個の環員を有する前記 ($\text{C}_3\sim\text{C}_8$ シクロアルキル) $\text{C}_1\sim\text{C}_6$ アルキルのシクロアルキル部分の環員炭素の1又は2個は、酸素若しくは硫黄によって、又は $-\text{NR}_{14}$ (このとき R_{14} は水素又は $\text{C}_1\sim\text{C}_4$ アルキルである)によって置換されていてもよく；及び前述の R_2 基の各々は、クロロ、フルオロ及び $\text{C}_1\sim\text{C}_4$ アルキルから独立して選択される3個以下の置換基によって、又はブロモ、ヨード、シアノ、ニトロ、 $\text{C}_1\sim\text{C}_6$ アルコキシ、 $-\text{O}-\text{CO}-$ ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{O}-\text{CO}-\text{N}$ ($\text{C}_1\sim\text{C}_4$ アルキル) ($\text{C}_1\sim\text{C}_2$ アルキル)、 $-\text{CO}_2$ ($\text{C}_1\sim\text{C}_4$ アルキル)、($\text{C}_1\sim\text{C}_4$ アルキル)スルファニル、($\text{C}_1\sim\text{C}_4$ アルキル)スルフィニル及び($\text{C}_1\sim\text{C}_4$ アルキル)スルホニルから選択される1個の置換基によって置換されていてもよく、このとき前記 $\text{C}_1\sim\text{C}_4$ アルキル及び前述の R_2 基の $\text{C}_1\sim\text{C}_6$ アルキル部分は、1個の炭素-炭素二重結合又は三重結合を含んでもよく；又は前記 $-\text{NR}_1\text{R}_2$ 及び前記 $-\text{CR}_1\text{R}_2\text{R}_{11}$ の R_1 及び R_2 は一緒に飽和若しくは部分飽和の5～8員環を形成し、このとき前記環は1又は2個の炭素-炭素二重結合を含んでもよく、及びこのとき環員炭素の1又は2個はO、S及びNから選択されるヘテロ原子により置き換えられていてもよく； R_3 は水素、 $\text{C}_1\sim\text{C}_6$ アルキル、フルオロ、クロロ、ブロモ、ヨード、ヒドロキシ、アミノ、SH、 $-\text{NH}$ ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{N}$ ($\text{C}_1\sim\text{C}_4$ アルキル) ($\text{C}_1\sim\text{C}_2$ アルキル)、 $-\text{CH}_2\text{OH}$ 、 $-\text{CH}_2\text{OCH}_3$ 、 $-\text{O}$ ($\text{C}_1\sim\text{C}_4$ アルキル)、($\text{C}_1\sim\text{C}_4$ アルキル)スルファニル、($\text{C}_1\sim\text{C}_4$ アルキル)スルホニル又は($\text{C}_1\sim\text{C}_4$ アルキル)スルフィニルであり、このとき前記 $\text{C}_1\sim\text{C}_6$ アルキル及び前述の R_3 基の $\text{C}_1\sim\text{C}_4$ アルキル部分は1個の二重結合又は三重結合を含んでもよく、及びヒドロキシ、アミノ、 $\text{C}_1\sim\text{C}_3$ アルコキシ、 $-\text{NH}$ ($\text{C}_1\sim\text{C}_2$ アルキル)、 $-\text{N}$ ($\text{C}_1\sim\text{C}_2$ アルキル) $_2$ 、 $-\text{HCOCH}_3$ 、フルオロ、クロロ及び $\text{C}_1\sim\text{C}_3$ チオアルキルから独立して選択される1～3個の置換基によって置換されていてもよく； R_4 は水素、 $\text{C}_1\sim\text{C}_6$ アルキル、フルオロ、クロロ、ブロモ、ヨード、 $\text{C}_1\sim\text{C}_6$ アルコキシ、ホルミル、トリフルオロメトキシ、 $-\text{CH}_2\text{OCH}_3$ 、 $-\text{CH}_2\text{OC}$ H_2CH_3 、 $-\text{CH}_2\text{CH}_2\text{OCH}_3$ 、 $-\text{CH}_2\text{CF}_3$ 、 CF_3 、アミノ、ニトロ、 $-\text{NH}$ ($\text{C}_1\sim\text{C}_4$ アルキル)、 $-\text{N}$ (CH_3) $_2$ 、 $-\text{NHCO}$ H_3 、 $-\text{NHCONHCH}_3$ 、($\text{C}_1\sim\text{C}_4$ アルキル)スルファニル、($\text{C}_1\sim\text{C}_4$ アルキル)スルフィニル、($\text{C}_1\sim\text{C}_4$ アルキル)

スルホニル、シアノ、ヒドロキシ、 $-\text{CO}$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{CHO}$ 又は $-\text{CO}_2$ ($\text{C}_1 \sim \text{C}_4$ アルキル) であり、このとき前述の R_4 基の前記 $\text{C}_1 \sim \text{C}_6$ アルキル、 $\text{C}_1 \sim \text{C}_6$ アルコキシ及び $\text{C}_1 \sim \text{C}_4$ アルキル部分は、1個の二重結合又は三重結合を含んでもよく、及びヒドロキシ、アミノ、 $-\text{NHCO}$ CH_3 、 NH ($\text{C}_1 \sim \text{C}_2$ アルキル)、 N ($\text{C}_1 \sim \text{C}_2$ アルキル) $_2$ 、 $-\text{CO}_2$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{CO}$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $\text{C}_1 \sim \text{C}_3$ アルコキシ、($\text{C}_1 \sim \text{C}_3$ アルキル) スルファニル、フルオロ、クロロ、シアノ及びニトロから選択される1個の置換基で置換されていてもよく； R_5 はフェニル、ナフチル、チエニル、ベンゾチエニル、ビリジル、キノリル、ピラジノリル、ピリミジル、イミダゾリル、フラニル、ベンゾフラニル、ベンゾチアゾリル、イソチアゾリル、ベンゾイソチアゾリル、チアゾリル、イソキサゾリル、ベンズイソキサゾリル、ベンズイミダゾリル、トリアゾリル、ピラゾリル、ピロリル、インドリル、アザインドリル、ベンズオキサゾリル、オキサゾリル、ピロジニル、チアゾリジニル、モルホリニル、ビリジニル、テトラゾリル又は3～8員シクロアルキル環又は9～12員シクロアルキル環システムであり、このとき前記シクロアルキル環及び前記シクロアルキル環システムは、1又は2個のO、S又は $-\text{N}-\text{G}$ (このときGは水素、 $\text{C}_1 \sim \text{C}_4$ アルキル、 $\text{C}_1 \sim \text{C}_4$ アルカノイル、フェニル又はベンジルである) であり、このとき前述の R_5 基の各々は、フルオロ、クロロ、 $\text{C}_1 \sim \text{C}_6$ アルキル、 $\text{C}_1 \sim \text{C}_6$ アルコキシ及びトリフルオロメチルから独立して選択される3個以下の置換基によって、又はブromo、ヨード、シアノ、ニトロ、アミノ、 $-\text{NH}$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{N}$ ($\text{C}_1 \sim \text{C}_4$ アルキル) ($\text{C}_1 \sim \text{C}_2$ アルキル)、 $-\text{CO}_2$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{CO}$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{SO}_2\text{NH}$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{SO}_2\text{N}$ ($\text{C}_1 \sim \text{C}_4$ アルキル) ($\text{C}_1 \sim \text{C}_2$ アルキル)、 $-\text{SO}_2\text{NH}_2$ 、 $-\text{NHSO}_2$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{S}$ ($\text{C}_1 \sim \text{C}_4$ アルキル) 及び $-\text{SO}_2$ ($\text{C}_1 \sim \text{C}_4$ アルキル) から選択される1個の置換基によって置換されていてもよく、このとき前述の R_5 基の前記 $\text{C}_1 \sim \text{C}_4$ アルキル及び $\text{C}_1 \sim \text{C}_6$ アルキル部分は、1個の二重結合又は三重結合を含んでもよく、及びフルオロ、クロロ、ヒドロキシ、アミノ、メチルアミノ、ジメチルアミノ及びアセチルから独立して選択される1又は2個の置換基によって置換されていてもよく； R_6 は水素又は $\text{C}_1 \sim \text{C}_6$ アルキルであり、このとき前記 $\text{C}_1 \sim \text{C}_6$ アルキルは1個のヒドロキシ、メトキシ、エトキシ又はフルオロ基により置換されていてもよく； R_7 は水素、 $\text{C}_1 \sim \text{C}_4$ アルキル、フルオロ、クロロ、ブromo、ヨード、シアノ、ヒドロキシ、 $\text{C}_1 \sim \text{C}_4$ アルコキシ、 $-\text{CO}$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{CO}_2$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{OCF}_3$ 、 CF_3 、 $-\text{CH}_2\text{OH}$ 、 $-\text{CH}_2\text{OCH}_3$ 又は $-\text{CH}_2\text{OCH}_2\text{CH}_3$ であり； R_8 及び R_9 は各々独立して水素、ヒドロキシ、メチル、エチル、メトキシ又はエトキシであるか；又は R_8 及び R_9 は一緒にオキソ (=O) 基を形成し； R_{10} は水素、 $\text{C}_1 \sim \text{C}_6$ アルキル、フルオロ、クロロ、ブromo、ヨード、 $\text{C}_1 \sim \text{C}_6$ アル

コキシ、ホルミル、アミノ、 $-\text{NH}$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{N}$ ($\text{C}_1 \sim \text{C}_4$ アルキル) ($\text{C}_1 \sim \text{C}_2$ アルキル)、シアノ、カルボキシ、アミド又は $-\text{SO}_n$ ($\text{C}_1 \sim \text{C}_4$ アルキル) (このときnは0、1又は2である) であり、このとき前述の R_{10} 基の前記 $\text{C}_1 \sim \text{C}_6$ アルキル及び $\text{C}_1 \sim \text{C}_4$ アルキル部分は1個のヒドロキシ、トリフルオロメチル、アミノ、カルボキシ、アミド、 $-\text{NHCO}$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{NH}$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $-\text{N}$ ($\text{C}_1 \sim \text{C}_4$ アルキル) ($\text{C}_1 \sim \text{C}_2$ アルキル)、 $-\text{CO}_2$ ($\text{C}_1 \sim \text{C}_4$ アルキル)、 $\text{C}_1 \sim \text{C}_3$ アルコキシ、 $\text{C}_1 \sim \text{C}_3$ チオアルキル、フルオロ、ブromo、クロロ、ヨード、シアノ又はニトロによって置換されていてもよく；並びに R_{11} は水素、ヒドロキシ、フルオロ又はメトキシである] の化合物、又はその医薬として許容できる塩である、請求項1記載の薬剤。

【請求項3】 B が $-\text{NR}_1\text{R}_2$ 、 $-\text{NHCHR}_1\text{R}_2$ 、 $-\text{CR}_1\text{R}_2\text{R}_{11}$ 、 $-\text{SCHR}_1\text{R}_2$ 又は $-\text{OCHR}_1\text{R}_2$ であり； R_1 が、1個のヒドロキシ、フルオロ又は $\text{C}_1 \sim \text{C}_2$ アルコキシ基で置換されていてもよく、及び1個の炭素-炭素二重結合又は三重結合を含んでもよい、 $\text{C}_1 \sim \text{C}_6$ アルキルであり； R_2 がベンジル又は1個の炭素-炭素二重結合又は三重結合を含んでもよい $\text{C}_1 \sim \text{C}_6$ アルキルであり、このとき前記 $\text{C}_1 \sim \text{C}_6$ アルキル及び前記ベンジルのフェニル部分がフルオロ、 $\text{C}_1 \sim \text{C}_2$ アルキル又は $\text{C}_1 \sim \text{C}_2$ アルコキシにより置換されていてもよく；並びに R_{11} が水素又はフルオロである、請求項2記載の薬剤。

【請求項4】 R_2 が (アリール) $\text{C}_1 \sim \text{C}_4$ アルキル又は (ヘテロアリール) $\text{C}_1 \sim \text{C}_4$ アルキルであり、このとき前記アリール部分がフェニル、フラニル又はベンゾフラニルであり、及び前記ヘテロアリール部分がチエニル、ベンゾチエニル、チアゾリル、ビリジル又はベンゾチアゾリルである、請求項2記載の薬剤。

【請求項5】 B が NR_1R_2 又は CHR_1R_2 であり、このとき R_1 及び R_2 がN又はCHと一緒に5又は6員環を形成し、このとき前記環が硫黄、酸素、又は B が NR_1R_2 であるときはさらに窒素を有してもよい、請求項2記載の薬剤。

【請求項6】 B が $-\text{NHCHR}_1\text{R}_2$ 又は $-\text{OCHR}_1\text{R}_2$ であり、このとき CHR_1R_2 部分が1個の酸素又は硫黄を含んでもよい5又は6員環である、請求項2記載の薬剤。

【請求項7】 R_3 がメチル、クロロ又はメトキシであり； R_4 がメチル、 $-\text{CH}_2\text{OH}$ 、シアノ、トリフルオロメトキシ、メトキシ、トリフルオロメチル、クロロ、 $-\text{CO}_2\text{C}_2\text{H}_5$ 、 $-\text{CH}_2\text{OCH}_3$ 、 $-\text{CH}_2\text{Cl}$ 、 $-\text{CH}_2\text{F}$ 、アミノ又はニトロであり； R_6 が水素、メチルスルフィニル、メチルスルファニル、メチルスルホニル又はエチルであり；並びに R_6 がフェニル又はビリジルであり、このとき前記フェニル又はビリジルがフルオロ、クロロ、ブromo、ヨード、 $\text{C}_1 \sim \text{C}_4$ アルコキシ、トリフルオロメチル、 $\text{C}_1 \sim \text{C}_3$ ヒドロキシルアルキル、 $-\text{CO}_2$ ($\text{C}_1 \sim \text{C}_2$ アルキル)、 $\text{C}_1 \sim \text{C}_2$ アルキルアミノ、 $-\text{CO}$ ($\text{C}_1 \sim \text{C}_4$ アルキル) 及び $\text{C}_1 \sim \text{C}_6$ アルキルから独立して選択される1個の置換基によって置換され、

このとき前記 $C_1 \sim C_6$ アルキル及び前記 $C_1 \sim C_4$ アルキルが、1個のヒドロキシ又はフルオロ基によって置換されていてもよく、及び1個の炭素-炭素二重結合又は三重結合を含んでもよい；請求項2記載の薬剤。

【請求項8】 式I又はIIの化合物が、以下から選択される、請求項2記載の薬剤：4-(1-エチルプロポキシ)-2,5-ジメチル-6-(2,4,6-トリメチルベンジル)-ピリミジン；2-(4-ブromo-2,6-ジメチルフェノキシ)-4-(1-エチルプロポキシ)-3,6-ジメチルピリジン；2-(4-エチル-2,6-ジメチルフェノキシ)-4-(1-エチルプロポキシ)-3,6-ジメチルピリジン；3-エチル-4-(1-エチルプロポキシ)-6-メチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン；2-(2,6-ジメチル-4-プロピルフェノキシ)-4-(1-エチルプロポキシ)-3,6-ジメチルピリジン；4-(1-エチルプロポキシ)-2-(4-メトキシ-2,6-ジメチルフェノキシ)-3,6-ジメチルピリジン；2-(4-エトキシ-2,6-ジメチルフェノキシ)-4-(1-エチルプロポキシ)-3,6-ジメチルピリジン；2-(4-クロロ-2,6-ジメチルフェノキシ)-4-(1-エチルプロポキシ)-3,6-ジメチルピリジン；4-(1-メトキシメチルプロポキシ)-3,6-ジメチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン；[3,6-ジメチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン-4-イル]-ジエチルアミン；[3,6-ジメチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン-4-イル]-エチルプロピルアミン；[2,5-ジメチル-6-(2,4,6-トリメチルフェノキシ)ピリミジン-4-イル](1-エチルプロピル)アミン；ブチル-[3,6-ジメチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン-4-イル]-エチルアミン；4-(1-エチルプロポキシ)-3,6-ジメチル-2-(2,4,6-トリメチルフェニルスルファニル)-ピリジン；ブチル-[2-(4-クロロ-2,6-ジメチルフェノキシ)-3,6-ジメチルピリジン-4-イル]-エチルアミン；4-(1-エチルプロピルアミノ)-6-メチル-2-(2,4,6-トリメチルフェノキシ)-ニコチン酸メチルエステル；[3,6-ジメチル-2-(2,4,6-トリメチルフェニルスルファニル)-ピリジン-4-イル]-エチルプロピルアミン；[4-(1-エチルプロピルアミノ)-6-メチル-2-(2,4,6-トリメチルフェノキシ)ピリジン-3-イル]メタノール；[2-(4-クロロ-2,6-ジメチルフェノキシ)-3,6-ジメチルピリジン-4-イル]-エチルプロピルアミン；1-(エチルプロピル)-[6-メチル-3-ニトロ-2-(2,4,6-トリメチルフェノキシ)-ピリジン-4-イル]-アミン；N4-(1-エチルプロピル)-6-メチル-3-ニトロ-N2-(2,4,6-トリメチル

フェニル)-ピリジン-2,4-ジアミン；N4-(1-エチルプロピル)-6-メチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン-3,4-ジアミン；N4-(1-エチルプロピル)-6-メチル-N2-(2,4,6-トリメチルフェニル)-ピリジン-2,3,4-トリアミン；[3,6-ジメチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン-4-イル]-エチル-2,2,2-トリフルオロエチルアミン；[3-クロロメチル-6-メチル-2-(2,4,6-トリメチルフェノキシ)ピリジン-4-イル]-1-(エチルプロピル)アミン；[3,6-ジメチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン-4-イル]-1-(エチルプロピル)アミン；[1-エチルプロピル]-[2-メチル-5-ニトロ-6-(2,4,6-トリメチルピリジン-3-イルオキシ)-ピリミジン-4-イル]アミン；(1-エチルプロピル)-[3-メトキシメチル-6-メチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン-4-イル]アミン；N-(1-エチルプロピル)-2-メチル-5-ニトロ-N-(2,4,6-トリメチルピリジン-3-イル)-ピリミジン-4,6-ジアミン；[2-(4-クロロ-2,6-ジメチルフェノキシ)-3,6-ジメチルピリジン-4-イル]-ジエチルアミン；4-(1-エチルプロポキシ)-3,6-ジメチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン；ブチル-[2,5-ジメチル-7-(2,4,6-トリメチルフェニル)-6,7-ジヒドロ-5H-ビロロ[2,3-d]ピリミジン-4-イル]-エチルアミン；4-(ブチルエチルアミノ)-2,5-ジメチル-7-(2,4,6-トリメチルフェニル)-5,7-ジヒドロビロロ[2,3-d]ピリミジン-6-オン；4-(1-エチルプロポキシ)-2,5-ジメチル-6-(2,4,6-トリメチルフェノキシ)-ピリミジン；N-ブチル-N-エチル-2,5-ジメチル-N-(2,4,6-トリメチルフェニル)-ピリミジン-4,6-ジアミン；(1-エチルプロピル)-[5-メチル-3-(2,4,6-トリメチルフェニル)-3H-イミダゾ[4,5-b]ピリジン-7-イル]アミン；[2,5-ジメチル-3-(2,4,6-トリメチルフェニル)-3H-イミダゾ[4,5-b]ピリジン-7-イル]-1-(エチルプロピル)アミン；N4-(1-エチルプロピル)-6,N3-ジメチル-2-(2,4,6-トリメチルフェノキシ)-ピリジン-3,4-ジアミン；N4-(1-エチルプロピル)-6,N3,N3-トリメチル-2-(2,4,6-トリメチルフェノキシ)ピリジン-3,4-ジアミン；6-(1-エチルプロポキシ)-2-メチル-N4-(2,4,6-トリメチルフェニル)-ピリミジン-4,5-ジアミン；[4-(1-エチルプロポキシ)-3,6-ジメチルピリジン-2-イル]-(2,4,6-トリメチルフェニル)アミン；及び6-(エチルプロピルアミノ)-2,7-ジメチル-9-(2,4,6-トリメチルフェニル)-7,9-

ジヒドロブリン-8-オン。

【請求項9】 化合物が、以下から選択される、請求項2記載の方法：ブチル-〔3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-b〕ピリジン-4-イル〕-エチルアミン；3, 6-ジメチル-4-

(テトラヒドロフラン-3-イルオキシ)-1-(2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-b〕ピリジン；〔3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-b〕ピリジン-4-イル〕- (1-メトキシメチルプロピル)-アミン；4- (1-メトキシメチルプロピル)-3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-b〕ピリジン；(1-エチルプロピル)-〔3, 5, 6-トリメチル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-b〕ピリジン-4-イル〕-アミン；4- (1-エチルプロピル)-2, 5-ジメチル-7- (2, 4, 6-トリメチルフェニル)-7H-ピロロ〔2, 3-b〕ピリジン；4- (1-エチルプロピル)-2, 5, 6-トリメチル-7- (2, 4, 6-トリメチルフェニル)-7H-ピロロ〔2, 3-b〕ピリジン；4- (1-エチルプロピル)-2, 5-ジメチル-7- (2, 6-ジメチル-4-プロモフェニル)-7H-ピロロ〔2, 3-b〕ピリジン；3- (4-メチルベンジル)-〔3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミノ}-プロパン-1-オール；ジエチル-〔6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；2-〔ブチル-〔6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミノ}-エタノール；ジブチル-〔6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；ブチル-エチル-〔6-メチル-3-メチルスルホニル-1- (2, 4, 6-トリクロロフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；ブチル-シクロプロピルメチル-〔6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；ジ-1-プロピル-〔6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；ジアリル-〔6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；ブチル-エチル-〔6-クロロ-3-メチルスルファニル-1- (2, 4, 6-トリ

クロロフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；ブチル-エチル-〔6-メトキシ-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；プロピル-エチル-〔3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；4- (1-エチルプロピル)-6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン；2-〔3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イルアミン〕-ブタン-1-オール；〔3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン-4-イル〕-アミン；4- (1-メチルプロピル)-アミン；4- (1-メトキシメチルプロピル)-3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル)-1H-ピラゾロ〔3, 4-d〕ピリミジン；n-ブチル-エチル-〔2, 5-ジメチル-7- (2, 4, 6-トリメチルフェニル)-7H-ピロロ〔2, 3-d〕ピリミジン-4-イル〕-アミン；4- (1-メチルプロピル)-2, 5-ジメチル-7- (2, 4, 6-トリメチルフェニル)-7H-ピロロ〔2, 3-d〕ピリミジン-4-イルアミノ}-ブタン-1-オール；2- (S)-〔7- (4-プロモ-2, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ〔2, 3-d〕ピリミジン-4-イルアミノ〕-ブタン-1-オール；4- (1-エチルプロピル)-2, 5, 6-トリメチル-7- (2, 4, 6-トリメチルフェニル)-7H-ピロロ〔2, 3-d〕ピリミジン；4- (1-メトキシメチルプロピル)-2, 5, 6-トリメチル-7- (2, 4, 6-トリメチルフェニル)-7H-ピロロ〔2, 3

-d] ピリミジン; 4-(1-エチル-ブチル)-2, 5, 6-トリメチル-7-(2, 4, 6-トリメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン; [7-(4-ブromo-2, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イル]- (1-メトキシメチル プロピル) アミン; 2 [7-(2-ブromo-4, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イルアミノ]-ブタン-1-オール; 2-[7-(4-エチル-2, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イルアミノ]-ブタン-1-オール; 2-[7-(2-エチル-4, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イルアミノ]-ブタン-1-オール; 及び2-[7-(2-フルオロメチル-4, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イルアミノ]-ブタン-1-オール。

【請求項10】 突然死のリスクが、動物における疾患状態の存在に関連し、このとき前記疾患状態が、心臓疾患、高血圧、頻脈、うっ血性心不全、脳卒中、骨粗鬆症、早産、心理的萎縮、ストレス性発熱、潰瘍、下痢、術後腸閉塞、精神的不安及びストレスに関連する腸の過敏、糖尿病、神経性疾患、脳障害、ギランバレー症候群、小児突然死症候群、先天性過換気症候群又は尿毒症性神経障害である、請求項1記載の薬剤。

【発明の詳細な説明】

【0001】

【産業上の利用分野】 本発明は、臨床上有効な量のコルチコトロピン放出因子 (CRF) アンタゴニストを投与することによる、特定の患者における突然死の発生を減じる方法に関する。CRFアンタゴニストは、QT分散 (dispersion) 及び心拍変動 (heart rate variability) を改善することによって、患者における突然死の発生を減じると考えられる。

【0002】

【従来技術】 突然の予期しない死が、中程度の心疾患にかかっていた患者の50%、重篤な心疾患を経験している患者の25%に起こっている (Barrら、Lancet、343 (8893) : 327-29 (1994))。心室再分極 (ventricular repolarization) における局所変化は、不整脈の電気神経学的基質を示すものであるが、QTインターバル (分散) の内部誘導 (interlead) 変動によって検出される。QTインターバル分散は、急性心筋梗塞の後の心室不整脈 (ventricular tachyarrhythmias)、長期のQT症候群、慢性心疾患及び肥大型心筋症にかかった患者に見られる (例えば、Potratzら、Eur. Heart J.、14: 254 (1993) ; Dayら、Br. Heart J.、63: 342-44 (1990) ; 及びBujaら、Am. J. Cardiol.、72: 973-976 (1993) を参照せよ)。

【0003】 本明細書に記載されたような式I及びIIの

化合物及びそれらの医薬として許容できる塩、並びにそのような化合物の塩の製造方法は、欧州特許出願EP0773 023A1、並びに、より詳細にはPCT国際特許出願PCT/IB95/00373 (公開番号W095/34563)、PCT/IB95/00439 (公開番号W095/33750)、PCT/US93/11333 (公開番号W094/13677) 及び PCT/US93/10715 (公開番号W094/13676) 中に開示されている。上記のこれらの欧州特許出願及びPCT国際特許出願の内容はすべて、引用により本明細書中に取り込まれる。

【0004】 前述のPCT国際特許出願は、CRFにより引き起こされ又は促進される病気の治療における、また、不安、鬱、倦怠症候群 (fatigue syndrome)、胃腸疾患、頭痛、痛み、ガン、免疫機能不全、出血性ストレス (hemorrhagic stress)、薬物中毒、薬物及びアルコールの中断症状、不妊 (fertility problems)、ストレスに起因する精神病性挿入 (stress-induced psychotic episodes)、アルツハイマー病のような神経変性性疾患、クローン病、痙攣性結腸及び過敏結腸のような過敏腸症候群 (irritable bowel syndrome)、食欲不振 (anorexia nervosa) のような摂食疾患、関節炎のような炎症性疾患、喘息及びアレルギー等の治療における、式I及びIIの化合物の使用についても言及している。

【0005】 本発明の方法において列記されている疾患の治療のために用いることのできる他のCRFアンタゴニストは、PCT国際特許出願PCT/IB95/00318 (国際公開番号W095/33727)、PCT/IB97/00918 (国際公開番号W098/05661)、PCT/IB97/00904 (国際公開番号W098/08846) 及びPCT/IB97/00922 (国際公開番号W098/08847)、PCT/EP98/02267 (国際公開番号W098/47874)、PCT/EP98/02268 (国際公開番号W098/47903)、PCT/US98/09861 (国際公開番号W098/51312)、PCT/US98/13840 (国際公開番号W099/01439)、PCT/US98/13913 (国際公開番号W099/01454) 並びに米国特許5,063,245、5,109,111、5,132,111、5,245,009、5,464,847、5,493,006、5,510,458、5,605,642、5,644,057、5,663,292、5,668,145、5,705,646及び5,712,303に記載されている。ここで引用したPCT国際特許出願及び米国特許は、それらの全体を本明細書に援用する。

【0006】 CRFアンタゴニストの重要性は文献、例えば米国特許5,063,245に論じられている。この内容はすべて、引用により本明細書中に取り込まれる。CRFアンタゴニストによって示される異なる活性の最近のアウトラインを、M. J. Owensら、Pharm. Rev.、43: 425-73 (1991) に見ることができる。この内容もすべて、引用により本明細書中に取り込まれる。

【0007】 PCT国際特許出願PCT/US98/07831 (国際公開番号W098/47899) は、炎症性疾患の治療における置換ピロロピリジンの有効性を開示する。開示された化合物は、特定の炎症性サイトカイン、即ちTNF- α 及び

L-1 β の産生を阻害する。掲げられている炎症性サイトカインに関連した炎症性疾患の一つは、うっ血性心疾患である。しかしながら、QT分散又は心拍変動については言及されていない。

【0008】

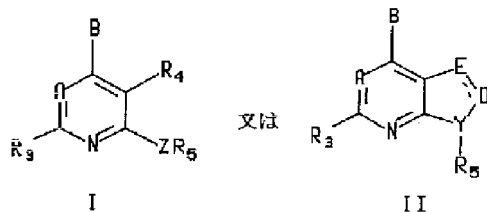
【課題を解決するための手段】本発明は、動物における突然死を予防する方法であって、前記動物、好ましくはヒトに、臨床上有効量のコルチコトロピン放出因子アンタゴニストを投与することを含む前記方法を提供する。

【0009】本発明の方法は、特定の患者、詳細には、心臓血管又は心臓に関連した疾患、例えば、高血圧(hypertension)、頻脈(tachycardia)、うっ血性心不全(congestive heart failure)等に苦しむ患者における突然死を予防する際に最も有用である。また、他の疾患、例えば、脳卒中(stroke)、骨粗鬆症(osteoporosis)、早産(premature birth)、心理的萎縮(psychosocial dwarfism)、ストレス性発熱(stress-induced fever)、潰瘍(ulcer)、下痢(diarrhea)、術後腸閉塞(post-operative ileus)、精神的不安及びストレスに関連する腸の過敏(colonic hypersensitivity associated with psychopathological disturbance and stress)等の患者においても同様に有用である。本発明の方法はまた、糖尿病患者における突然死を予防する際にも有用である。また、多くの神経疾患(neurological disorders)、例えば、脳障害(brain damage)、ギランバレー症候群(Guillain-Barre syndrome)、小児突然死症候群(sudden infant death syndrome)、先天性過換気症候群(congenital hypoventilation syndrome)又は尿毒症性神経障害(uremic neuropathy)等の患者においても同様に有用である。

【0010】好ましい実施態様において、本発明は式I又はIIの化合物を用いる。

【0011】

【化2】



【0012】式中、点線は所望により存在する二重結合を表し、Aは-CR₇又はNであり、Bは-NR₁R₂、-CR₁R₂R₁₁、C(=CR₁R₂)R₂、NHCR₁R₁R₂、OCR₁R₁R₂、-SCR₁R₁R₂、-CR₁R₂OR₁、-CR₁R₂SR₁、-C(S)R₂、-NHR₁R₂、-CR₂R₁NHR₁又は-C(O)R₂であり；DはEとDとが二重結合で結合し、及びEが-CR₄であるとき、N又は-CR₁であり；EとDとが二重結合で結合し、及びEがNであるとき、-CR₁₀であり；又はEとDとが単結合で結合しているとき、-CR₆R₉、-CHR₁₀、-C=O、-

C=S、-C=NH又は-C=NCH₃であり；EはEとDとが二重結合で結合しているとき、-CR₄又はNであり；又はEとDとが単結合で結合しているとき、-CR₄R₆又は-NR₆であり；YはN又は-CHであり；ZはNH、O、S、-N(C₁~C₂アルキル)又は-CR₂R₁₃であり、このときR₁₂及びR₁₃は各々独立に水素、トリフルオロメチル若しくはメチルであるか、又はR₁₂及びR₁₃の一方がシアノ、他方が水素若しくはメチルであり；R₁は水素又はC₁~C₆アルキルであり、このとき前記C₁~C₆アルキルはヒドロキシ、シアノ、ニトロ、フルオロ、クロロ、ブロモ、ヨード、C₁F₃、C₁~C₄アルコキシ、-O-CO-(C₁~C₄アルキル)、-O-CO-NH(C₁~C₄アルキル)、-O-CO-N(C₁~C₄アルキル)(C₁~C₂アルキル)、-NH(C₁~C₄アルキル)、-N(C₁~C₂アルキル)(C₁~C₄アルキル)、-S(C₁~C₄アルキル)、-N(C₁~C₄アルキル)CO(C₁~C₄アルキル)、-NHCO(C₁~C₄アルキル)、-CO₂(C₁~C₄アルキル)、-CONH(C₁~C₄アルキル)、-CON(C₁~C₄アルキル)(C₁~C₂アルキル)、(C₁~C₄アルキル)スルフィニル、(C₁~C₄アルキル)スルホニル及び(C₁~C₄アルキル)スルファニルから独立して選択される2個以下の置換基で置換されていてもよく、このとき前述のR₁基中の前記C₁~C₆アルキル、C₁~C₄アルコキシ及びC₁~C₄アルキル部分は、1個の二重結合又は三重結合を含んでもよく；R₂はC₁~C₃アルキル、ヘテロアリール、アリール、ヘテロアリール(C₁~C₄アルキル)又はアリール(C₁~C₄アルキル)であり、このとき前記アリール及び前記(アリール)C₁~C₄アルキルのアリール部分はフェニル及びナフチルから選択され、及び前記ヘテロアリール及び前記(ヘテロアリール)C₁~C₄アルキルのヘテロアリール部分はチエニル、ベンゾチエニル、ピリジル、チアゾリル、キノリル、ピラジニル、ピリミジル、イミダゾリル、フラニル、ベンゾフラニル、ベンゾチアゾリル、イソチアゾリル、ベンズイソチアゾリル、ベンズイソキサゾリル、ベンズイミダゾリル、インドリル及びベンゾキサゾリルから選択されるか；又はR₂はC₃~C₈シクロアルキル又は(C₃~C₈シクロアルキル)C₁~C₆アルキルであり、このとき少なくとも4つの環員を有する前記シクロアルキル及び少なくとも4つの環員を有する前記(C₃~C₈シクロアルキル)C₁~C₆アルキルのシクロアルキル部分の環炭素の1又は2個は、酸素若しくは硫黄によって、又は-NR₁₄(このときR₁₄は水素又はC₁~C₄アルキルである)によって置換されていてもよく；及び前述のR₂基は、クロロ、フルオロ及びC₁~C₄アルキルから独立して選択される3個以下の置換基によって、又はブロモ、ヨード、シアノ、ニトロ、C₁~C₆アルコキシ、-O-CO-(C₁~C₄アルキル)、-O-CO-N(C₁~C₄アルキル)(C₁~C₂アルキル)、-CO₂(C₁~C₄アルキル)、(C₁~C₄アルキル)スルファニル、(C₁~C₄アルキル)スルフィニル及び(C₁~C₄アルキル)スルホニルから選択される1個の置換基によって

置換されていてもよく、このとき前記 $C_1 \sim C_4$ アルキル及び前述の R_2 基の $C_1 \sim C_6$ アルキル部分は、1個の炭素-炭素二重結合又は三重結合を含んでもよく；又は前記 $-NR_1R_2$ 及び前記 $-CR_1R_2R_{11}$ の R_1 及び R_2 は、一緒になって飽和若しくは部分飽和の5～8員環を形成し、このとき前記環は1又は2個の炭素-炭素二重結合又は二重結合を含んでもよく、及びこのとき環員炭素の1又は2個は、O、S及びNから選択されるヘテロ原子により置き換えられていてもよく； R_3 は水素、 $C_1 \sim C_6$ アルキル、フルオロ、クロロ、ブロモ、ヨード、ヒドロキシ、アミノ、SH、 $-NH(C_1 \sim C_4$ アルキル)、 $-N(C_1 \sim C_4$ アルキル)($C_1 \sim C_2$ アルキル)、 $-CH_2OH$ 、 $-CH_2OCH_3$ 、 $-O(C_1 \sim C_4$ アルキル)、($C_1 \sim C_4$ アルキル)スルフェニル、($C_1 \sim C_4$ アルキル)スルホニル又は($C_1 \sim C_4$ アルキル)スルフィニルであり、このとき前記 $C_1 \sim C_6$ アルキル及び前述の R_3 基の $C_1 \sim C_4$ アルキル部分は1個の二重結合又は三重結合を含んでもよく、及びヒドロキシ、アミノ、 $C_1 \sim C_3$ アルコキシ、 $-NH(C_1 \sim C_2$ アルキル)、 $-N(C_1 \sim C_2$ アルキル) $_2$ 、 $-NHC(=O)CH_3$ 、フルオロ、クロロ及び $C_1 \sim C_3$ チオアルキルから独立して選択される1～3個の置換基によって置換されていてもよく； R_4 は水素、 $C_1 \sim C_6$ アルキル、フルオロ、クロロ、ブロモ、ヨード、 $C_1 \sim C_6$ アルコキシ、ホルミル、トリフルオロメトキシ、 $-CH_2OCH_3$ 、 $-CH_2OC(=O)CH_3$ 、 $-CH_2CH_2OCH_3$ 、 $-CH_2CF_3$ 、 CF_3 、アミノ、ニトロ、 $-NH(C_1 \sim C_4$ アルキル)、 $-N(CH_3)_2$ 、 $-NHC(=O)CH_3$ 、 $-NHCONHCH_3$ 、($C_1 \sim C_4$ アルキル)スルフェニル、($C_1 \sim C_4$ アルキル)スルフィニル、($C_1 \sim C_4$ アルキル)スルホニル、シアノ、ヒドロキシ、 $-O(C_1 \sim C_4$ アルキル)、 $-CHO$ 又は $-CO_2(C_1 \sim C_4$ アルキル)であり、このとき前述の R_4 基の前記 $C_1 \sim C_6$ アルキル、 $C_1 \sim C_6$ アルコキシ及び $C_1 \sim C_4$ アルキル部分は、1個の二重結合又は三重結合を含んでもよく、及びヒドロキシ、アミノ、 $-NHCOCH_3$ 、 $-NH(C_1 \sim C_2$ アルキル)、 $-N(C_1 \sim C_2$ アルキル) $_2$ 、 $-CO_2(C_1 \sim C_4$ アルキル)、 $-CO(C_1 \sim C_4$ アルキル)、 $C_1 \sim C_3$ アルコキシ、($C_1 \sim C_3$ アルキル)スルフェニル、フルオロ、クロロ、シアノ及びニトロから選択される1個の置換基で置換されていてもよく； R_5 はフェニル、ナフチル、チエニル、ベンゾチエニル、ビリジル、キノリル、ピラジノリル、ピリミジル、イミダゾリル、フラニル、ベンゾフラニル、ベンゾチアゾリル、イソチアゾリル、ベンゾイソチアゾリル、チアゾリル、イソキサゾリル、ベンゾイソキサゾリル、ベンゾイミダゾリル、トリアゾリル、ピラゾリル、ピロリル、インドリル、アザインドリル、ベンゾオキサゾリル、オキサゾリル、ピロジニル、チアゾリジニル、モルホリニル、ビリジニル、テトラゾリル又は3～8員シクロアルキル環若しくは9～12員ビシクロアルキル環システムであり、このとき前記シクロアルキル環及び前記ビシクロアルキル環システムは、1又は2個のO、S又は $-N-G$ (このときGは水素、 $C_1 \sim C_4$ アルキル、 $C_1 \sim C_4$ アルカノイル、フェニル又はベンジルである)であり、このとき前述の R_5 基の各々は、フルオロ、クロロ、 $C_1 \sim C_6$ アルキル、 $C_1 \sim C_6$ アルコキシ及びトリフルオロメチルから独立して選択される3個以下の置換基によって、又はブロモ、ヨード、シアノ、ニトロ、アミノ、 $-NH(C_1 \sim C_4$ アルキル)、 $-N(C_1 \sim C_4$ アルキル)($C_1 \sim C_2$ アルキル)、 $-CO_2(C_1 \sim C_4$ アルキル)、 $-CO(C_1 \sim C_4$ アルキル)、 $-SO_2NH(C_1 \sim C_4$ アルキル)、 $-SO_2N(C_1 \sim C_4$ アルキル)($C_1 \sim C_2$ アルキル)、 $-SO_2NH_2$ 、 $-NHSO_2(C_1 \sim C_4$ アルキル)、 $-S(C_1 \sim C_4$ アルキル)及び $-SO_2(C_1 \sim C_4$ アルキル)から選択される1個の置換基によって置換されていてもよく、このとき前述の R_5 基の前記 $C_1 \sim C_4$ アルキル及び $C_1 \sim C_6$ アルキル部分は、1個の二重結合又は三重結合を含んでもよく、及びフルオロ、クロロ、ヒドロキシ、アミノ、メチルアミノ、ジメチルアミノ及びアセチルから独立して選択される1又は2個の置換基によって置換されていてもよく； R_6 は水素又は $C_1 \sim C_6$ アルキルであり、このとき前記 $C_1 \sim C_6$ アルキルは1個のヒドロキシ、メトキシ、エトキシ又はフルオロ基により置換されていてもよく； R_7 は水素、 $C_1 \sim C_4$ アルキル、フルオロ、クロロ、ブロモ、ヨード、シアノ、ヒドロキシ、 $C_1 \sim C_4$ アルコキシ、 $-CO(C_1 \sim C_4$ アルキル)、 $-CO_2(C_1 \sim C_4$ アルキル)、 $-OCF_3$ 、 CF_3 、 $-CH_2OH$ 、 $-CH_2OCH_3$ 又は $-CH_2OCH_2CH_3$ であり； R_8 及び R_9 は各々独立して水素、ヒドロキシ、メチル、エチル、メトキシ又はエトキシであるか；又は R_8 及び R_9 は一緒にオキソ(=O)基を形成し； R_{10} は水素、 $C_1 \sim C_6$ アルキル、フルオロ、クロロ、ブロモ、ヨード、 $C_1 \sim C_6$ アルコキシ、ホルミル、アミノ、 $-NH(C_1 \sim C_4$ アルキル)、 $-N(C_1 \sim C_4$ アルキル)($C_1 \sim C_2$ アルキル)、シアノ、カルボキシ、アミド又は $-SO_n(C_1 \sim C_4$ アルキル)(このときnは0、1又は2である)であり、このとき前述の R_{10} 基の前記 $C_1 \sim C_6$ アルキル及び $C_1 \sim C_4$ アルキル部分は1個のヒドロキシ、トリフルオロメチル、アミノ、カルボキシ、アミド、 $-NHCO(C_1 \sim C_4$ アルキル)、 $-NH(C_1 \sim C_4$ アルキル)、 $-N(C_1 \sim C_4$ アルキル)($C_1 \sim C_2$ アルキル)、 $-CO_2(C_1 \sim C_4$ アルキル)、 $C_1 \sim C_3$ アルコキシ、 $C_1 \sim C_3$ チオアルキル、フルオロ、ブロモ、クロロ、ヨード、シアノ又はニトロによって置換されていてもよく；並びに R_{11} は水素、ヒドロキシ、フルオロ又はメトキシである。

【0013】好ましくは、Bが $-NR_1R_2$ 、 $-NHCHR_1R_2$ 、 $-CR_1R_2R_{11}$ 、 $-SCHR_1R_2$ 又は $-OCHR_1R_2$ であり； R_1 が、1個のヒドロキシ、フルオロ又は $C_1 \sim C_2$ アルコキシ基で置換されていてもよく、及び1個の炭素-炭素二重結合又は三重結合を含んでもよい $C_1 \sim C_6$ アルキルであり； R_2 がベンジル又は1個の炭素-炭素二重結合又は三重結合を含んでもよい $C_1 \sim C_6$ アルキルであり、このとき前記 $C_1 \sim C_6$ アルキル及び前記ベンジルのフェニル部分がフルオロ、 $C_1 \sim C_2$ アルキル又は $C_1 \sim C_2$ アルコキシにより置換されていてもよく；並びに R_{11} が水素又はフルオロである。

【0013】好ましくは、Bが $-NR_1R_2$ 、 $-NHCHR_1R_2$ 、 $-CR_1R_2R_{11}$ 、 $-SCHR_1R_2$ 又は $-OCHR_1R_2$ であり； R_1 が、1個のヒドロキシ、フルオロ又は $C_1 \sim C_2$ アルコキシ基で置換されていてもよく、及び1個の炭素-炭素二重結合又は三重結合を含んでもよい $C_1 \sim C_6$ アルキルであり； R_2 がベンジル又は1個の炭素-炭素二重結合又は三重結合を含んでもよい $C_1 \sim C_6$ アルキルであり、このとき前記 $C_1 \sim C_6$ アルキル及び前記ベンジルのフェニル部分がフルオロ、 $C_1 \sim C_2$ アルキル又は $C_1 \sim C_2$ アルコキシにより置換されていてもよく；並びに R_{11} が水素又はフルオロである。

【0014】また好ましくは、 R_2 が(アリール) $C_1 \sim C_4$ アルキル又は(ヘテロアリール) $C_1 \sim C_4$ アルキルであり、このとき前記アリール部分がフェニル、フラニル又はベンゾフラニルであり、及び前記ヘテロアリール部分がチエニル、ベンゾチエニル、チアゾリル、ピリジル又はベンゾチアゾリルである。

【0015】また好ましくは、 B が NR_1R_2 又は CHR_1R_2 であり、このとき R_1 及び R_2 が N 又は CH と一緒にあって5又は6員環を形成し、このとき前記環が硫黄、酸素、又は B が N R_1R_2 であるときはさらに窒素を有してもよい。

【0016】また好ましくは、 B が $-NHCHR_1R_2$ 又は $-OCH$ R_1R_2 であり、このとき CHR_1R_2 部分が1個の酸素又は硫黄を含んでもよい5又は6員環である。

【0017】また好ましくは、 R_3 がメチル、クロロ又はメトキシであり； R_4 がメチル、 $-CH_2OH$ 、シアノ、トリフルオロメトキシ、メトキシ、トリフルオロメチル、クロロ、 $-CO_2CH_3$ 、 $-CH_2OCH_3$ 、 $-CH_2Cl$ 、 $-CH_2F$ 、アミノ又はニトロであり； R_6 が水素、メチルスルフィニル、メチルスルファニル、メチルスルホニル又はエチルであり；並びに R_5 がフェニル又はピリジルであり、このとき前記フェニル又はピリジルがフルオロ、クロロ、ブロモ、ヨード、 $C_1 \sim C_4$ アルコキシ、トリフルオロメチル、 $C_1 \sim C_3$ ヒドロキシアルキル、 $-CO_2$ ($C_1 \sim C_2$ アルキル)、 $C_1 \sim C_2$ アルキルアミノ、 $-CO$ ($C_1 \sim C_4$ アルキル)及び $C_1 \sim C_6$ アルキルから独立して選択される1個の置換基によって置換され、このとき前記 $C_1 \sim C_6$ アルキル及び前記 $C_1 \sim C_4$ アルキルは1個のヒドロキシ又はフルオロ基によって置換されていてもよく、及び1個の炭素-炭素二重結合又は三重結合を含んでもよい。

【0018】式I又はIIの化合物は、以下の化合物を含む：4-(1-エチル-プロポキシ)-2,5-ジメチル-6-(2,4,6-トリメチル-ベンジル)-ピリミジン；2-(4-ブromo-2,6-ジメチル-フェノキシ)-4-(1-エチル-プロポキシ)-3,6-ジメチル-ピリジン；2-(4-エチル-2,6-ジメチル-フェノキシ)-4-(1-エチル-プロポキシ)-3,6-ジメチル-ピリジン；3-エチル-4-(1-エチル-プロポキシ)-6-メチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン；2-(2,6-ジメチル-4-プロピル-フェノキシ)-4-(1-エチル-プロポキシ)-3,6-ジメチル-ピリジン；4-(1-エチル-プロポキシ)-2-(4-メトキシ-2,6-ジメチル-フェノキシ)-3,6-ジメチル-ピリジン；2-(4-エトキシ-2,6-ジメチル-フェノキシ)-4-(1-エチル-プロポキシ)-3,6-ジメチル-ピリジン；2-(4-クロロ-2,6-ジメチル-フェノキシ)-4-(1-エチル-プロポキシ)-3,6-ジメチル-ピリジン；4-(1-メトキシメチル-プロポキシ)-3,6-ジメチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン；[3,6-ジメチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-4-

イル]-ジエチル-アミン；[3,6-ジメチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-4-イル]-エチル-プロピル-アミン；[2,5-ジメチル-6-(2,4,6-トリメチル-フェノキシ)-ピリミジン-4-イル](1-エチル-プロピル)アミン；ブチル

[3,6-ジメチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-4-イル]-エチル-アミン；4-(1-エチル-プロポキシ)-3,6-ジメチル-2-(2,4,6-トリメチル-フェニルスルファニル)-ピリジン；ブチル-[2-(4-クロロ-2,6-ジメチル-フェノキシ)-3,6-ジメチル-ピリジン-4-イル]-エチル-アミン；4-(1-エチル-プロピルアミノ)-6-メチル-2-(2,4,6-トリメチル-フェノキシ)-ニコチン酸メチルエステル；[3,6-ジメチル-2-(2,4,6-トリメチル-フェニルスルファニル)-ピリジン-4-イル]-エチル-プロピル-アミン；[4-(1-エチル-プロピルアミノ)-6-メチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-3-イル]-メタノール；[2-(4-クロロ-2,6-ジメチル-フェノキシ)-3,6-ジメチル-ピリジン-4-イル]-エチル-プロピル-アミン；1-(エチル-プロピル)-[6-メチル-3-ニトロ-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-4-イル]-アミン； N_4 -(1-エチル-プロピル)-6-メチル-3-ニトロ- N_2 -(2,4,6-トリメチル-フェニル)-ピリジン-2,4-ジアミン； N_4 -(1-エチル-プロピル)-6-メチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-3,4-ジアミン； N_4 -(1-エチル-プロピル)-6-メチル- N_2 -(2,4,6-トリメチル-フェニル)-ピリジン-2,3,4-トリアミン；[3,6-ジメチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-4-イル]-エチル-(2,2,2-トリフルオロ-エチル)-アミン；[3-クロロメチル-6-メチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-4-イル]-(1-エチル-プロピル)-アミン；

[3,6-ジメチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-4-イル](1-エチル-プロピル)-アミン；(1-エチル-プロピル)-[2-メチル-5-ニトロ-6-(2,4,6-トリメチル-ピリジン-3-イルオキシ)-ピリミジン-4-イル]-アミン；(1-エチル-プロピル)-[3-メトキシメチル-6-メチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン-4-イル]-アミン； N_4 -(1-エチル-プロピル)-2-メチル-5-ニトロ- N -(2,4,6-トリメチル-ピリジン-3-イル)-ピリミジン-4,6-ジアミン；[2-(4-クロロ-2,6-ジメチル-フェノキシ)-3,6-ジメチル-ピリジン-4-イル]-ジエチル-アミン；4-(1-エチル-プロポキシ)-3,6-ジメチル-2-(2,4,6-トリメチル-フェノキシ)-ピリジン；ブチル-[2,5-ジメチル-7-(2,4,6-トリメチル

フェニル) -6, 7-ジヒドロ-5H-ピロロ [2, 3-d] ピリミジン-4-イル] -エチル-アミン; 4- (ブチル-エチルアミノ) -2, 5-ジメチル-7- (2, 4, 6-トリメチルフェニル) -5, 7-ジヒドロ-ピロロ [2, 3-d] ピリミジン-6-オン; 4- (1-エチルプロボキシ)

2, 5-ジメチル-6- (2, 4, 6-トリメチルフェノキシ) -ピリミジン; N-ブチル-N-エチル-2, 5-ジメチル-N- (2, 4, 6-トリメチルフェニル) -ピリミジン-4, 6-ジアミン; (1-エチル-プロピル) - [5-メチル-3- (2, 4, 6-トリメチルフェニル) -3H-イミダゾ [4, 5-b] ピリジン-7-イル] -アミン; [2, 5-ジメチル-3- (2, 4, 6-トリメチルフェニル) -3H-イミダゾ [4, 5-b] ピリジン-7-イル] - (1-エチル-プロピル) -アミン; N4- (1-エチル-プロピル) -6, N3-ジメチル-2- (2, 4, 6-トリメチルフェノキシ) -ピリジン-3, 4-ジアミン; N4- (1-エチル-プロピル) -6, N3, N3-トリメチル-2- (2, 4, 6-トリメチルフェノキシ) -ピリジン-3, 4-ジアミン; 6- (1-エチル-プロボキシ) -2-メチル-N4- (2, 4, 6-トリメチルフェニル) -ピリミジン-4, 5-ジアミン; [4- (1-エチル-プロボキシ) -3, 6-ジメチル-ピリジン-2-イル] - (2, 4, 6-トリメチルフェニル) -アミン; 及び6- (エチル-プロピル-アミノ) -2, 7-ジメチル-9- (2, 4, 6-トリメチルフェニル) -7, 9-ジヒドロ-プリン-8-オン。

【0019】式I又はIIの化合物は、さらに以下の化合物を含む: ブチル- [3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-b] ピリジン-4-イル] -エチルアミン; 3, 6-ジメチル-4- (テトラヒドロフラン-3-イルオキシ) -1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-b] ピリジン; [3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-b] ピリジン-4-イル] - (1-メトキシメチルプロピル) -アミン; 4- (1-メトキシメチルプロボキシ) -3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-b] ピリジン; (1-エチルプロピル) - [3, 5, 6-トリメチル-1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-b] ピリジン-4-イル] -アミン; 4- (1-エチルプロボキシ) -2, 5-ジメチル-7- (2, 4, 6-トリメチルフェニル) -7H-ピロロ [2, 3-b] ピリジン; 4- (1-エチルプロボキシ) -2, 5-ジメチル-7- (2, 6-ジメチル-4-プロモフェニル) -7H-ピロロ [2, 3-b] ピリジン; 3- (4-メチル-ベンジル) - [3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミノ} -ブ

ロパン-1-オール; ジエチル- [6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; 2- [ブチル- [6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミノ} -エタノール; ジブチル- [6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; ブチル-エチル- [6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; ブチル-エチル- [6-メチル-3-メチルスルホニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; ブチル-シクロプロピルメチル- [6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; ジ-1-プロピル- [6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; ジアリル- [6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; ブチル-エチル- [6-クロロ-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; ブチル-エチル- [6-メトキシ-3-メチルスルファニル-1- (2, 4, 6-トリクロロフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; プロピル-エチル- [3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; 4- (1-エチル-プロピル) -6-メチル-3-メチルスルファニル-1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン; 2- [3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン-4-イル] -アミン; 4- (1-メトキシメチルプロボキシ) -3, 6-ジメチル-1- (2, 4, 6-トリメチルフェニル) -1H-ピラゾロ [3, 4-d] ピリミジン; n-ブチル-エチル- [2, 5-ジメチル-7- (2, 4, 6-トリメチルフェニル) -7H-ピロロ [2, 3-d] ピリミジン-4-イル] -アミン; ジn-プロピル- [2, 5-ジメチル-7- (2, 4, 6-トリメチルフェニル) -7H-ピロロ [2, 3-d] ピリミジン-4-イル] -アミン; エチル-n-プロピル- [2, 5-ジメチル-7- (2, 4, 6-トリメチルフェニル) -7H-ピロロ [2, 3-d] ピリミジン-4-イル] -アミン; ジエチル-2, 5-

ジメチル-7-(2, 4, 6-トリメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン-4-イル] アミン; n-ブチル-エチル- [2, 5, 6-トリメチル-7-(2, 4, 6-トリメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン-4-イル] アミン; 2-[N-n-ブチル-N-[2, 5-ジメチル-7-(2, 4, 6-トリメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン-4-イル] アミノ]-エタノール; 4-(1-エチル-プロピル)-2, 5, 6-トリメチル-7-(2, 4, 6-トリメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン; n-ブチル-エチル- [2, 5-ジメチル-7-(2, 4-ジメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン-4-イル] アミン; 2, 5-ジメチル-7-(2, 4, 6-トリメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン-4-イル]- (1-エチル-プロピル) アミン; 2-[7-(4-プロモ-2, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イルアミノ]-ブタン-1-オール; 2-(S)-[7-(4-プロモ-2, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イルアミノ]-ブタン-1-オール; 4-(1-エチル-プロポキシ)-2, 5, 6-トリメチル-7-(2, 4, 6-トリメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン; 4-(1-メトキシメチルプロポキシ)-2, 5, 6-トリメチル-7-(2, 4, 6-トリメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン; 4-(1-エチル-ブチル)-2, 5, 6-トリメチル-7-(2, 4, 6-トリメチルフェニル)-7H-ピロロ [2, 3-d] ピリミジン; [7-(4-プロモ-2, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イル]- (1-メトキシメチル-プロピル) アミン; 2-[7-(2-プロモ-4, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イルアミノ]-ブタン-1-オール; 2-[7-(2-エチル-4, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イルアミノ]-ブタン-1-オール; 及び2-[7-(2-フルオロメチル-4, 6-ジメチルフェニル)-2, 5-ジメチル-7H-ピロロ [2, 3-d] ピリミジン-4-イルアミノ]-ブタン-1-オール。

【0020】CRFアンタゴニストによる、心臓での副交感神経及び交感神経の電気的作用の正常化によるQT分散における改善は、致命的な心室不整脈の発生を減少させる結果になり、そして死亡率の低減の結果になると考えられる。同様に、CRFアンタゴニストは同じメカニズムを介して、心拍変動の改善により死亡率を低減すると考えられる。心拍変動は中間心拍の周囲の揺動 (fluctuations around the mean heart rate) であり、心臓

性呼吸のコントロール系 (cardiorespiratory control system) の相関として用いることができる。低い心拍の変動、即ち副交感神経又は交感神経の何れかの支配は、糖尿病、心疾患及び骨折後 (post-infarction) の患者における、並びに多くの神経障害 (例えば、脳障害、ギランバレー症候群、小児突然死症候群、先天性過換気症候群) 又は尿毒症性神経障害等における突然の心臓死に関係する (例えば、Malpasら、Diabetes, 39: 1177-1181 (1990); Wooら、J. Am. Coll. Cardiol., 23: 565-569 (1994); Biggerら、Circulation, 85: 164-171 (1992); 及びvan Ravenswaaij-ArtsらのAnnals of Internal Medicine, 118: 436-447 (1993)) を参照せよ。)

【0021】CRFアンタゴニストは、多くの疾患において、心臓及び呼吸器系への中枢神経の拍出 (neural outflow) のバランスをとることによって突然死の発生を減じる。リスクのある患者は、心電図QT分散及び心拍変動という手段で、容易かつ安価にモニターされて、そのような治療が有効であるかどうかを決定することができる。

【0022】疾患の状態に加えて、他の症状を緩和するために患者に投与されるある薬物は、QT分散及び/又は心拍変動を引き起こすか、又はそのような結果になりうる。このような薬物の例として、フェノチアジン及び非特異的抗精神病薬 (例えば、クロルプロマジン、レスピラドン)、クラス1A及びクラスIII不整脈薬 (例えば、キニジン及びソトロール)、並びに麻酔薬 (例えば、エンフルラン、イソフルラン) 等を挙げることができる。このような場合もまた、心臓における副交感神経及び交感神経の電気的作用を正常化し、患者のQT分散及び心拍変動を改善するために、CRFアンタゴニストを投与することが有効であると考えられる。

【0023】CRFアンタゴニストは単独で、特定の患者における突然死を減じる一方で、CRFアンタゴニストを他の薬物と組み合わせることも好ましいと考えられる。例えば、心臓及び/又は呼吸器系への神経の拍出のバランスをとることができる他の薬物は、CRFアンタゴニストの有効性を相互作用によって改善しうる。

【0024】本発明の方法において使用するための好ましい式I及びIIの化合物、並びにそれらの医薬として許容できる塩は、容易に製造することができる。式IIの化合物 (A、D、及びYはNであり、二重結合でEとDとが結合し、Eが- CR_4 である) は、上記のPCT公開W094/13677に記載された1又はそれ以上の合成方法によって製造できる。式IIの化合物 (A及びYはNであり、二重結合でEとDとが結合し、Eが- CR_4 であり、Dが- CR_{10} である) は、上記のPCT公開W094/13676に記載された1又はそれ以上の合成方法によって製造できる。式IIの化合物 (Aが- CR_7 であり、二重結合でEとDとが結合し、Eが- CR_4 であり、D及びNが- CR_{10} であり、YがNである) は、上記のPC

T公開W095/34563に記載された1又はそれ以上の合成方法によって製造できる。式IIの他の化合物及び式Iの化合物は、上記のPCT公開W095/33750に記載された1又はそれ以上の合成方法によって製造できる。好ましい化合物のあるものの製造に関する更なる有用な情報は、PCT/IB95/00437（公開番号W096/39388）中に与えられている。これは特定の中間体の生産を記載するものである。

【0025】式I又はIIの化合物の医薬として許容できる塩は、酸性又は塩基性の基の塩を含む。例えば、医薬として許容できる塩は、 R_1 置換基がカルボキシである場合のような酸性の基の、ナトリウム、カルシウム又はカリウム塩を含む。そのような塩は、一般的には式I又はIIの化合物の1を、適当な溶媒中で、1モル等量のNaOH又はKOHと組み合わせることにより製造される。アミノ基のような塩基性の基の医薬として許容できる酸付加塩は、式I又はIIの化合物の塩基形態のものと適当な酸を反応させることにより形成される。塩基性の基の医薬として許容できる塩は、塩酸塩、シュウ酸塩、硫酸塩、リン酸塩、リン酸水素塩、リン酸二水素塩、酢酸塩、コハク酸塩、クエン酸塩、酒石酸塩、乳酸塩、マンデル酸塩、メタンスルホン酸（メシラート；mesylate）塩及びp-トルエンスルホン酸（トシラート；tosylate）塩等を含む。1価の酸の塩（例えば、塩酸塩、シュウ酸塩、p-トルエンスルホン酸塩、酢酸塩）の場合、少なくとも1モル等量、そして通常はモル過剰の酸を用いる。しかし、そのような塩が、硫酸塩、ヘミコハク酸塩（hemisuccinate）、リン酸水素塩又はリン酸塩として所望される場合、一般的には適切かつ正確な化学等量の酸が用いられる。

【0026】フリーの塩基及び酸は、通常、所望の塩の沈殿が期待できる共溶媒中で組み合わせられ、そうでなければ濃縮するか又は非溶媒を添加することによって単離される。

【0027】本明細書において3～8員のシクロアルキル環、又は9～12員のビシクロアルキル環システムに言及するときは、それらの各々は所望により1又は2個のO、S、又はN-Gを有してもよく、シクロアルキル環又はビシクロアルキル環システムにおいては酸素及び硫黄原子が互いに結合しないことが理解される。シクロアルキル環が3員であるとき、1個のO、S又はN-Gを含んでもよい。例えば、O及びNHを有する6員のシクロアルキル環は、モルホリニルである。 R_2 又は R_5 がヘテロサイクリック基である場合、基は、1個の炭素原子を介して連結することができる。

【0028】上記の式I及びIIには、表される群類に含まれるすべての化合物のラセミ体及びすべての立体異性体（例えば、すべての幾何異性体、及びすべての光学異性体）が含まれることが意図されている。

【0029】本発明の方法においては、式I及びIIの化

合物、並びにそれらの医薬として許容できる塩は、単独で又は医薬として許容できるキャリアと組み合わせられて、単回又は複数回の何れかで投与される。好ましい医薬として許容できるキャリアは、不活性固体希釈剤又は充填剤、滅菌水性溶液、及び種々の有機溶媒を含む。そして、活性化合物及び医薬として許容できるキャリアとの組み合わせにより形成された医薬組成物は、種々の投与型、例えば、錠剤、粉末剤、トローチ剤（lozenge）、シロップ剤、注射用液剤等で容易に投与される。これらの医薬組成物は、適切であれば、芳香剤、結合剤、賦形剤等を含みうる。そして、経口投与の目的のためには、錠剤は、クエン酸ナトリウム、炭酸カルシウム及びリン酸カルシウムのような種々の賦形剤を含むことができ、でんぷん、アルギン酸及び特定の複合ケイ酸塩のような種々の崩壊剤と、ポリビニルピロリドン、ショ糖、ゼラチン及びアラビアガムのような結合剤とを一緒に含んでもよい。加えて、ステアリン酸マグネシウム、ラウリル硫酸ナトリウム及びタルクのような滑剤は、錠剤化の目的のためにしばしば有用である。同一タイプの固体組成物はまた、軟質及び硬質ゼラチンカプセル中に充填物として用いることができる。これは、好ましくはラクトース（乳糖）及び高分子量のポリエチレングリコールを含む。水性懸濁剤又はエリキシル剤が経口投与のために求められるときは、水、エタノール、プロピレングリコール、グリセリン及びそれらの組み合わせのような希釈剤とともに、その剤の中の本質的な活性成分は、種々の甘味剤又は芳香剤、着色料又は染料及び、望ましければ、乳化剤又は懸濁化剤と組み合わせることができる。一般的には経口投与が好ましい。しかし、患者が嘔下することができないとき、又はそれ以外の理由で経口投与に支障があるときは、直腸、腸管外（parenteral）又は局所投与のような他の投与経路が適するであろう。

【0030】腸管外投与のためには、ゴマ油若しくは落花生油、水溶性プロピレングリコール又は滅菌水溶液中に溶解した活性成分の液剤を用いることができる。そのような液剤は、必要であれば緩衝化することが好ましく、また、そのような液体は最初に十分な塩類又はグルコースで等張化する。このような特別の液剤は、静脈中、筋中、皮下又は腹腔内に投与するのに特に好ましい。用いられる滅菌水性溶媒は当業者に知られた標準的な技術によって得ることができる。

【0031】経皮的（例えば局所）投与の目的のためには、希釈され、滅菌された水性又は部分的に水性の液剤（通常約0.1%～5%濃度）、そうでなければ上記の腸管外投与用液剤と同様のものを用いることができる。

【0032】ある量の活性成分を有する種々の医薬組成物の製造方法は、当業者に知られており、又は本明細書の開示により明らかである。他には、例えば、Remington's Pharmaceutical Sciences, Mack Publishing社、Eaton, Pa.、第15版（1975）を参照せよ。

【0033】本発明の方法において、式I及びIIの化合物並びにそれらの医薬として許容できる塩の効果的な投与量は、医者には一般的に知られているように、意図する投与経路及び他の要素（例えば、患者の年齢及び体重）に依存する。一般的には、一日あたりの投与量は、約0.1mg/kg～約50mg/kg（治療されるべき患者の体重）が好ましい。より好ましくは、一日あたりの投与量は、約1.0mg/kg～約20mg/kg（体重）である。この一日あたりの投与量は、単回又は分割投与により与えることができる。

【0034】式I及びIIの化合物並びにそれらの医薬として許容できる塩の、CRFアンタゴニスト活性の検定方法は、Wynnら、Endocrinology、116：1653-1659（1985）及びGrigoriadisら、Peptides、10：179-188（1989）に記載されている。これらの方法は、試験されるされる化合物のCRF受容体に対する結合親和性（CRFアンタゴニストとして期待される活性に非常に関係がある）を測定するものである。IC₅₀値として表現される活性化化合物の結合親和性は、概して、約0.2ナノモル濃度～約10マイクロモル濃度である。

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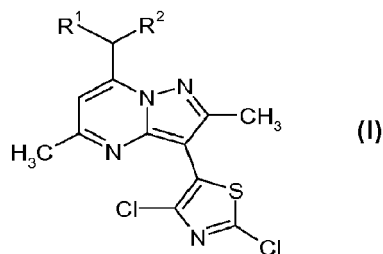
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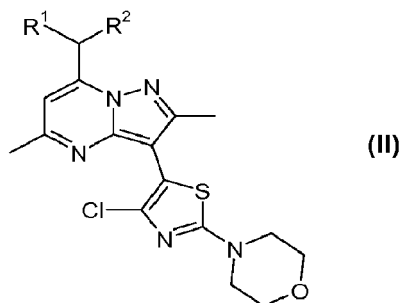
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(54) Title: THIAZOLYL-PYRAZOLOPYRIMIDINE COMPOUNDS AS SYNTHETIC INTERMEDIATES AND RELATED SYNTHETIC PROCESSES



(57) Abstract: The present invention relates to a synthetic intermediate of the formula (I) and its use in a synthetic process to make compounds of the formula (II).





GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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THIAZOLYL-PYRAZOLOPYRIMIDINE COMPOUNDS AS SYNTHETIC
INTERMEDIATES AND RELATED SYNTHETIC PROCESSES

5 This invention relates to novel intermediates and processes in the synthesis of thiazolyl-pyrazolopyrimidine compounds, which final compounds are useful as CRF-1 receptor antagonists for the treatment of certain psychiatric and neuroendocrine disorders.

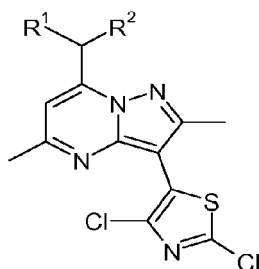
Corticotropin releasing factor (CRF) is a peptide hypothalamic hormone which also has a broad spectrum of physiological effects consistent with a neurotransmitter or neuromodulator role in the brain. CRF has been implicated in a wide range of psychiatric
10 disorders and neurological diseases including alcohol and substance abuse and associated withdrawal symptoms, as well as depression and anxiety. CRF-1 receptor antagonists have particularly been implicated for the treatment of alcohol dependency and depression.

Certain thiazolyl-pyrazolo[1,5-*a*]pyrimidines have been found to be particularly useful as CRF-1 antagonists, as for example 7-(1-ethyl-propyl)-3-(4-chloro-2-morpholin-
15 4-yl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine and 7-(1-propyl-butyl)-3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine, as described in WO 2008/036579. Recent advances in copper catalyzed arylation reactions with different substrates have been described in J. Am. Chem. Soc., vol. 130, pg. 1128-1129 (2008).

20 The present invention provides novel synthetic intermediates and synthetic processes using said intermediates to make thiazolyl-pyrazolo[1,5-*a*]pyrimidine CRF-1 receptor antagonists more economically and in higher yields. Certain embodiments of the presently claimed processes also avoid the use of mutagenic materials and environmentally toxic catalysts, as compared to previously known synthetic methods, as
25 for example, those disclosed in WO 2008/036579.

In one aspect of the present invention, there is provided a compound of Formula I

2



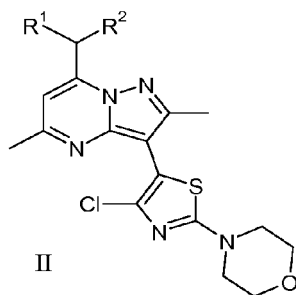
Formula I

wherein:

R^1 and R^2 are independently ethyl or *n*-propyl.

- 5 In one preferred embodiment, R^1 and R^2 are each ethyl. In a second preferred embodiment, R^1 and R^2 are each *n*-propyl.

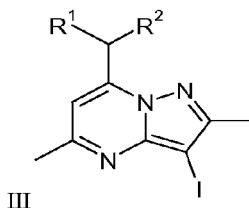
In another aspect of the present invention, there is provided a synthetic process for preparing a compound of the Formula II



II

- 10 wherein R^1 and R^2 are independently ethyl or *n*-propyl, comprising the steps of

i) reacting a compound of Formula III,



III

- 15 or a salt thereof, with 2,4-di-chlorothiazole, or a salt thereof, in the presence of a copper halide catalyst, a ligand selected from 1,10-phenanthroline and bipyridine,

and a base selected from K_3PO_4 and Cs_2CO_3 , to form a compound of Formula I (above); and

ii) reacting the compound of Formula I, with morpholine, or a salt thereof, in the presence of a suitable inorganic base.

5

In one preferred embodiment of this aspect of the invention, R^1 and R^2 are each ethyl. In a second preferred embodiment of this aspect of the invention, R^1 and R^2 are each *n*-propyl.

Suitable copper halide catalysts include CuI, CuBr, and CuCl. CuCl is preferred.

10

Use of a copper ligand facilitates the efficient catalysis by the copper ion. Both 1,10-phenanthroline and bipyridine are effective ligands in the present process. 1,10-phenanthroline is preferred. When bipyridine is used, higher mole percents of bipyridine and copper are needed for high yields, as for example from about 0.9 to about 1.1 equivalents, say about 1 equivalent bipyridine, and from about 0.5 to about 1.1

15

equivalents of the copper source, say about 1 equivalent of the copper source, with CuCl being preferred.

When 1,10-phenanthroline is used, it may be effectively used in catalytic amounts, and with catalytic amounts of the copper source, as for example from about 5-50 mole percent each (0.05-0.50 equivalents), with CuCl being the preferred copper source. In one
20 embodiment, the 1,10-phenanthroline and the copper catalyst, say CuCl, are used at about 5-50 mole percent (0.05-0.50 equivalents). In another embodiment, the 1,10-phenanthroline and the copper catalyst, say CuCl, are used at about 10 ± 1 mole percent (0.10 equivalents). In yet another embodiment, the 1,10-phenanthroline and the copper catalyst, say CuCl, are used at about 30 ± 1 mole percent (0.30 equivalents).

25

The reaction of step i) is base driven and is sensitive to the base selected. The dichlorothiazole reagent is base and temperature labile and is susceptible to degradation if the selected base is too strong and/or the reaction temperature is too high, as for example with *t*-butoxy lithium or *t*-butoxy potassium and/or at temperatures higher than about 120° C. As such, suitable bases for step i) are K_3PO_4 and Cs_2CO_3 , with Cs_2CO_3 being
30 preferred. Likewise, it is preferred that the reaction of step i) be run at a temperature of from about 80 to 120° C.

The reaction of step i) is less sensitive to the solvent selected with suitable solvents being aprotic solvents, as for example 1,4-dioxane, 2-MeTHF, DMAC, CPME, propanenitrile, 2-MeTHF/DMAC, *n*-BuOAc and DMF. The co-solvent system of 2-MeTHF/DMAC with proportions in the range of 70/30 to 80/20 being preferred, as for
 5 example 80/20 2-MeTHF/DMAC.

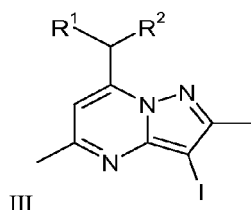
The reaction of step ii) is less sensitive to the inorganic base and solvent selected. Suitable bases include K_2CO_3 , Cs_2CO_3 , Na_2CO_3 , K_3PO_4 , NaOH, and the like. K_2CO_3 and K_3PO_4 are preferred with K_2CO_3 being particularly preferred.

Suitable solvents for the reaction of step ii) include most polar solvents, such as
 10 methanol, ethanol, *n*-propanol, 2-propanol, *n*-butanol, acetonitrile, 2-MeTHF, and morpholine. Morpholine is preferred in that it is an excellent solvent and a reactant in the reaction of step ii).

Preferred reaction temperatures for the reaction of step ii) are from about 60 - 120° C.

15 In one preferred embodiment of the present invention, the synthetic process comprises the steps of:

i) reacting a compound of Formula III,



or a salt thereof, wherein R^1 and R^2 are independently ethyl or *n*-propyl, with 2,4-di-chlorothiazole, or a salt thereof, in the presence of CuCl, 1,10-phenanthroline,
 20 and a base selected from K_3PO_4 and Cs_2CO_3 , to form a compound of Formula I (above); and

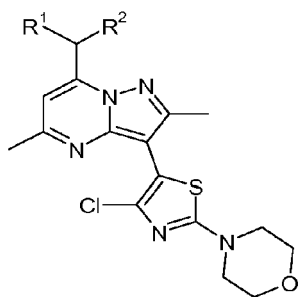
ii) reacting the compound of Formula I, with morpholine, or a salt thereof, in the presence of K_2CO_3 .

25 It is further preferred in this embodiment of the process that the reaction of step i) use from about 5-50 mole percent, particularly about 30 mole percent, CuCl and from about 5-50 mole percent, particularly about 30 mole percent, 1,10-phenanthroline with 2-MeTHF/DMAC (particularly about 80/20 2-MeTHF/DMAC) as a solvent system and

5

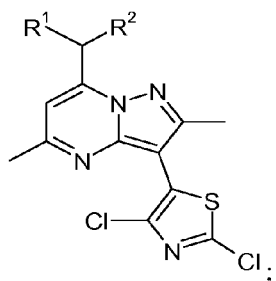
that the reaction be run at from about 80 to 120° C, and that the reaction of step ii) use morpholine as a solvent and K₂CO₃ as the base.

In a another aspect of the invention, there is provided a synthetic process for
5 preparing a compound of the formula



wherein R¹ and R² are independently ethyl or *n*-propyl,
comprising the steps of

i) reacting a compound of the formula

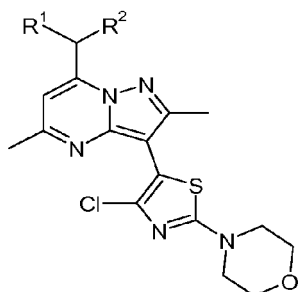


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or a salt thereof, with an excess of morpholine or salt thereof, in the presence of a suitable solvent.

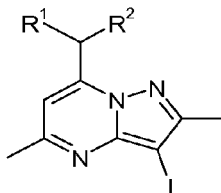
One embodiment of this aspect of the invention provides a synthetic process for preparing a compound of the formula

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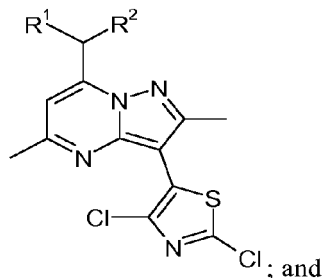
wherein R^1 and R^2 are independently ethyl or *n*-propyl,
comprising the steps of

i) reacting a compound of the formula



5

or a salt thereof, with 2,4-di-chlorothiazole, or a salt thereof, in the presence of a copper halide catalyst, a ligand selected from 1,10-phenanthroline and bipyridine, and a base selected from K_3PO_4 and Cs_2CO_3 , to form a compound of the formula



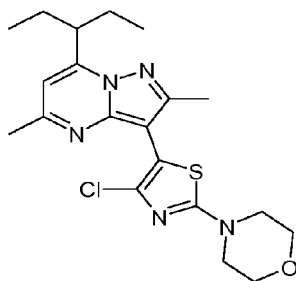
10 ii) reacting the product of step i), with an excess of morpholine, or a salt thereof, in a suitable solvent.

In this aspect of the invention, various aprotic solvents may be suitable, but preferred solvents are 2-MeTHF and 2-propanol. In a preferred embodiment, 2-propanol
15 is used as solvent. It is surprisingly found that use of 2-propanol reduces reaction times about three fold over prior solvent systems tested. It is also surprisingly found that use of

2-propanol provides the desired amination product in high purity even in the presence of competing nucleophilic alcohol. Use of 2-propanol also further simplifies the work up of the final product by allowing crystallization directly from this solvent, thus avoiding a further solvent exchange prior to crystallization.

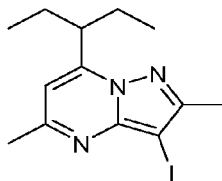
- 5 In this aspect of the invention, it has also been surprisingly found that morpholine can function as a suitable base for the amination reaction, thus eliminating the need for an additional base to drive the reaction. Additionally, yields are improved while maintaining quality of final product. Reduction of the amount of morpholine used is also of benefit in the final workup of the product. In one embodiment wherein 2-MeTHF is used as the
- 10 solvent, the reaction is run with between about 4.0 and about 6.0 equivalents, say about 5.35 equivalents, of morpholine, or salt thereof. In another embodiment wherein 2-propanol is used as the solvent, the reaction is run with between about 3.0 and about 4.0 equivalents, say about 3.3 equivalents, of morpholine, or salt thereof.

- Thus in one embodiment of the invention, there is provided a synthetic process for
- 15 preparing a compound of the formula



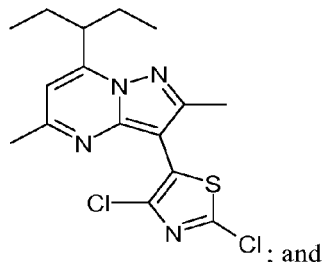
comprising the steps of

- i) reacting a compound of the formula



- 20 or a salt thereof, with 2,4-di-chlorothiazole, or a salt thereof, in the presence of copper chloride, 1,10-phenanthroline, and Cs_2CO_3 , to form a compound of the formula

8



ii) reacting the product of step i), with an excess of morpholine, or a salt thereof, in 2-propanol as a solvent.

5 Abbreviations use in this application are as follows:

CPME means cyclopentyl methyl ether.

DMAC means N,N-dimethylacetamide.

DMF means dimethylformamide.

EtOAc means ethylacetate.

10 2-MeTHF means 2-methyltetrahydrofuran.

2-MeTHF/DMAC system means 2-methyltetrahydrofuran and N,N-dimethylacetamide in a co-solvent system in proportions between about 70/30 and about 80/20.

n-BuOAc means *n*-butylacetate.

15 XRPD means X-ray powder diffraction.

DSC means differential scanning calorimetry.

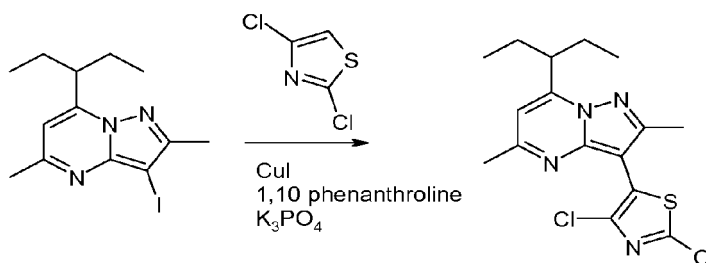
Further preferred embodiments for the synthetic process of the present invention are as described in the examples below.

20

EXAMPLES

Example 1: 7-(1-ethyl-propyl)-3-(2,4-dichloro-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine

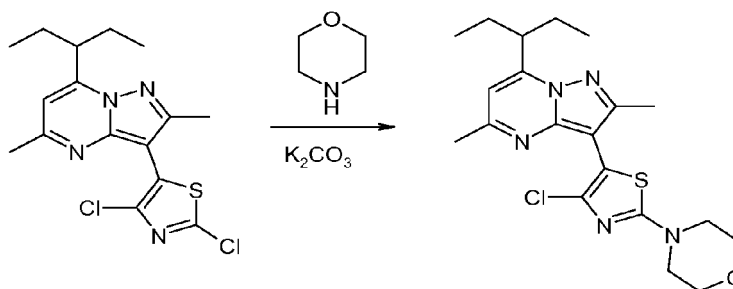
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Charge 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (1.03 g, 3.00 mmoles), K_3PO_4 (1.95 g, 9.00 mmoles), 2,4-dichlorothiazole (0.58 g, 3.75 mmoles), 1,10 phenanthroline (0.05 g, 0.30 mmoles) and anhydrous DMAC (5 mL) to a round
 5 bottom flask equipped with a magnetic stir bar, thermal couple and N_2 inlet. Degas the yellow heterogeneous reaction mixture with N_2 (gas) for 30 min. and then add CuI (0.06 g, 0.30 mmoles) in one portion followed by additional 30 min. degassing with N_2 (gas). Stir the reaction mixture at 120 °C for about 6 hr. Cool the reaction mixture to room temperature overnight, add toluene (10 mL) and stir for 1 hr. Purify the mixture through
 10 silica gel eluting with toluene (10ml). Extract with 1 M HCl (10 mL), water (10 mL), brine (10 mL) and concentrate under reduced pressure to give a yellow solid. Recrystallize the solid from methanol (5ml) to yield the title compound as a yellow crystalline solid. (0.78 g, 70% yield, >99% pure by LC) MS(ES) = 369 (M+1). ^1H NMR ($CDCl_3$) = 6.5 (1H, s); 3.6 (1H, m); 2.6 (3H, s); 2.5 (3H, s); 1.9 (4H, m); 0.9 (6H, t).

15

Example 2: 7-(1-ethyl-propyl)-3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine



20

Charge 7-(1-ethyl-propyl)-3-(2,4-dichloro-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (0.37 g, 1.00 mmoles), K_2CO_3 (0.28 g, 2.00 mmoles) and anhydrous morpholine (3 mL) to a round bottom flask equipped with a magnetic stir bar

10

and N₂ inlet. Stir the yellow mixture at 100 °C for about 4 hr., during which time the reaction becomes homogeneous. Cool the reaction mixture to room temperature, add H₂O (10 mL) and stir the heterogeneous reaction mixture overnight at room temperature. Collected the yellow solid by filtration, wash with H₂O and allowed to air dry overnight to give the crude title compound (391mg). Recrystallize from isopropyl alcohol (3 mL) to yield the title compound as a light yellow crystalline solid (380 mg, 90.6% yield, >99% by LC). MS(ES) = 420 (M+1). ¹H NMR (CDCl₃)= 6.45 (1H, s); 3.81 (m, 4H); 3.62 (1H, m); 3.50 (m, 4H); 2.6 (3H, s); 2.45 (3H, s); 1.85 (4H, m); 0.9 (6H, t).

10 Example 3:

The reactions of Example 1 are run with various other catalysts, ligands, bases and solvents, which are found to have the following effects on yield of 7-(1-ethyl-propyl)-3-(2,4-dichloro-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine. (See Tables 1 - 4).

15 Table 1: Evaluation of different ligands

Ligand	% Product
2,2'-bipyridine	72.7
1,10-phenanthroline	84.7
L-proline	23.7
2-acetylcyclohexanone	19.0

(Reactions are carried out in parallel reactors with 1.2 mmol 2,4-dichlorothiazole, 1 mmol 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine, 0.5 mmol CuI, 0.5 mmol ligand and 2.1 mmol Cs₂CO₃ in 4 mL DMAC. The reactions are degassed under N₂ for 30 min. and then heated at between 80 and 100°C overnight under N₂. Percent product is measured as the percent of total area under the HPLC curve for the product peak. Longer reaction times are shown in parenthesis)

25 Table 2: Evaluation of various solvents

Solvent	% Product
DMAC	82.4
10% H ₂ O in DMAC	1.6
DMAC (under air)	8.5
DMF	76.9
1-methyl-2-pyrrolidinone (NMP)	77.3
Dioxane	69.5
THF	66.9
Toluene	23.0

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Ethylene glycol dimethyl ether (DME)	60.8
Dimethyl sulfoxide (DMSO)	76.7
Propanenitrile [CuCl catalyst]	99
Acetonitrile [CuCl catalyst]	8
2-MeTHF [48 hr, CuCl catalyst]	98
BuOAc [24 hr, CuCl catalyst]	98
CPME [127 hr, with CuCl catalyst]	87
2-MeTHF/DMAC (4:1) [14-24 hr, CuCl catalyst]	98 -100

- 5 (Reactions are carried out in parallel reactors with 1.2 mmol 2,4-dichlorothiazole 1 mmol 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine, 0.25 mmol CuI, 0.25 mmol 1,10-phenanthroline and 2.1 mmol Cs₂CO₃ in 3 mL specified solvent. The reactions are degassed under N₂ for 30 minutes and then heated at 100°C overnight under N₂. Percent product is measured as the percent of total area under the HPLC curve for the product peak.)

Table 3: Evaluation of different copper sources

Copper Source	% Product
CuCl	81.3
CuBr	78.2
CuI	78.8

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- 15 (Reactions are carried out in in parallel reactors with 1 mmol 2,4-dichlorothiazole 1 mmol 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine, 0.05 mmol CuX, 0.01 mmol 1,10-phenanthroline and 3 equivalents K₃PO₄ in 3 mL DMAC. The reactions are degassed under N₂ for 30 minutes and then heated at 100°C overnight under N₂. Percent product is measured as the percent of total area under the HPLC curve for the product peak.)

Table 4: Evaluation of various inorganic bases

Base	% Product
Cs ₂ CO ₃	87.0
Cs ₂ CO ₃ (1.1 eq.)	71.0
K ₂ CO ₃	25.6
Na ₂ CO ₃	6.0
Li ₂ CO ₃	1.8
KOAc	5.3
K ₃ PO ₄	83.8

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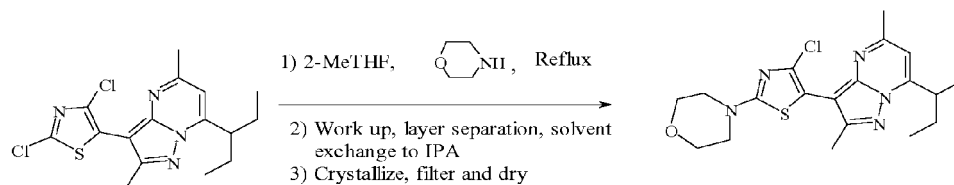
- (Reactions are carried out in in parallel reactors with 1 mmol 2,4-dichlorothiazole 1 mmol 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine, 0.1 mmol CuI, 0.1 mmol 1,10-phenanthroline and 2.1 mmol base and degassed for 30 minutes prior to the addition of 3 mL DMAC. The reactions are degassed under N₂ for 10 minutes and then heated at 100°C overnight under N₂. Percent product

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is measured as the percent of total area under the HPLC curve for the product peak.)

Example 4. Use of morpholine both as a reactant and base in 2-MeTHF as solvent.

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7-(1-ethyl-propyl)-3-(2,4-dichloro-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (15.2 g, 41.16 mmols) is charged into a 250 mL 3-necked round bottomed flask, followed by addition of 2-MeTHF (61 mL, 4.0 volumes), the yellowish brown slurry is stirred at about 20 °C for 5 min. Then morpholine (19 g, 218.18 mmols) is added over 2-5 minutes. Contents are heated to reflux and maintained at reflux for 12 hr. The slurry is cooled to 25 °C, followed by addition of 2-MeTHF (53 mL, 3.5 volumes) and water (38 mL, 2.5 volumes). The reaction mixture is warmed to 40 °C, where upon a homogenous solution with two distinct layers formed. The layers are separated, the organic layer is filtered and concentrated to ~3 volumes at atmospheric pressure. Four volumes 2-propanol (61 mL) are added. The solution is concentrated to ~3 volumes followed by addition of 4 volumes 2-propanol (61 mL), re-concentrated to ~3 volumes, followed by addition of another 6 volumes 2-propanol (91 mL), and refluxed for 15 min. The clear solution is gradually cooled to 75 °C, seeded with 0.45 g 7-(1-ethyl-propyl)-3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine slurried in 2 mL 2-propanol, rinsed with an additional 2 mL 2-propanol and transferred to a crystallization flask. The slurry is cooled to between 0-5 °C, maintained for 1 hr, filtered and the product rinsed with 2-propanol (30 mL, 2 volumes). The solid is dried at 60 °C in a vacuum oven to afford 16.92 g 7-(1-ethyl-propyl)-3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine. Purity of product by HPLC assay is 100.00 %. XRPD and DSC data of product is consistent with reference sample. MS(ES) = 420 (M+1).

Example 5. Use of morpholine as both reactant and base in 2-propanol as solvent.

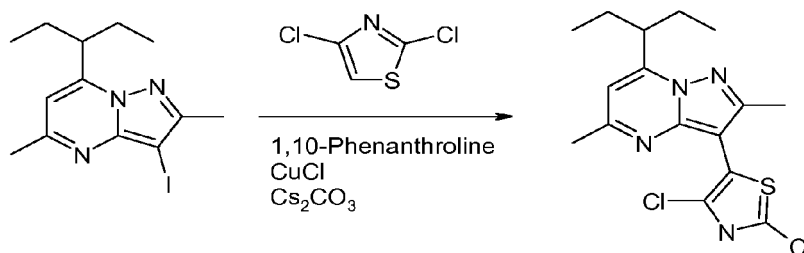
7-(1-ethyl-propyl)-3-(2,4-dichloro-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (11.64 mmoles) is charged into a 100 mL 3-necked round bottomed flask followed by addition of 2-propanol (16 mL, 3.72 volumes). The yellowish brown slurry is stirred at about 20 °C for 5 min. Then morpholine (3.3 g, 37.84 mmoles) is added over 2-5 minutes. Contents are refluxed for 6 hr. The slurry is cooled to 25 °C. 2-Propanol (32 mL, 7.44 volumes) and water (8.6 mL, 2.0 volumes) are added and the mixture warmed to 70-75 °C, filtered and concentrated to ~9 volumes at atmospheric pressure. The clear solution is gradually cooled to 55 °C, seeded with 0.06 g of crystalline 7-(1-ethyl-propyl)-3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine slurried in 0.5 mL 2-propanol, rinsed with additional 0.5 mL 2-propanol and added to crystallization flask. The slurry is cooled to 0-5 °C, maintained for 1 hr., filtered and the product rinsed with 2-propanol (9 mL, 2.1 volumes). Suctioned dried under vacuum at 60 °C to afford 4.6 g of dry 7-(1-ethyl-propyl)-3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (88.8 % yield, purity by HPLC assay is 99.88 %). MS(ES) = 420 (M+1).

Example 6: 7-(1-ethyl-propyl)-3-(2,4-dichloro-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine

7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (10 g, 29.17 mmoles), 2, 4-dichlorothiazole (5.2 g , 33.76 mmoles), cesium carbonate(19.9g, 61.07 mmoles) and 1,10-phenanthroline (1 g, 5.5 mmoles) are charged into a 250 mL 3-necked round bottomed flask, followed by 2-MeTHF (36 mL, 3.6 volumes). The reaction mixture is degassed with nitrogen and then evacuated. Cuprous chloride (0.57 g, 5.7 mmoles), DMAC (10 mL, 1 volume) and 2-MeTHF (4 mL, 0.4 volumes) are added in succession. The reaction mixture is degassed with nitrogen and then evacuated. The contents are refluxed for 20 hr. The reaction mixture is cooled to ~70 °C and 2-MeTHF (100 mL, 10 volumes) is added. The contents are filtered at ~70 °C and the residual cake is washed with 2-MeTHF (80 mL, 8 volumes) at about 65-72°C. The filtrate is transferred into a separatory funnel and extracted with water. The organic layer is separated and washed with dilute HCl. The resulting organic layer is treated with Darco G60, filtered hot (60°C). The filtrate is concentrated at atmospheric pressure to ~2.8

volumes. 25 mL 2-propanol is added, followed by re-concentration to ~2.8 volumes. An additional 25 mL 2-propanol is added, followed again by re-concentration to ~2.8 volumes. Finally, 48 mL 2-propanol is added. The contents are cooled to -7 °C, maintained at -7 °C for 1 hr., filtered and rinsed with 20 mL chilled 2-propanol. Product is suction dried and then vacuum dried at 60 °C to afford 9.41 g 7-(1-ethyl-propyl)-3-(2,4-dichloro-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (purity of product by HPLC assay is 95.88 %). MS(ES) = 369 (M+1).

Example 7. Synthesis of 7-(1-ethyl-propyl)-3-(2, 4-dichloro-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine using 1,4-Dioxane solvent and CuCl catalyst



Add dioxane (9.06X), Cs₂CO₃ (2.00X), 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (1.0 equivalent), 2,4-dichlorothiazole (0.54 equivalent) to a reactor under N₂. Purge the reactor with N₂ three times, degas with N₂ for 0.5~1 hr., and then add 1,10-phenanthroline (0.3 eq) and CuCl (0.3eq) under N₂, degassing with N₂ for 0.5~1 hr. Heat the reactor to 100 °C ~110 °C under N₂. Stir the mixture for 22~24 hr. at 100 °C ~110 °C. Cool to 10~20 °C and add water (10V) and CH₃OH (5V), stir the mixture for 1~1.5 hr. at 10~20 °C. Filter the suspension, resuspend the wet cake in water, stir for 1~1.5 hr. at 10~20 °C, and filter the suspension again. Charge the wet cake to n-heptane (16V) and EtOAc (2V) under N₂. Heat the reactor to 40 °C~50 °C under N₂.

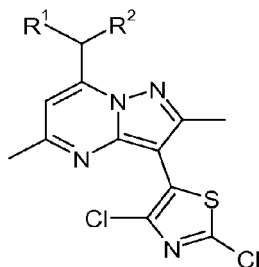
Active carbon (0.1X) is added at 40 °C~50 °C. The reactor is heated to 55 °C~65 °C under N₂ and stirred at 55 °C~65 °C for 1~1.5 hr. The suspension is filtered at 40~55 °C through diatomite (0.4 X). The cake is washed with n-heptane (2.5V). The filtrate is transferred to another reactor. EtOAc (10V) is added and the the organic layer washed with 2 N HCl (10V) three times, followed by washing two times with water (10X, 10V). The organic layer is concentrated to 3~4V below 50 °C. The mixture is heated to

80~90 °C. The mixture is stirred at this temperature for 40~60 min. The mixture is cooled to 0~5°C, stirred for 1~1.5 hr. at 0~5°C and filtered. The cake is washed with n-heptane (1V) and vacuum dried at 45~50 °C for 8~10 hr. The crude product is dissolved in 2-propanol (7.5V) under N₂, and re-crystallized with 2-propanol. The cake is dried in a vacuum oven at 45°C~50°C for 10-12 hr. (55~80% yield). ¹H NMR δ 6.537 (s, 1H) 3.591-3.659 (m, 1H, *J*=6.8Hz), 2.593 (s, 3H), 2.512 (s, 3H), 1.793-1.921(m, 4H), 0.885-0.903 (m, 6H).

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WE CLAIM:

1. A compound of the formula



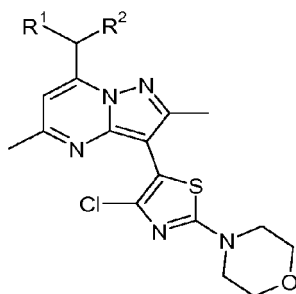
wherein:

- 5 R¹ and R² are independently ethyl or *n*-propyl;
or a salt thereof.

2. The compound according to claim 1, or a salt thereof, wherein R¹ and R²
are each ethyl.

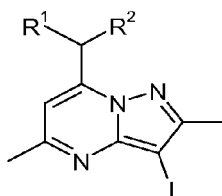
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3. A synthetic process for preparing a compound of the formula



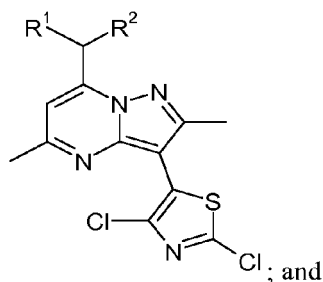
wherein R¹ and R² are independently ethyl or *n*-propyl,
comprising the steps of

- 15 i) reacting a compound of the formula



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or a salt thereof, with 2,4-di-chlorothiazole, or a salt thereof, in the presence of a copper halide catalyst, a ligand selected from 1,10-phenanthroline and bipyridine, and a base selected from K_3PO_4 and Cs_2CO_3 , to form a compound of the formula



- 5 ii) reacting the product of step i), with morpholine, or a salt thereof, in the presence of a suitable inorganic base.
4. The process according to Claim 3 wherein R^1 and R^2 are each ethyl.
- 10 5. The process according to either Claim 3 or Claim 4 wherein the copper halide is CuCl
6. The process according to any one of Claims 3-5 wherein the ligand is 1,10-phenanthroline.
- 15 7. The process according to any one of Claims 3-6 wherein the base in step i) is Cs_2CO_3 .
8. The process according to any one of Claims 3-7 wherein the base in step ii) is K_2CO_3 .
- 20 9. The process according to claim 3 wherein R^1 and R^2 are each ethyl, the copper halide is CuCl, the ligand is 1,10-phenanthroline, the base in step i) is Cs_2CO_3 , and the base in step ii) is K_2CO_3 .

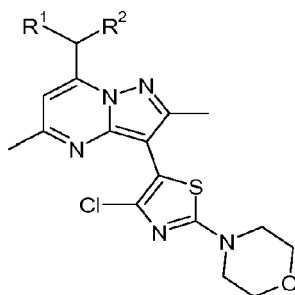
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10. The process according to Claim 9 wherein the solvent for step i) is 1, 4-Dioxane or 2-MeTHF/DMAC and the solvent for step ii) is morpholine, 2-MeTHF, or 2-propanol

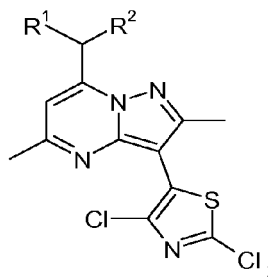
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11. A synthetic process for preparing a compound of the formula



wherein R¹ and R² are independently ethyl or *n*-propyl, comprising the steps of

10 i) reacting a compound of the formula



or a salt thereof, with excess morpholine or salt thereof, in the presence of a suitable solvent.

15 12. The process of Claim 11 wherein the solvent is 2-methyl tetrahydrofuran.

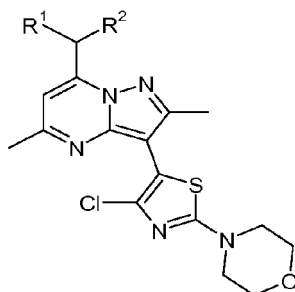
13. The process of Claim 11 wherein the solvent is 2-propanol.

14. The process of any one of Claims 11 – 13 wherein R¹ and R² are each ethyl.

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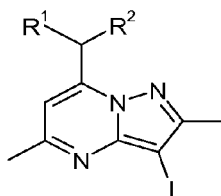
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15. A synthetic process for preparing a compound of the formula

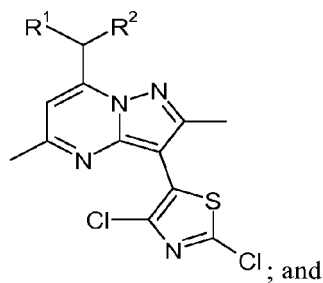


wherein R^1 and R^2 are independently ethyl or *n*-propyl,
comprising the steps of

5 i) reacting a compound of the formula



or a salt thereof, with 2,4-di-chlorothiazole, or a salt thereof, in the presence of a
copper halide catalyst, a ligand selected from 1,10-phenanthroline and bipyridine,
and a base selected from K_3PO_4 and CS_2CO_3 , to form a compound of the formula



10

ii) reacting the product of step i), with an excess of morpholine, or a salt thereof,
in in a suitable solvent.

16. The process of Claim 15 wherein the solvent is 2-methyl tetrahydrofuran.

15

17. The process of Claim 15 wherein the solvent is 2-propanol.

18. The process of any one of Claims 15 – 17 wherein R^1 and R^2 are each ethyl.

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/058722

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D487/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/036579 A1 (LILLY CO ELI [US]; CHEN ZHAOGEN [US]; HAMDOUCHI CHAFIQ HAMDOUCHI [US];) 27 March 2008 (2008-03-27) cited in the application Pages 30-32, 36-38 examples 4,14,16,16/alternate preparation,17 -----	1-18

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

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Lange, Tim

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2009/058722

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008036579 A1	27-03-2008	AR 062886 A1	10-12-2008
		AU 2007297421 A1	27-03-2008
		CA 2663511 A1	27-03-2008
		CL 26932007 A1	18-04-2008
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		EA 200970303 A1	30-10-2009
		EP 2094709 A1	02-09-2009
		KR 20090052366 A	25-05-2009
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Bibliographic data

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Pub/Issue Date: 2009-08-26
Inventor(s): IGOR RAKOV[US]| CASEY BOHL[US]|RAKOV IGOR |BOHL CASEY
Applicant(s): OSURF OHIO STATE UNIVERSITY RE[US]
Classification: C07C255/00A1; C07D207/00A1
Application number: CN20078034701 2007-07-19
Priority number: US20060831988P 20060719;

Abstract of CN101516835A

This invention provides new compounds and uses thereof in treating a variety of diseases or conditions in a subject, including, inter alia, prostate cancer, muscle wasting diseases and/or disorders or a bone-related diseases and/or disorders.

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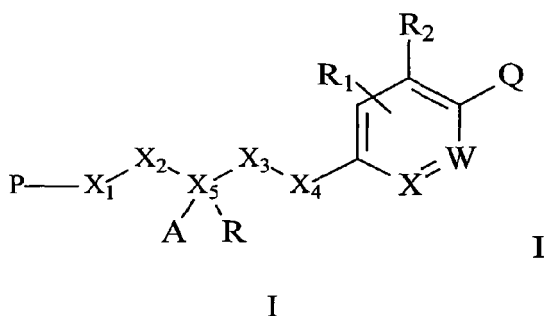
权利要求书 32 页 说明书 162 页 附图 19 页

[54] 发明名称
选择性雄激素受体调节剂、它们的类似物和
衍生物以及它们的用途

[57] 摘要

本发明提供新型化合物以及它们在治疗个体的各种疾病或病症中的用途，所述疾病或病症尤其包括前列腺癌、肌萎缩疾病和/或病症、或骨相关疾病和/或病症。

1. 化合物，其由式 I 的结构表示：

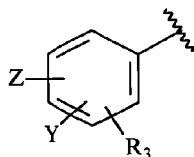


其中

X 是 C 或 N；W 是 C 或 N；

X₁ 是 N、NH、N(C₁₋₄ 烷基)、NAc、NCOOH 或键；或者 X₁ 和 X₃ 与 X₁ 和 X₃ 所连接的 X₂ 和 X₅ 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环；或者 X₁ 和 X₅ 与 X₁ 和 X₅ 所连接的 X₂ 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环，且 A 不存在；

P 是 H 或式 II：



X₂ 是 C₁₋₄ 亚烷基、SO₂、C(O)、CH-[CH₂-(C₄₋₈ 环)]、CH(C₁₋₄ 烷基)、CH(NH₂)、CH(OH)、CH(C₁₋₄ 卤代烷基)、键；或者 X₂ 和 R₃ 与 X₂ 所连接的 X₁ 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环；或者 X₂ 和 X₅ 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环，且 A 不存在；

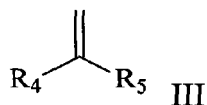
X₃ 是 C₁₋₄ 亚烷基、NH、N、CH(OH)、键；或者 X₁ 和 X₃ 与 X₁ 和 X₃ 所连接的 X₂ 和 X₅ 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环；或者 X₃ 和 X₅ 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环，且 A 不存在；或者 X₃ 和 X₄ 一起形成双键或一起形成饱和的或不饱和

的、取代的或未取代的 3-6 元环；

X_4 是 O、S、SO、C(O)、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键；或者 X_4 和 X_5 与 X_4 和 X_5 所连接的 X_3 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环，且 A 不存在；或者 X_3 和 X_4 一起形成双键或一起形成饱和的或不饱和的、取代的或未取代的 3-6 元环；

X_5 是碳或者 X_5 和 X_1 与 X_1 和 X_5 所连接的 X_2 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环，且 A 不存在；或者 X_5 和 X_2 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环，且 A 不存在；或者 X_5 和 X_3 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环，且 A 不存在；或者 X_5 和 X_4 与 X_5 和 X_4 所连接的 X_3 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环，且 A 不存在；

A 不存在，或是 H、OH、SH、NH₂、C₁₋₄ 烷基、CHF₂、CH₂F、CF₃、CN、CH₂OH、CH₂O(C₁₋₄ 烷基)、CH₂O(C₁₋₄ 酰基)、CH₂CN、CH₂N₃、CH₂NCS、CHO、C(O)O(C₁₋₄ 烷基)、CONHR'、C(O)N(R')₂、C(O)O(C₁₋₄ 烷基)、C(O)R'、CH₂CF₃、CF₂CF₃、CH₂OR'CH₂C(O)CH=CH₂ 或 C(O)CH=CH₂；或者 A 和 R 形成式 III 所表示的双键：



Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br；

Y 是 H、C₁₋₄ 烷基、CF₃、NO₂、CN、F、Cl、I 或 Br；

Q 是 H、CN、OH、NO₂、卤化物、CF₃、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NH₂SO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂]；

R 是 H、OH、C₁₋₄ 烷基、CF₃、CH₂OH、O(C₁₋₄ 烷基)或 O(C₁₋₄ 酰基)；

R₁ 是 H、OH、NH₂、F、Cl、Br 或 I；

R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基

或 $\text{CH}=\text{CH}-\text{O}-$ 对氰基苯基;

R_3 是 H 、 C_{1-4} 烷基、卤化物、 CN 、 NO_2 ; 或者 R_3 和 X_2 与 R_3 所连接的苯环和 X_2 所连接的 X_1 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环;

R' 是 NH_2 、 OH 或 CH_3 ;

R_4 和 R_5 独立地是 H 、 CH_3 、卤化物、 OH 、 C_{1-4} 烷基、 C_{1-6} 环烷基、卤代(C_{1-4} 烷基)、苯基、芳基、 C_{4-8} 杂环烷基或羟基(C_{1-4} 烷基);

R_6 是 CN 、 NO_2 、 $\text{NHC}(\text{O})\text{CH}_3$ 或卤化物;

其中

如果 P 是式 II, X 是 C , X_1 是 NH , X_2 是 $\text{C}(\text{O})$, X_3 是 CH_2 , R 是 OH 并且 A 是烷基或卤代烷基, 那么 X_4 是 $\text{S}(\text{O})[=\text{CHCH}_2\text{N}(\text{CH}_3)_2]$ 、 $\text{C}(\text{OH})[\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2]$ 、 $\text{C}[=\text{CHCH}_2\text{N}(\text{CH}_3)_2]$ 、 $\text{N}[\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2]$ 、 $\text{P}(\text{O})(\text{C}_{1-4}\text{烷基})$ 或 $\text{N}(\text{O})[\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2]$;

或者

如果 P 是式 II, X 是 C , X_1 是 NH , X_2 是 $\text{C}(\text{O})$, X_3 是 CH_2 , R 是 OH 或 OAlk 并且 X_4 是 O 、 S 、 NH 、 S 、 SO 、 SO_2 或亚烷基, 那么 A 是 CN 、 CH_2OH 、 $\text{CH}_2\text{O}(\text{C}_{1-4}\text{烷基})$ 、 $\text{CH}_2\text{O}(\text{C}_{1-4}\text{酰基})$ 、 CH_2CN 、 CH_2N_3 、 CH_2NCS 、 CHO 、 $\text{C}(\text{O})\text{O}(\text{C}_{1-4}\text{烷基})$ 、 CONHR' 、 $\text{C}(\text{O})\text{N}(\text{R}')_2$ 、 $\text{C}(\text{O})\text{O}(\text{C}_{1-4}\text{烷基})$ 、 $\text{C}(\text{O})\text{R}'$ 、 $\text{CH}_2\text{OR}'\text{CH}_2\text{C}(\text{O})\text{CH}=\text{CH}_2$ 或 $\text{C}(\text{O})\text{CH}=\text{CH}_2$;

或者

如果 P 是苯基, X 是 C , X_1 是 NH , X_2 是 $\text{C}(\text{O})$, R 是 OH 并且 A 是 COOH , X_3 是键, X_4 是 CH_2 , R_1 、 R_2 是 H , 那么 Q 不是 H ;

或者

如果 P 是式 II, X 是 C , X_3 是 CH_2 , X_4 是 O 或 NH , R_1 、 R_2 和 R_3 是 H , 并且 X_5 和 X_1 形成 5 元环噁唑烷-2-酮, 那么 Q 不是卤素或 H ;

或者

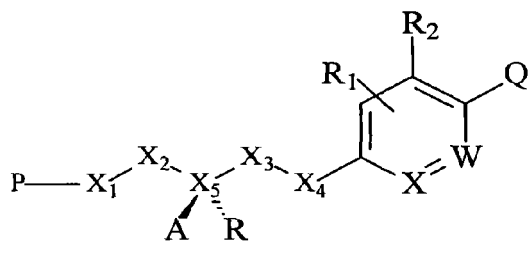
如果 P 是 H , X 是 C , X_1 是 NH , X_2 是 $\text{C}(\text{O})$, R 是 OH , A 是烷基, X_3 是键或 CH_2 并且 X_4 是键或 CH_2 , R_1 和 R_2 是 H , 那么 Q 不是 H 或卤素;

或者

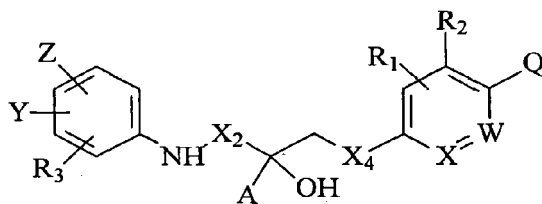
如果 P 是式 II, R_3 是 H , X 和 W 是 C , X_1 和 X_5 与 X_1 和 X_5 所连接的

X_2 一起形成噁唑烷二酮, R 是烷基, X_3 是 CH_2 并且 X_4 是 O 、 NH 、 S 、 SO_2 或 CH_2 , 那么 Q 不是卤化物。

2. 权利要求 1 的化合物, 其由以下结构表示:



3. 权利要求 1 的化合物, 其中所述化合物由式 IV 的结构表示:



IV

其中

X 是 C 或 N ; W 是 C 或 N ;

X_2 是 C_{1-4} 亚烷基、 SO_2 、 $CH-[CH_2-(C_{4-8} \text{ 环})]$ 、 $CH(C_{1-4} \text{ 烷基})$ 、 $CH(NH_2)$ 、 $CH(OH)$ 、 $CH(C_{1-4} \text{ 卤代烷基})$ 、键;

X_4 是 O 、 S 、 SO 、 $C(O)$ 、 $S(O)[=CHCH_2N(CH_3)_2]$ 、 SO_2 、 NH 、 NHR' 、 NO 、 C_{1-4} 亚烷基、 $C(OH)[CH_2CH_2N(CH_3)_2]$ 、 $C[=CHCH_2N(CH_3)_2]$ 、 $PO(C_{1-4} \text{ 烷基})$ 、 $N[CH_2CH_2N(CH_3)_2]$ 、 $NO[CH_2CH_2N(CH_3)_2]$ 或键;

A 是 H 、 OH 、 SH 、 NH_2 、 C_{1-4} 烷基、 CHF_2 、 CH_2F 、 CF_3 、 CN 、 CH_2OH 、 $CH_2O(C_{1-4} \text{ 烷基})$ 、 $CH_2O(C_{1-4} \text{ 酰基})$ 、 CH_2CN 、 CH_2N_3 、 CH_2NCS 、 CHO 、 $C(O)O(C_{1-4} \text{ 烷基})$ 、 $CONHR'$ 、 $C(O)N(R')_2$ 、 $C(O)O(C_{1-4} \text{ 烷基})$ 、 $C(O)R'$ 、 CH_2CF_3 、 CF_2CF_3 、 $CH_2OR'CH_2C(O)CH=CH_2$ 或 $C(O)CH=CH_2$;

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br;

Y 是 H、C₁₋₄ 烷基、CF₃、CN、NO₂、F、Cl、I 或 Br;

Q 是 H、CN、OH、NO₂、CF₃、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NHSO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂];

R 是 H、OH、C₁₋₄ 烷基、CF₃、CH₂OH、O(C₁₋₄ 烷基)或 O(C₁₋₄ 酰基);

R₁ 是 H、OH、F、Cl、Br 或 I;

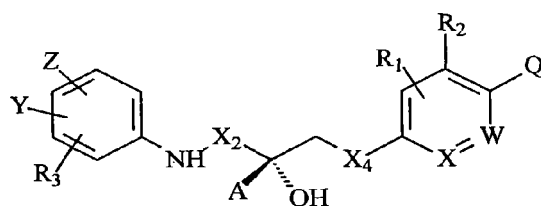
R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基;

R₃ 是 H、C₁₋₄ 烷基、卤化物、CN 或 NO₂;

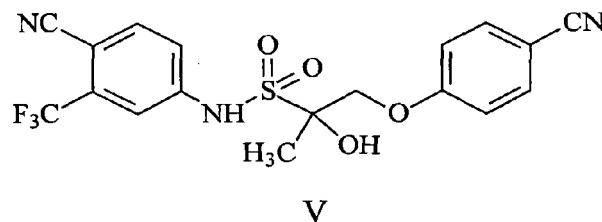
R' 是 NH₂、OH 或 CH₃; 并且

R₆ 是 CN、NO₂、NHC(O)CH₃ 或卤化物。

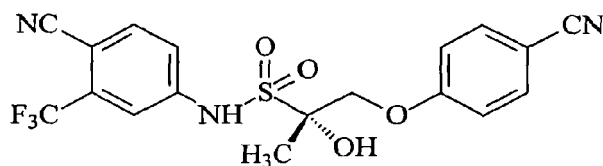
4. 权利要求 3 的化合物, 其由以下结构表示:



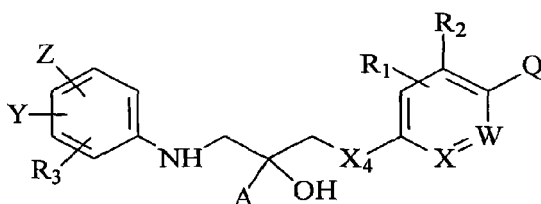
5. 权利要求 2 的化合物, 其中所述化合物由式 V 的结构表示:



6. 权利要求 5 的化合物, 其由以下结构表示:



7. 权利要求 2 的化合物，其中所述化合物由式 VI 的结构表示：



VI

其中

X 是 C 或 N；W 是 C 或 N；

X₄ 是 O、S、SO、C(O)、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键；

A 是 H、OH、SH、NH₂、C₁₋₄ 烷基、CHF₂、CH₂F、CF₃、CN、CH₂OH、CH₂O(C₁₋₄ 烷基)、CH₂O(C₁₋₄ 酰基)、CH₂CN、CH₂N₃、CH₂NCS、CHO、C(O)O(C₁₋₄ 烷基)、CONHR'、C(O)N(R')₂、C(O)O(C₁₋₄ 烷基)、C(O)R'、CH₂CF₃、CF₂CF₃、CH₂OR'CH₂C(O)CH=CH₂ 或 C(O)CH=CH₂；

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br；

Y 是 H、C₁₋₄ 烷基、CF₃、CN、NO₂、F、Cl、I 或 Br；

Q 是 H、CN、OH、NO₂、CF₃、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NHCO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂]；

R 是 H、OH、C₁₋₄ 烷基、CF₃、CH₂OH、O(C₁₋₄ 烷基)或 O(C₁₋₄ 酰基)；

R₁ 是 H、OH、F、Cl、Br 或 I；

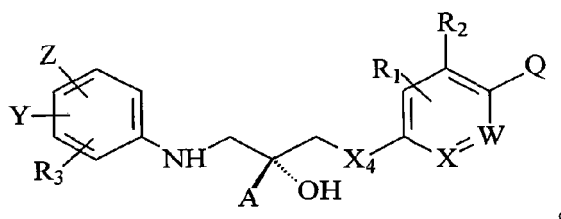
R_2 是 H、由 R_6 取代的苄基、 $(CH_2)_2$ -O-对氰基苯基、 CH_2 -O-对氰基苯基或 $CH=CH$ -O-对氰基苯基；

R_3 是 H、 C_{1-4} 烷基、卤化物、CN 或 NO_2 ；

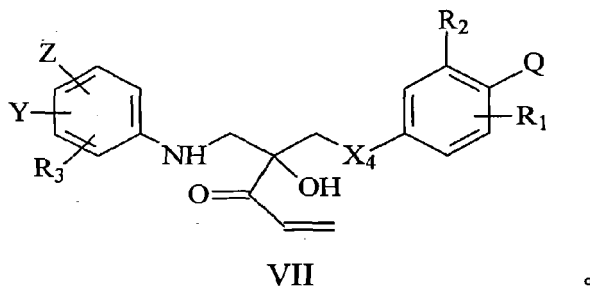
R' 是 NH_2 、OH 或 CH_3 ；并且

R_6 是 CN、 NO_2 、 $NHC(O)CH_3$ 或卤化物。

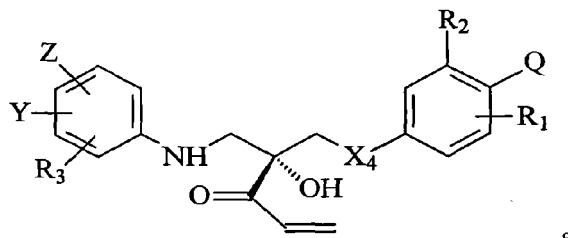
8. 权利要求 7 的化合物，其由以下结构表示：



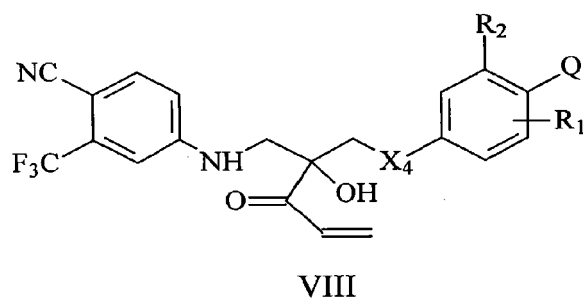
9. 权利要求 7 的化合物，其中所述化合物由式 VII 的结构表示：



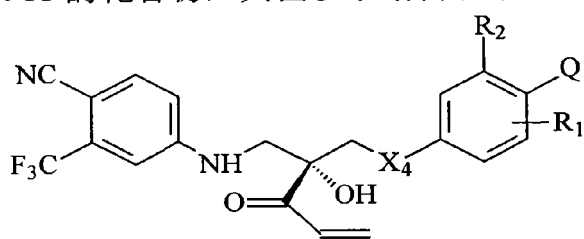
10. 权利要求 9 的化合物，其由以下结构表示：



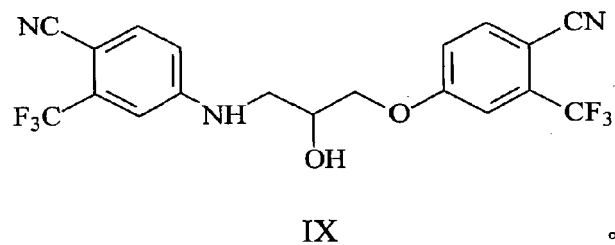
11. 权利要求 9 的化合物，其中所述化合物由式 X 的结构表示：



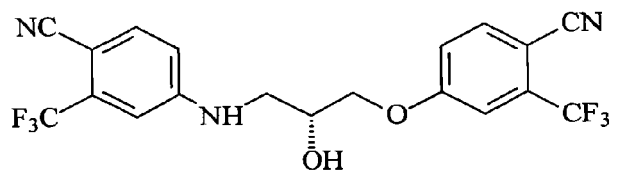
12. 权利要求 11 的化合物，其由以下结构表示：



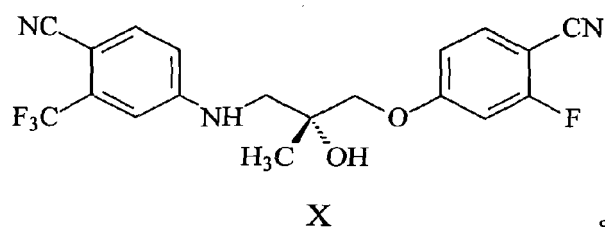
13. 权利要求 7 的化合物，其中所述化合物由式 IX 的结构表示：



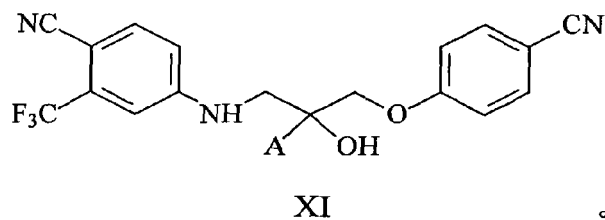
14. 权利要求 13 的化合物，其由以下结构表示：



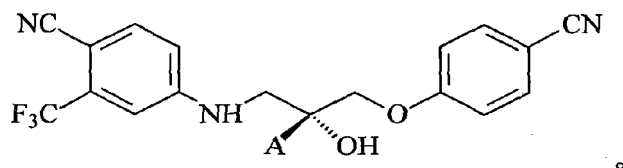
15. 权利要求 7 的化合物，其中所述化合物由式 X 的结构表示：



16. 权利要求 7 的化合物，其中所述化合物由式 X 的结构表示：



17. 权利要求 16 的化合物，其由以下结构表示：



18. 权利要求 15 的化合物，其中 A 是 CH_2OH 。

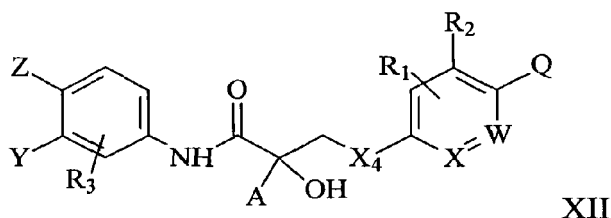
19. 权利要求 17 的化合物，其中 A 是 CH_2OMe 。

20. 权利要求 17 的化合物，其中 A 是 CF_3 。

21. 权利要求 17 的化合物，其中 A 是 CH_3 。

22. 权利要求 17 的化合物，其中 A 是 CN 。

23. 权利要求 1 的化合物，其中所述化合物由式 XII 的结构表示：



其中

X 是 C 或 N; W 是 C 或 N;

X₄ 是 O、S、SO、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键;

A 是 CN、CH₂OH、CH₂O(C₁₋₄ 烷基)、CH₂O(C₁₋₄ 酰基)、CH₂CN、CH₂N₃、CH₂NCS、CHO、C(O)O(C₁₋₄ 烷基)、CONHR'、C(O)N(R')₂、C(O)O(C₁₋₄ 烷基)、C(O)R'、CH₂OR'CH₂C(O)CH=CH₂ 或 C(O)CH=CH₂;

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br;

Y 是 H、C₁₋₄ 烷基、CF₃、CN、NO₂、F、Cl、I 或 Br;

Q 是 H、CN、OH、NO₂、CF₃、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NHCO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂];

R₁ 是 H、OH、F、Cl、Br 或 I;

R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基;

R₃ 是 H、C₁₋₄ 烷基、卤化物、CN 或 NO₂;

R' 是 NH₂、OH 或 CH₃; 并且

R₆ 是 CN、NO₂、NHC(O)CH₃ 或卤化物。

24. 权利要求 23 的化合物, 其中 X₄ 是 O。

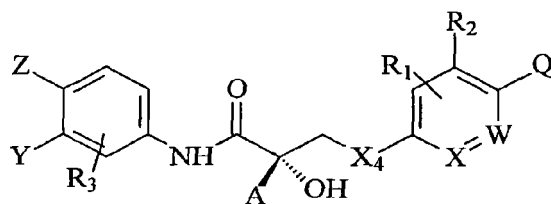
25. 权利要求 24 的化合物, 其中 A 是 CH₂OH。

26. 权利要求 24 的化合物, 其中 A 是 CN。

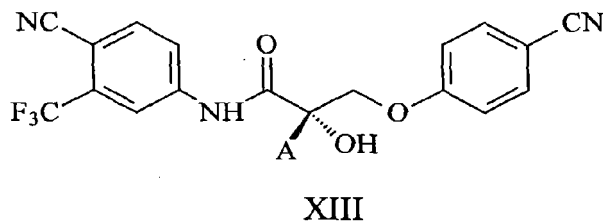
27. 权利要求 23 的化合物, 其中 Q 是 CN, R_1 和 R_2 是 H。

28. 权利要求 23 的化合物, 其中 Q 是 CN, Z 是 CN, Y 是 CF_3 , 并且 R_1 、 R_2 和 R_3 是 H。

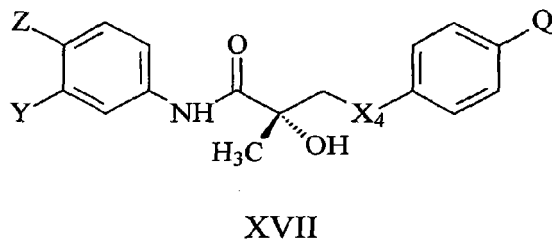
29. 权利要求 23 的化合物, 其由以下结构表示:



30. 权利要求 23 的化合物, 其中所述化合物由式 XIII 的结构表示:



31. 权利要求 1 的化合物, 其中所述化合物由式 XVII 的结构表示:



其中

X_4 是 $S(O)[=CHCH_2N(CH_3)_2]$ 、 $C(OH)[CH_2CH_2N(CH_3)_2]$ 、 $C[=CHCH_2N(CH_3)_2]$ 、 $PO(C_{1-4} \text{ 烷基})$ 、 $N[CH_2CH_2N(CH_3)_2]$ 、

$\text{NO}[\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2]$;

Q 是 H、CN、OH、 NO_2 、卤化物、 C_{2-6} 烯基、 C_{2-6} 炔基、 $\text{C}(\text{O})\text{CH}_2\text{NH}_2$ 、 $\text{C}(\text{O})\text{NH}(\text{C}_{1-4} \text{ 烷基})$ 、 $\text{C}(\text{O})\text{N}(\text{C}_{1-4} \text{ 烷基})_2$ 、 $\text{C}(\text{O})\text{NH}_2$ 、 $\text{NH}(\text{C}_{1-4} \text{ 烷基})$ 、 $\text{NHC}(\text{O})\text{NH}(\text{C}_{1-4} \text{ 烷基})$ 、 $\text{CH}_2\text{C}(\text{O})\text{NH}_2$ 、 $\text{NHC}(\text{O})\text{NH}_2$ 、 $\text{NHCO}(\text{C}_{1-4} \text{ 烷基})$ 、 $\text{NHSO}_2(\text{C}_{1-4} \text{ 烷基})$ 、 $\text{O}[\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2]$ 、 $\text{O}[\text{CH}_2\text{CH}_2(\text{C}_{4-8} \text{ 环烷基})]$ 、 $\text{O}[\text{CH}_2\text{CH}_2(\text{C}_{4-8} \text{ 杂环烷基})]$ 或 $\text{O}[\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2]$;

Z 是 H、CN、 NO_2 、 $\text{NHC}(\text{O})\text{CH}_3$ 、 C_{1-4} 烷基、F、Cl、I 或 Br; 并且

Y 是 H、 C_{1-4} 烷基、 CF_3 、CN、 NO_2 、F、Cl、I 或 Br。

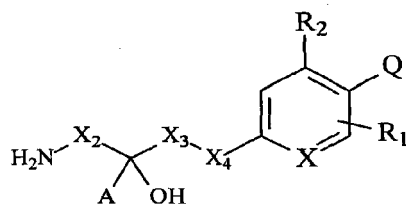
32. 权利要求 31 的化合物, 其中 Q 是 F。

33. 权利要求 31 的化合物, 其中 Q 是 CN。

34. 权利要求 31 的化合物, 其中 Z 是 CN 且 Y 是 CF_3 。

35. 权利要求 31 的化合物, 其中 Q 是 CN, Z 是 CN 且 Y 是 CF_3 。

36. 权利要求 1 的化合物, 其中所述化合物由式 XIX 的结构表示:



XIX

其中

X 是 C 或 N; W 是 C 或 N;

X_2 是 C_{1-4} 亚烷基、 SO_2 、 $\text{C}(\text{O})$ 、 $\text{CH}[\text{CH}_2-(\text{C}_{4-8} \text{ 环})]$ 、 $\text{CH}(\text{C}_{1-4} \text{ 烷基})$ 、 $\text{CH}(\text{NH}_2)$ 、 $\text{CH}(\text{OH})$ 、 $\text{CH}(\text{C}_{1-4} \text{ 卤代烷基})$ 、键;

X_3 是 C_{1-4} 亚烷基、NH、N、 $\text{CH}(\text{OH})$ 或键;

X_4 是 O、S、 SO 、 $\text{C}(\text{O})$ 、 $\text{S}(\text{O})[=\text{CHCH}_2\text{N}(\text{CH}_3)_2]$ 、 SO_2 、NH、 NHR' 、 NO 、 C_{1-4} 亚烷基、 $\text{C}(\text{OH})[\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2]$ 、 $\text{C}[=\text{CHCH}_2\text{N}(\text{CH}_3)_2]$ 、 $\text{PO}(\text{C}_{1-4}$

烷基)、 $N[CH_2CH_2N(CH_3)_2]$ 、 $NO[CH_2CH_2N(CH_3)_2]$ 或键;

A 是 H、OH、SH、 NH_2 、 C_{1-4} 烷基、 CHF_2 、 CH_2F 、 CF_3 、CN、 CH_2OH 、 $CH_2O(C_{1-4}$ 烷基)、 $CH_2O(C_{1-4}$ 酰基)、 CH_2CN 、 CH_2N_3 、 CH_2NCS 、CHO、 $C(O)O(C_{1-4}$ 烷基)、 $CONHR'$ 、 $C(O)N(R')_2$ 、 $C(O)O(C_{1-4}$ 烷基)、 $C(O)R'$ 、 CH_2CF_3 、 CF_2CF_3 、 $CH_2OR'CH_2C(O)CH=CH_2$ 或 $C(O)CH=CH_2$;

Z 是 H、CN、 NO_2 、 $NHC(O)CH_3$ 、 C_{1-4} 烷基、F、Cl、I 或 Br;

Y 是 H、 C_{1-4} 烷基、 CF_3 、 NO_2 、CN、F、Cl、I 或 Br;

Q 是 H、CN、OH、 NO_2 、 CF_3 、卤化物、 C_{2-6} 烯基、 C_{2-6} 炔基、 $C(O)CH_2NH_2$ 、 $C(O)NH(C_{1-4}$ 烷基)、 $C(O)N(C_{1-4}$ 烷基) $_2$ 、 $C(O)NH_2$ 、 $NH(C_{1-4}$ 烷基)、 $NHC(O)NH(C_{1-4}$ 烷基)、 $CH_2C(O)NH_2$ 、 $NHC(O)NH_2$ 、 $NHCO(C_{1-4}$ 烷基)、 $NHSO_2(C_{1-4}$ 烷基)、 $O-[CH_2CH_2CH(CH_3)_2]$ 、 $O-[CH_2CH_2(C_{4-8}$ 环烷基)]、 $O-[CH_2CH_2(C_{4-8}$ 杂环烷基)]或 $O-[CH_2CH_2N(CH_3)_2]$;

R_1 是 H、OH、F、Cl、Br 或 I;

R_2 是 H、由 R_6 取代的苄基、 $(CH_2)_2$ -O-对氰基苯基、 CH_2 -O-对氰基苯基或 $CH=CH$ -O-对氰基苯基;

R' 是 NH_2 、OH 或 CH_3 ; 并且

R_6 是 CN、 NO_2 、 $NHC(O)CH_3$ 或卤化物;

其中

如果 X 是 C, X_1 是 NH, X_2 是 $C(O)$, R 是 OH, A 是烷基, X_3 是键或 CH_2 , 并且 X_4 是键或 CH_2 , R_1 和 R_2 是 H, 那么 Q 不是 H 或卤素。

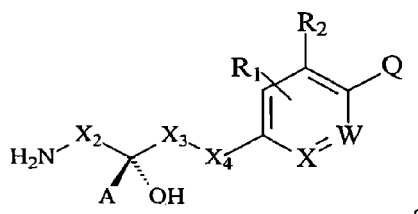
37. 权利要求 36 的化合物, 其中 X_2 是 CH_2 。

38. 权利要求 36 的化合物, 其中 X_2 是 $C(O)$ 。

39. 权利要求 36 的化合物, 其中 X_3 是 CH_2 。

40. 权利要求 36 的化合物, 其中 X_4 是 O。

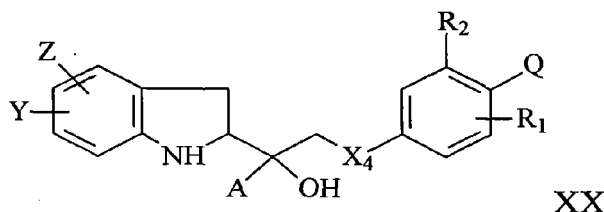
41. 权利要求 36 的化合物, 其由以下结构表示:



42. 权利要求 1 的化合物, 其中 P 是式 II, 并且 X_2 和 X_3 与 X_1 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环。

43. 权利要求 42 的化合物, 其中所述饱和的或不饱和的、取代的或未取代的 5-或 6-元环是吡咯烷、吡咯、吗啉、哌啶-4-酮或哌啶。

44. 权利要求 42 的化合物, 其中所述化合物由式 XX 的结构表示:



其中

X_4 是 O、S、SO、C(O)、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键;

A 是 H、OH、SH、NH₂、C₁₋₄ 烷基、CHF₂、CH₂F、CF₃、CN、CH₂OH、CH₂O(C₁₋₄ 烷基)、CH₂O(C₁₋₄ 酰基)、CH₂CN、CH₂N₃、CH₂NCS、CHO、C(O)O(C₁₋₄ 烷基)、CONHR'、C(O)N(R')₂、C(O)O(C₁₋₄ 烷基)、C(O)R'、CH₂CF₃、CF₂CF₃、CH₂OR'CH₂C(O)CH=CH₂ 或 C(O)CH=CH₂;

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br;

Y 是 H、C₁₋₄ 烷基、CF₃、NO₂、CN、F、Cl、I 或 Br;

Q 是 H、CN、OH、CF₃、NO₂、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、

NHSO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂];

R₁ 是 H、OH、F、Cl、Br 或 I;

R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基;

R' 是 NH₂、OH 或 CH₃; 并且

R₆ 是 CN、NO₂、NHC(O)CH₃ 或卤化物。

45. 权利要求 44 的化合物, 其中 A 是 CH₃。

46. 权利要求 44 的化合物, 其中 X₄ 是 O。

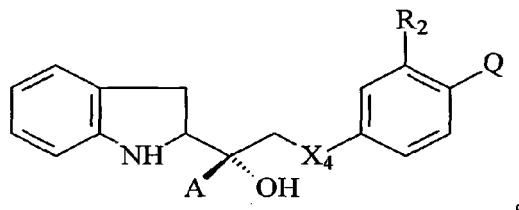
47. 权利要求 44 的化合物, 其中 Q 是 CN。

48. 权利要求 44 的化合物, 其中 Z 是 CN 且 Y 是 CF₃。

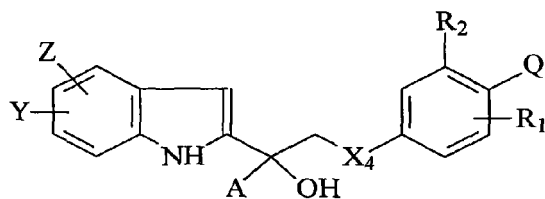
49. 权利要求 44 的化合物, 其中 Z 是 CN, Q 是 CN 且 Y 是 CF₃。

50. 权利要求 44 的化合物, 其中 A 是 CH₃, X₄ 是 O, Z 是 CN, Q 是 CN, R₁ 和 R₂ 是 H, 并且 Y 是 CF₃。

51. 权利要求 44 的化合物, 其由以下结构表示:



52. 权利要求 42 的化合物, 其中所述化合物由式 XXI 的结构表示:



XXI

53. 权利要求 52 的化合物, 其中 A 是 CH₃。

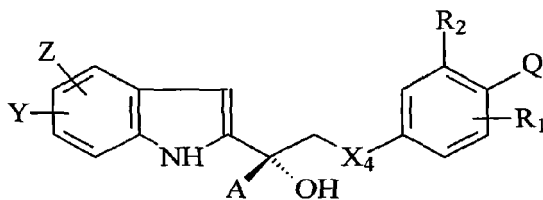
54. 权利要求 52 的化合物, 其中 X₄ 是 O。

55. 权利要求 52 的化合物, 其中 Q 是 CN。

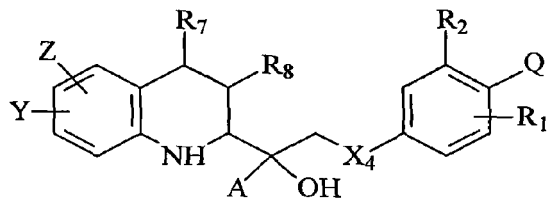
56. 权利要求 52 的化合物, 其中 Z 是 CN 且 Y 是 CF₃。

57. 权利要求 52 的化合物, 其中 A 是 CH₃, X₄ 是 O, Z 是 CN, Q 是 CN, R₁ 和 R₂ 是 H, 并且 Y 是 CF₃。

58. 权利要求 52 的化合物, 其由以下结构表示:



59. 权利要求 42 的化合物, 其中所述化合物由式 XXII 的结构表示:



XXII

其中 A、Z、Y、X₄、Q、R₁ 和 R₂ 如式 XX 中所述, R₇ 是 H 或氧代,

并且 R_8 是 H 或 $=CH_2$ 。

60. 权利要求 58 的化合物, 其中 A 是 CH_3 。

61. 权利要求 58 的化合物, 其中 X_4 是 O。

62. 权利要求 58 的化合物, 其中 R_7 和 R_8 是 H。

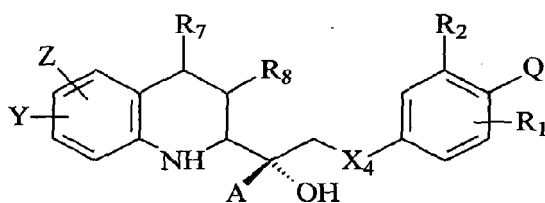
63. 权利要求 58 的化合物, 其中 R_7 是 $=CH_2$ 且 R_8 是氧代。

64. 权利要求 58 的化合物, 其中 Q 是 CN。

65. 权利要求 58 的化合物, 其中 Z 是 CN 且 Y 是 CF_3 。

66. 权利要求 58 的化合物, 其中 A 是 CH_3 , X_4 是 O, Z 是 CN, Q 是 CN, R_1 和 R_2 是 H, 并且 Y 是 CF_3 。

67. 权利要求 58 的化合物, 其由以下结构表示:

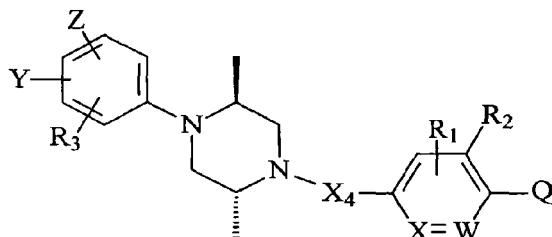


68. 权利要求 1 的化合物, 其中 P 是式 II, 并且 X_1 和 X_3 与 X_1 和 X_3 所连接的 X_2 和 X_5 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环。

69. 权利要求 68 的化合物, 其中所述饱和的或不饱和的、取代的或未取代的 5-或 6-元环是哌嗪、哌嗪-2,6-二酮、哌啶、吡咯烷、环己酮、环己烷、环己烯、环戊酮、环戊烷、环戊烯、琥珀酰亚胺、噁唑烷、噁唑烷

二酮或噁唑烷酮。

70. 权利要求 69 的化合物, 其中所述化合物由式 XXIII 的结构表示:



XXIII

X 是 C 或 N; W 是 C 或 N;

X₄ 是 O、S、SO、C(O)、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键;

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br;

Y 是 H、C₁₋₄ 烷基、CF₃、NO₂、CN、F、Cl、I 或 Br;

Q 是 H、CN、OH、CF₃、NO₂、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NHCO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂];

R₁ 是 H、OH、F、Cl、Br 或 I;

R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基;

R₃ 是 H、C₁₋₄ 烷基、卤化物、CN 或 NO₂;

R' 是 NH₂、OH 或 CH₃; 并且

R₆ 是 CN、NO₂、NHC(O)CH₃ 或卤化物。

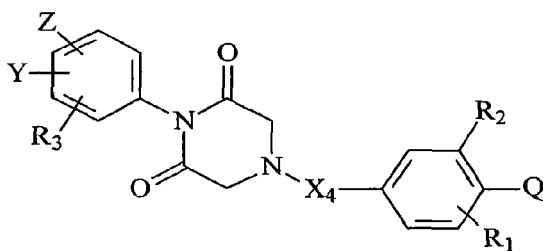
71. 权利要求 70 的化合物, 其中 X₄ 是 O。

72. 权利要求 70 的化合物, 其中 Q 是 CN。

73. 权利要求 70 的化合物, 其中 Z 是 CN 且 Y 是 CF₃。

74. 权利要求 70 的化合物, 其中 X₄ 是 O, Z 是 CN, Q 是 CN, R₁、R₂ 和 R₃ 是 H 且 Y 是 CF₃。

75. 权利要求 68 的化合物, 其中所述化合物由式 XXXIII 的结构表示:



XXXIII

76. 权利要求 75 的化合物, 其中 X₄ 是 O。

77. 权利要求 75 的化合物, 其中 Q 是 CN。

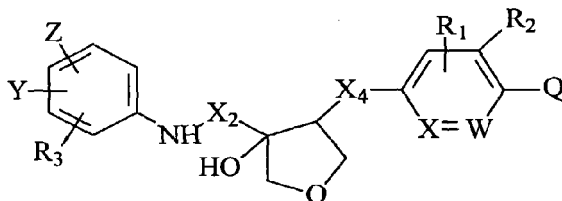
78. 权利要求 75 的化合物, 其中 Z 是 CN 且 Y 是 CF₃。

79. 权利要求 75 的化合物, 其中 X₄ 是 O, Z 是 CN, Q 是 CN, R₁、R₂ 和 R₃ 是 H 且 Y 是 CF₃。

80. 权利要求 1 的化合物, 其中 P 是式 II, X₃ 和 X₅ 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环。

81. 权利要求 80 的化合物, 其中所述饱和的或不饱和的、取代的或未取代的 5-或 6-元环是四氢呋喃、吡咯烷、哌啶、环己酮、环己烷、环己烯、环己烯酮、环戊酮、环戊烯酮、环戊烷、环戊烯、琥珀酰亚胺、噁唑烷、噁唑烷二酮、噁唑烷酮。

82. 权利要求 80 的化合物, 其中所述化合物由式 XXXIV 的结构表示:



XXXIV

X 是 C 或 N; W 是 C 或 N;

X₂ 是 C₁₋₄ 亚烷基、SO₂、C(O)、CH-[CH₂-(C₄₋₈ 环)]、CH(C₁₋₄ 烷基)、CH(NH₂)、CH(OH)、CH(C₁₋₄ 卤代烷基)、键;

X₄ 是 O、S、SO、C(O)、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键;

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br;

Y 是 H、C₁₋₄ 烷基、CF₃、NO₂、CN、F、Cl、I 或 Br;

Q 是 H、CN、OH、NO₂、CF₃、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NHSO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂];

R₁ 是 H、OH、F、Cl、Br 或 I;

R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基;

R₃ 是 H、C₁₋₄ 烷基、卤化物、CN 或 NO₂;

R' 是 NH₂、OH 或 CH₃; 并且

R₆ 是 CN、NO₂、NHC(O)CH₃ 或卤化物。

83. 权利要求 82 的化合物, 其中 X₄ 是 O。

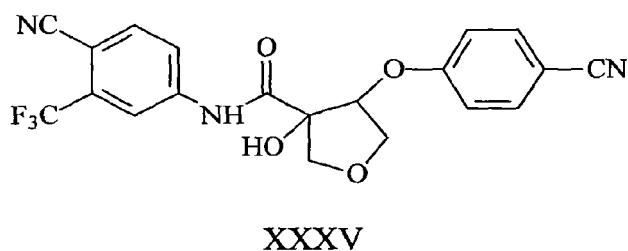
84. 权利要求 82 的化合物, 其中 Q 是 CN。

85. 权利要求 92 的化合物, 其中 X_2 是 CH_2 。

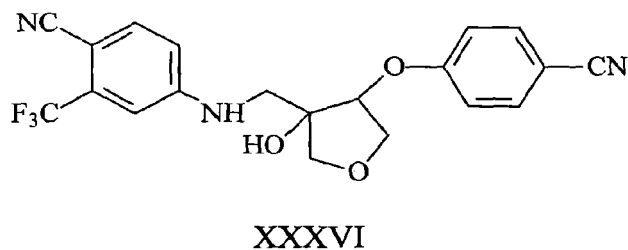
86. 权利要求 82 的化合物, 其中 X_2 是 $C(O)$ 。

87. 权利要求 82 的化合物, 其中 Z 是 CN 且 Y 是 CF_3 。

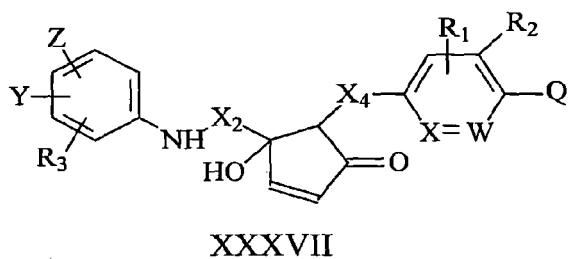
88. 权利要求 82 的化合物, 其中所述化合物由式 XXXV 的结构表示:



89. 权利要求 82 的化合物, 其中所述化合物由式 XXXVI 的结构表示:



90. 权利要求 80 的化合物, 其中所述化合物由式 XXXVII 的结构表示:



91. 权利要求 90 的化合物, 其中 X_2 是 CH_2 。

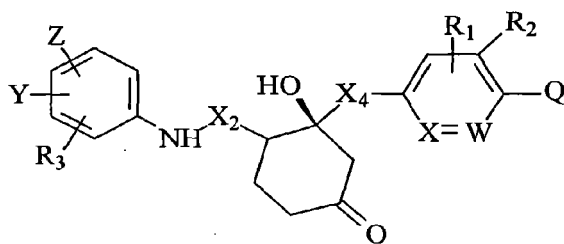
92. 权利要求 90 的化合物, 其中 X_2 是 $C(O)$ 。

93. 权利要求 90 的化合物, 其中 X_4 是 O 。

94. 权利要求 90 的化合物, 其中 Q 是 CN 。

95. 权利要求 90 的化合物, 其中 Z 是 CN 且 Y 是 CF_3 。

96. 权利要求 80 的化合物, 其中所述化合物由式 XXXVIII 的结构表示:



XXXVIII

97. 权利要求 96 的化合物, 其中 X_2 是 CH_2 。

98. 权利要求 96 的化合物, 其中 X_2 是 $C(O)$ 。

99. 权利要求 96 的化合物, 其中 X_4 是 O 。

100. 权利要求 96 的化合物, 其中 Q 是 CN 。

101. 权利要求 96 的化合物, 其中 Z 是 CN 且 Y 是 CF_3 。

102. 权利要求 1 的化合物, 其中 P 是式 II, 并且 X_1 和 X_5 与 X_1 和 X_5 所连接的 X_2 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环;

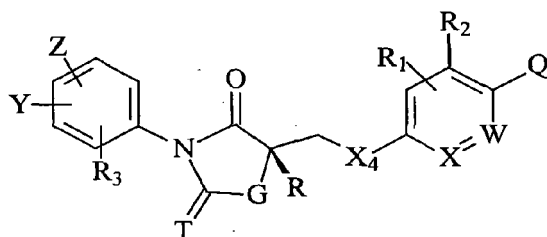
其中

如果 P 是式 II, X 是 C, X_3 是 CH_2 , X_4 是 O 或 NH, R_1 、 R_2 和 R_3 是 H, 并且 X_5 和 X_1 形成 5 元环噁唑烷-2-酮, 那么 Q 不是卤素或 H; 或者

如果 P 是式 II, R_3 是 H, X 和 W 是 C, X_1 和 X_5 与 X_1 和 X_5 所连接的 X_2 一起形成噁唑烷二酮, R 是烷基, X_3 是 CH_2 , 并且 X_4 是 O、NH、S、 SO_2 或 CH_2 , 那么 Q 不是卤化物。

103. 权利要求 102 的化合物, 其中所述饱和的或不饱和的、取代的或未取代的 5-或 6-元环是吡咯烷、哌啶、吗啉、琥珀酰亚胺、噁唑烷、噁唑烷二酮、噁唑烷酮。

104. 权利要求 102 的化合物, 其中所述化合物由式 XXXIX 的结构表示:



XXXIX

其中:

X 是 C 或 N; W 是 C 或 N;

G 是 O、NH、 NC_{1-4} 烷基、 NC_{1-4} 酰基、S、 CHC_{1-4} 烷基、 CHC_{1-4} 酰基、 $C(C_{1-4} \text{ 酰基})_2$ 、 $C(C_{1-4} \text{ 烷基})_2$ 或 $(CH_2)_n$, 其中 n 是 1-3;

T 是 S 或 O;

X_4 是 O、S、 SO 、 $C(O)$ 、 $S(O)[=CHCH_2N(CH_3)_2]$ 、 SO_2 、NH、 NHR' 、 NO 、 C_{1-4} 亚烷基、 $C(OH)[CH_2CH_2N(CH_3)_2]$ 、 $C[=CHCH_2N(CH_3)_2]$ 、 $PO(C_{1-4} \text{ 烷基})$ 、 $N[CH_2CH_2N(CH_3)_2]$ 、 $NO[CH_2CH_2N(CH_3)_2]$ 或键;

Z 是 H、CN、 NO_2 、 $NHC(O)CH_3$ 、 C_{1-4} 烷基、F、Cl、I 或 Br;

Y 是 H、 C_{1-4} 烷基、 CF_3 、 NO_2 、CN、F、Cl、I 或 Br;

Q 是 H、CN、OH、 CF_3 、 NO_2 、卤化物、 C_{2-6} 烯基、 C_{2-6} 炔基、 $C(O)CH_2NH_2$ 、

C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NHSO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂];

R 是 H、OH、C₁₋₄ 烷基、CF₃、CH₂OH、O(C₁₋₄ 烷基)或 O(C₁₋₄ 酰基);

R₁ 是 H、OH、F、Cl、Br 或 I;

R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基;

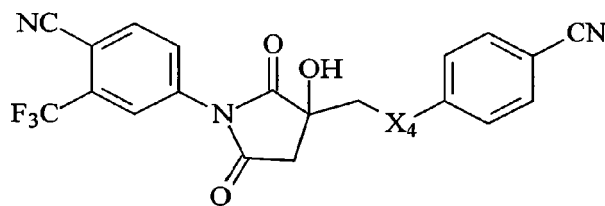
R₃ 是 H、C₁₋₄ 烷基、卤化物、CN 或 NO₂;

R' 是 NH₂、OH 或 CH₃; 并且

R₆ 是 CN、NO₂、NHC(O)CH₃ 或卤化物;

其中如果 R₃ 是 H, X 和 W 是 C, G 是 O, T 是 O, R 是烷基, X₃ 是 CH₂, 并且 X₄ 是 O、NH、S、SO₂ 或 CH₂, 那么 Q 不是卤化物。

105. 权利要求 104 的化合物, 其中所述化合物由式 XLV 的结构表示:

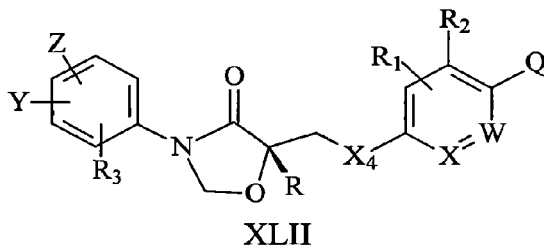


XLV

其中 X₄ 如以上关于化合物 XXXIV 所述。

106. 权利要求 105 的化合物, 其中 X₄ 是 O。

107. 权利要求 102 的化合物, 其中所述化合物由式 XLII 的结构表示:



XLII

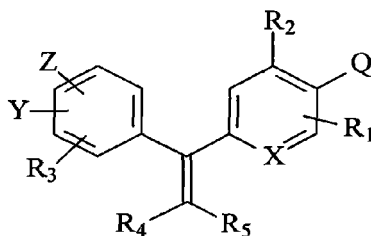
其中:

X、X₄、R、R₁、R₂、R₃、Z、Y 和 Q 如以上关于化合物 XXXIV 所述;

其中

如果 X 是 C, X₄ 是 O、NH 或 S, R₁、R₂ 和 R₃ 是 H 并且 R 是 H, 那么 Q 不是卤素、H 或 CN。

108. 权利要求 1 的化合物, 其中所述化合物由式 XLVII 的结构表示:



XLVII

其中

X 是 N 或 CH;

Z 是 H、CN、NO₂、NHC(O)CH₃、CH₃、F、Cl、I 或 Br;

Y 是 H、CH₃、CF₃、CN、F、Cl、I 或 Br;

Q 是 H、O-(CH₂)₂CH(CH₃)₂ 或 O-(CH₂)₂N(CH₃)₂;

R₁ 是 H、OH、F、Cl、Br 或 I;

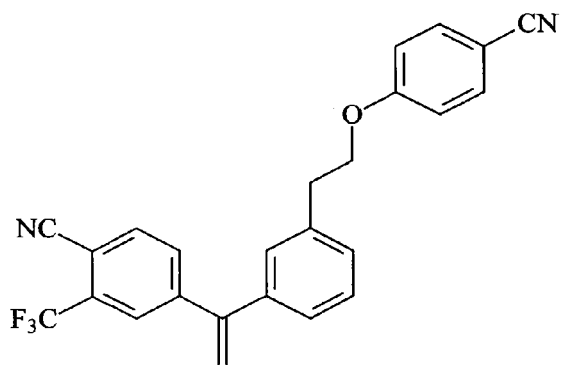
R₂ 是由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基;

R₃ 是 H、CH₃、卤化物、CN 或 NO₂;

R₄ 和 R₅ 独立地是 H、CH₃、卤化物、OH、C₁₋₄ 烷基、C₁₋₆ 环烷基、卤代-C₁₋₄ 烷基、苯基、芳基、杂环或羟基-C₁₋₄ 烷基; 并且

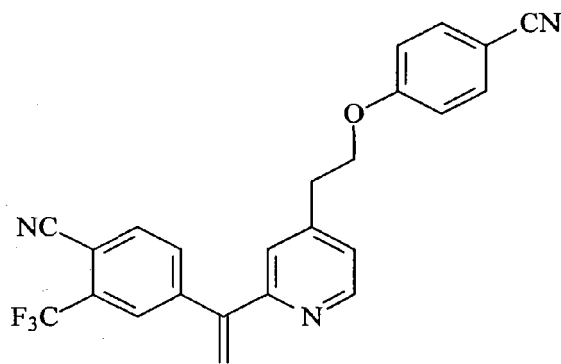
R₆ 是 CN、NO₂、NHC(O)CH₃ 或卤化物。

109. 权利要求 108 的化合物, 其中所述化合物由式 XLVIII 的结构表示:



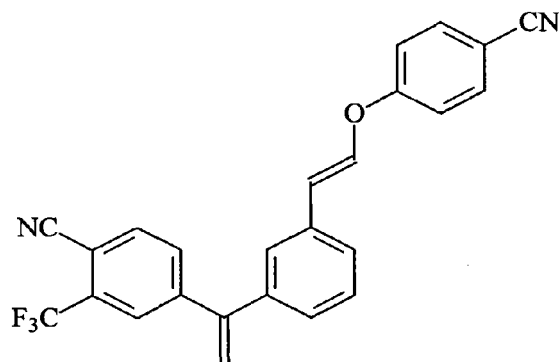
XLVIII

110. 权利要求 108 的化合物,其中所述化合物由式 XLIX 的结构表示:



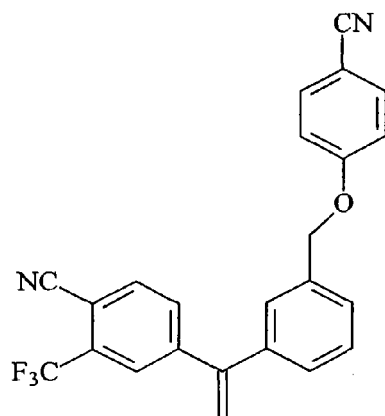
XLIX

111. 权利要求 108 的化合物,其中所述化合物由式 L 的结构表示:



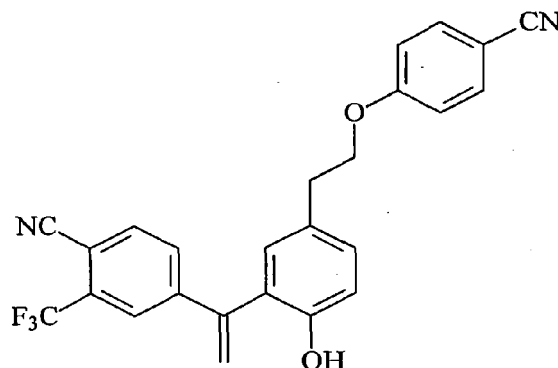
L

112. 权利要求 108 的化合物, 其中所述化合物由式 LI 的结构表示:



LI

113. 权利要求 108 的化合物, 其中所述化合物由式 LII 的结构表示:



LII

114. 药物组合物, 其包含权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、N-氧化物、水合物或它们的任意组合; 以及合适的载体或稀释剂。

115. 药物组合物, 其包含有效量的权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、N-氧化物、水合物或它们的任意组合; 以及药学上可接受的载体、稀释剂或盐。

116. 在雄性个体中避孕的方法,所述方法包括向所述个体给药有效抑制所述个体中精子产生的量的权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤,由此实现在所述个体中的避孕。

117. 激素治疗方法,所述方法包括使个体的雄激素受体与有效实现改变雄激素依赖性病症的量的权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合接触的步骤。

118. 治疗罹患前列腺癌的个体的方法,所述方法包括向所述个体给药有效治疗所述个体中的前列腺癌的量的权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

119. 延缓罹患前列腺癌的个体的前列腺癌的进展的方法,所述方法包括向所述个体给药有效延缓所述个体中前列腺癌的进展的量的权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

120. 治疗个体的骨相关的病症、或增加个体的骨量、促进个体的骨形成的方法,所述方法包括向所述个体给药有效治疗所述骨相关的病症的量的权利要求 1 的化合物或其异构体、药学上可接受的盐、药品、晶体、水合物、*N*-氧化物或它们的任意组合的步骤。

121. 权利要求 120 的方法,其中所述个体罹患骨质疏松症、骨质减少、骨吸收增加、骨折、骨脆弱、骨矿物质密度(BMD)丢失或它们的任意组合。

122. 权利要求 120 的方法,其中所述方法增加所述个体的骨强度。

123. 权利要求 120 的方法,其中所述化合物促进或增强成骨细胞生成。

124. 权利要求 120 的方法,其中所述化合物抑制破骨细胞增殖。

125. 治疗个体的肌萎缩症、降低其发病率、延缓其进展、减轻其严重性或减轻与其相关的症状的方法,所述方法包括向所述个体给药有效治疗所述个体的所述肌萎缩症的量的权利要求 1 的化合物或其异构体、药学上可接受的盐、药品、晶体、水合物、*N*-氧化物或它们的任意组合的步骤。

126. 权利要求 125 的方法,其中所述肌萎缩症归因于病状、疾患、疾病或病症。

127. 权利要求 126 的方法,其中所述病状、疾患、疾病或病症是神经性的、感染性的、慢性的或遗传性的。

128. 权利要求 126 的方法,其中所述病状、疾患、疾病或病症是肌营养不良症、肌萎缩、伴 X 染色体的脊延髓肌萎缩症(SBMA)、恶病质、营养不良、麻风病、糖尿病、肾脏病、慢性阻塞性肺疾病(COPD)、癌症、终末期肾功能衰竭、少肌症、肺气肿、骨软化症、HIV 感染、AIDS 或心脏病。

129. 权利要求 126 的方法,其中所述肌萎缩症是年龄相关的肌萎缩症,废用性失调相关的肌萎缩症;或者所述肌萎缩症归因于慢性下腰痛、烧伤、中枢神经系统(CNS)损伤或损害、周围神经损伤或损害、脊髓损伤或损害、化学性损伤或损害、或者酒精中毒。

130. 治疗人类个体的糖尿病、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法,所述方法包括向所述个体给药权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

131. 治疗患有的人类个体的葡萄糖耐受不良、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法,所述方法包括向所述个体给药权利要求1的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

132. 治疗人类个体的高胰岛素血症、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法,所述方法包括向所述个体给药权利要求1的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

133. 治疗人类个体的胰岛素抵抗、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法,所述方法包括向所述个体给药权利要求1的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

134. 治疗与糖尿病相关的疾病、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法,所述方法包括向所述个体给药权利要求1的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

135. 治疗人类个体的脂肪肝症、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法,所述方法包括向所述个体给药权利要求1的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

136. 治疗人类个体的心血管疾病、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法,所述方法包括向所述个体给药权利要求1的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

137. 治疗个体的恶病质、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法，所述方法包括向所述个体给药权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

138. 权利要求 137 的方法，其中所述恶病质与所述个体的癌症相关。

139. 治疗个体的类风湿性关节炎、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法，所述方法包括向所述个体给药权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

140. 治疗个体的慢性肾病、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法，所述方法包括向所述个体给药权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

141. 治疗个体的终末期肾病、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法，所述方法包括向所述个体给药权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

142. 治疗个体的脆弱、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法，所述方法包括向所述个体给药权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

143. 治疗个体的性腺功能减退症、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法，所述方法包括向所述个体给药权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水

合物或它们的任意组合的步骤。

144. 治疗个体的年龄相关的功能减退、减轻其严重性、降低其发病率、延缓其发作或减少其发病的方法,所述方法包括向所述个体给药权利要求 1 的化合物和/或其异构体、药学上可接受的盐、药品、晶体、*N*-氧化物、水合物或它们的任意组合的步骤。

选择性雄激素受体调节剂、它们的类似物和衍生物以及它们的用途

交叉引用相关申请

本申请要求2006年7月19日提交的美国临时专利申请60/831880和2006年7月19日提交的美国临时专利申请60/831988的利益；这两件申请以其全部通过引用结合于此。

发明领域

本发明提供细胞核激素受体结合化合物、包含它们的组合物以及它们在治疗个体的各种疾病或病症中的使用方法，所述疾病或病症尤其包括前列腺癌、肌萎缩症和与此相关的疾病或病症、和/或骨相关的疾病或病症。

发明背景

细胞核激素受体超家族是最大的一类转录因子，涉及广泛的生理过程。该家族的48个成员分为三类，第1类包括雄激素（AR）、雌激素（ER- α 和ER- β ）、糖皮质激素（GR）、孕酮（PR）和盐皮质激素（MR）的受体。第2类包括类视黄醇、甲状腺和维生素D的受体，而第3类包括其配体有待鉴定的受体（孤儿（orphans））。细胞核激素受体具有功能少有表征的N末端域（NTD）、负责受体与DNA应答元件结合的DNA结合域（DBD）、含有核定位信号的铰链区和结合配体并活化或抑制受体功能的配体结合域（LBD）。而且，存在两个活化功能域，一个位于NTD（AF-1）中，另一个位于LBD（AF-2）中。第I类受体，由于DBD高度的氨基酸序列同源性、LBD的中等同源性以及相似的二级和三级结构特征、和类固醇配体共同的化学特征，经常能与其它第I类受体的配体结合（即交叉反应）。例如，早期对AR和ER的研究表明，类固醇由类固醇A-环与螺旋-3残基接触并且D-环与螺旋-11残基接触而在LBD中的定位，可能对所有类固醇激素受体都是普遍的。

雄激素受体（“AR”）是配体激活的转录调节蛋白，它通过其对内源性雄

激素的活性介导雄性性发育和性功能的诱导。雄激素通常被称为雄性性激素。雄激素是在体内由睾丸和肾上腺皮质产生的类固醇，或者可以在实验室中合成。雄激素类固醇在许多生理过程中起重要作用，包括雄性性征例如肌肉和骨量、前列腺生长、精子发生和雄性毛发模式的产生和维持(Matsumoto, *Endocrinol. Met. Clin. N. Am.* 23:857-75 (1994))。内源性类固醇雄激素包括睾酮和双氢睾酮(“DHT”)。睾酮是睾丸分泌的主要类固醇，而且是雄性血浆中发现的主要循环雄激素。在许多外周组织中，睾酮由 5 α -还原酶转化为 DHT。因此，DHT 被认为是大多数雄激素作用的细胞内介质(Zhou 等人, *Molec. Endocrinol.* 9:208-18 (1995))。其它类固醇雄激素包括睾酮的酯，例如环戊烷丙酸酯(cypionate)、丙酸酯、苯基丙酸酯(phenylpropionate)、环戊基丙酸酯(cyclopentylpropionate)、异己酸酯(isocarporate)、庚酸酯和癸酸酯，以及其它合成雄激素，例如 7-甲基-去甲睾酮(“MENT”)及其醋酸酯(Sundaram 等人, "7 Alpha-Methyl-Nortestosterone(MENT): The Optimal Androgen For Male Contraception," *Ann. Med.*, 25:199-205 (1993) ("Sundaram"))。因为 AR 涉及雄性性发育和性功能，所以 AR 可能是实现雄性避孕或其它形式的激素替代疗法的靶标。

人类孕酮受体(PR)存在三种不同的亚型：PR-A、PR-B 和 PR-C(Kastner 等人, *EMBO J* 9:1603-1614, 1990; Wei 等人, *Mol Endo* 10:1379-1387, 1996)，其中，PR-A 和 PR-B 是最丰富的。然而，PR-A 亚型对 PR-B 亚型的比率在靶组织中不恒定，这会改变细胞应答，因为各亚型的活性可以变化。

很少有化合物在很多种条件下都显示出部分孕激素活性。RU-486，最普遍使用的抗孕激素，仅在选定的条件下显示出部分激动剂活性。具有部分激动剂活性的抗孕激素化合物对治疗各种孕激素调节的疾病和病症有用，然而，这些很少的已知抗孕激素仅具有有限的部分激动剂活性，本领域仍需要具有广泛的部分激动剂活性的抗孕激素。

世界人口增长和计划生育的社会意识已经促进了大量的避孕研究。避孕无论如何都是一个困难的课题。它伴有文化和社会的烙印，牵涉宗教，最肯定地，它伴有重要的健康关切。当该课题的焦点集中在雄性避孕时，情况只会恶化。尽管存在合适的避孕器械，但是在历史上，社会期望妇女作出避孕的决定并承担后果。虽然对性传播疾病的忧虑使男人更加认识到需要培养安

全和负责的性习惯，但是妇女仍然经常承担避孕选择的主要压力。妇女们有一些选择，从临时的机械装置（例如海绵和子宫帽）到临时的化学方法（例如杀精子药）。妇女们还有一些可支配的更持久的选择，例如物理装置包括宫内节育器和宫颈帽，以及更持久的化学处理，例如避孕丸和皮下埋植剂。然而，至今，男人们仅有的选择包括使用避孕套和输精管切除术。然而，许多男人不喜欢使用避孕套，因为这会减弱性敏感性，干扰性自发，并很有可能因破裂或使用不当而导致怀孕。输精管切除术也不受青睐。如果有更方便的控制生育的方法供男人使用，特别是在性行为之前无需准备活动的长期方法，那么这些方法就能显著地增加男人承担更多的避孕责任的可能性。

雄性类固醇（例如睾酮及其衍生物）的给药在这方面显得特别有潜力，因为这些化合物具有组合的抑制促性腺激素和代替雄激素的性质(Steinberger 等人, "Effect of Chronic Administration of Testosterone Enanthate on Sperm Production and Plasma Testosterone, Follicle Stimulating Hormone, and Luteinizing Hormone Levels: A Preliminary Evaluation of a Possible Male Contraceptive", *Fertility and Sterility* 28:1320- 28 (1977))。长期给药大剂量的睾酮完全停止精子的产生（无精子）或将精子减少至很低的水平（精子减少）。造成不育所需的抑制精子产生的程度还不清楚。然而，世界卫生组织最近的一篇报道显示，每周肌肉注射庚酸睾酮造成 98%的接受治疗的男性无精子或严重的精子减少（即每毫升小于三百万个精子）和不育(World Health Organization Task Force on Methods And Regulation of Male Fertility, "Contraceptive Efficacy of Testosterone-Induced Azoospermia and Oligospermia in Normal Men," *Fertility and Sterility* 65:821-29 (1996))。

已经开发出多种睾酮酯，它们在肌肉注射后被吸收得更慢，由此产生更大的雄激素效应。庚酸睾酮是这些酯类中使用最广泛的。尽管庚酸睾酮在确定激素药剂用于雄性避孕的可行性方面有很大价值，但是它具有几个缺点，包括需要每周注射和在肌肉内注射后立即出现睾酮的超生理性最高水平(Wu, "Effects of Testosterone Enanthate in Normal Men: Experience From a Multicenter Contraceptive Efficacy Study," *Fertility and Sterility* 65:626-36 (1996))。

雄性和雌性的骨矿物质密度（BMD）都随着年龄而降低。骨矿物质含量

(BMC) 和 BMD 降低的量与骨强度的减小相关, 并容易使患者骨折。

骨质疏松症是全身性骨骼疾病, 其特征是骨量少、骨组织退化并导致骨脆弱 (bone fragility) 和骨折敏感性增加。在美国, 该病症影响超过 0.25 亿人口, 每年造成超过 130 万例骨折, 包括每年 50 万例脊柱骨折、25 万例髋骨折、24 万例腕骨骨折。髋骨折是骨质疏松症最严重的后果, 其中 5-20% 的患者在一年内死亡, 超过 50% 的存活者残疾。老人罹患骨质疏松症的风险最大, 因此, 随着人口老龄化, 预计该问题会显著增加。在接下来的 60 年内, 全世界的骨折发生率预计将增加三倍, 而一项研究估计, 2050 年世界上将有 450 万例髋骨折。

妇女罹患骨质疏松症风险比男性更大。在绝经期后的五年中, 妇女骨丢失急剧加速。增加风险的其它因素包括吸烟、酒精滥用、久坐的生活习惯和低的钙摄入。然而, 骨质疏松症也经常发生于男性身上。十分确定, 男性的骨矿物质密度随着年龄而减少。骨矿物质含量和密度的降低与骨强度的减小相关并容易造成骨折。性激素在非生殖组织中的多效性作用的潜在的分子机制仅仅刚开始为人们所认识, 但是, 清楚的是, 生理浓度的雄激素和雌激素在维持整个生命周期中的骨稳态上起重要作用。因此, 出现雄激素和雌激素剥夺时, 结果是骨重建速度增加, 这使吸收和成形的平衡倾向吸收, 这导致全身性的骨量丢失。在男性, 成熟期性激素的自然减少 (雄激素的直接减少以及由雄激素的外周芳构化产生的雌激素的低水平) 与骨脆弱相关。在去势的男性中也观察到了这种效应。

肌萎缩是指肌肉量的进行性损失和/或肌肉的进行性衰弱和退化, 包括控制运动的骨骼肌或随意肌、控制心脏 (心肌病) 的心肌和平滑肌。慢性肌萎缩是以肌肉量的进行性损失、肌肉衰弱和退化为特征的慢性病症。

肌萎缩过程中发生的肌肉量损失可以通过分解代谢造成肌肉蛋白的降解来表征。发生蛋白质分解代谢的原因是异常地高速度的蛋白质降解、异常地低速度的蛋白质合成或两者的组合。无论是高程度的蛋白质降解还是低程度的蛋白质合成引起的肌肉蛋白分解代谢都会导致肌肉量的减少和肌萎缩。

肌萎缩与慢性的、神经性的、遗传性的或传染性的病状、疾病、疾患或病症相关。它们包括肌营养不良, 例如迪谢内肌营养不良和肌强直性营养不良; 肌萎缩, 例如小儿麻痹症后肌萎缩 (PPMA); 恶病质, 例如心源性恶

病质、艾滋病恶病质和癌症恶病质、营养不良、麻风病、糖尿病、肾脏病、慢性阻塞性肺疾病（COPD）、癌症、终末期肾功能衰竭、少肌症、肺气肿、骨软化症、HIV 感染、AIDS 和心肌病。

此外，其它的情况和病症也与肌萎缩有关并能导致肌萎缩。它们包括慢性下腰痛、高龄（advanced age）、中枢神经系统（CNS）损伤、周围神经损伤、脊髓损伤、化学性损伤、中枢神经系统（CNS）损害、周围神经损害、脊髓损害、化学性损害、烧伤、在四肢固定和因疾病或损伤而长期住院时发生的废用性失调（disuse deconditioning）和酒精中毒。

完整的雄激素受体（AR）信号转导途径对于骨骼肌的适当发育十分关键。而且，完整的 AR 信号转导途径增加瘦肌肉量、肌肉强度和肌肉蛋白的合成。

肌萎缩如果不减弱，会造成可怕的健康后果。例如，在肌萎缩过程中发生的变化会导致身体状况变弱，这对个体的健康有害，导致骨折易感性增强和体能状况虚弱。而且，肌萎缩是患恶病质和 AIDS 的病人发病率和死亡率的强烈预兆。

在基础科学和临床水平都急需新的创新方法以开发用于以下的化合物：

a) 男性避孕；b) 治疗各种激素相关的病症，例如与衰老男性雄激素减退（ADAM）相关的病症，例如疲劳、抑郁、性欲降低、性功能障碍、勃起功能障碍、性腺功能减退症、骨质疏松症、脱发、贫血、肥胖症、少肌症、骨质减少、骨质疏松症、良性前列腺增生、情绪和认知改变以及前列腺癌；c) 治疗与 ADIF 相关的病症，例如性功能障碍、性欲降低、性腺功能减退症、少肌症、骨质减少、骨质疏松症、认知和情绪改变、抑郁、贫血、脱发、肥胖症、子宫内膜异位症、乳腺癌、子宫癌和卵巢癌；d) 治疗和/或预防慢性肌萎缩或少肌症；e) 减少前列腺癌的发病率、中止前列腺癌或引起前列腺癌消退；f) 口服雄激素替代和/或其它临床治疗和/或诊断领域。

很多种疾病和/或病症都受到性腺功能减退症和分解代谢作用的影响，包括肾病、中枢神经系统损伤、烧伤和慢性伤口。

在美国，肾功能衰竭的发病率上升且十分普遍。登记终末期肾脏疾病（ESRD）医疗保险基金项目的患者数量从 1973 年的约 1 万受益人增加至 1983 年的 86354 人，至 2002 年 12 月 3 日达到 431284 人。单是 2002 年，就有 100359 名患者参加美国 ESRD 项目。慢性肾病（CKD）是 ESRD 的先兆，

它在肾脏不能完全从体内排除废物时发生。CKD 是一种缓慢地进行性疾病，其中，糖尿病、高血压和贫血可能是并发的病症。

CKD 使用表示可用肾功能的分期系统来诊断（第 1 期=正常肾功能），而且患者在早期通常不会出现症状。CKD 的第 5 期是 ESRD，它是完全或接近完全的肾衰竭，而且通常发生在肾功能低于基线的 10%时。

与 ESRD 相关的伴随症状包括性腺功能减退症、非意愿体重减轻、疲劳等等。

烧伤导致睾酮减少、氮水平下降和骨矿物质密度（BDM）减少，这些在损伤后甚至可能要持续一年之久，并且与伤口愈合不良、感染风险增大、瘦体重减少、复原受阻和延缓烧伤幸存者重返社会有关。因烧伤而引起的分解代谢作用造成显著的非意愿体重减轻，并进一步使问题复杂化。

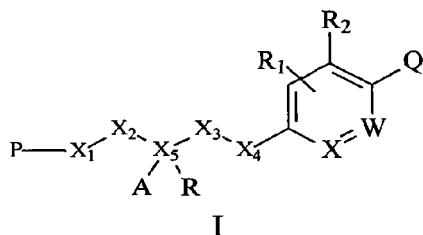
脊髓损伤（SCI）可能会导致改变中枢神经递质的分泌和产生，这继而可能造成下丘脑-垂体-肾上腺轴功能障碍，导致睾酮和其它激素水平的下降。SCI 或其它急性疾病或创伤在特征上包括分解代谢增强伴随合成代谢活动减弱，引发易于损失瘦身体组织的病症。只要分解代谢过程不中断，那么紊乱的营养利用将继续下去。损失瘦体重的影响包括伤口扩展和愈合机制受损。由于营养不良和蛋白缺乏加上固定，脊髓损伤的患者处于高度的患褥疮的风险中。

慢性伤口可以由任意种病症引起，包括糖尿病、循环问题、固定等等。使疾病复杂化（例如在糖尿病中）的是出现神经病，其增加足溃疡的风险。

虽然有许多这些病症的处理和治疗，但是都不理想。由于已显示雄激素受体（AR）信号转导途径增加瘦肌肉量、肌肉强度和肌肉蛋白合成，并且由于性腺功能减退症伴随这些病症，所以靶向 AR 信号转导途径的分子可以用于治疗这些疾病和/或病症。

发明内容

在一个实施方案中，本发明提供由式 I 的结构表示的化合物：

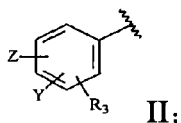


其中

X 是 C 或 N; W 是 C 或 N;

X₁ 是 N、NH、N(C₁₋₄ 烷基)、NAc、NCOOH 或键; 或者 X₁ 和 X₃ 与 X₁ 和 X₃ 所连接的 X₂ 和 X₅ 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环; 或者 X₁ 和 X₅ 与 X₁ 和 X₅ 所连接的 X₂ 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环, 且 A 不存在;

P 是 H 或式 II:



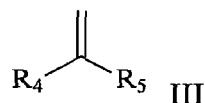
X₂ 是 C₁₋₄ 亚烷基、SO₂、C(O)、CH-[CH₂-(C₄₋₈ 环)]、CH(C₁₋₄ 烷基)、CH(NH₂)、CH(OH)、CH(C₁₋₄ 卤代烷基)、键; 或者 X₂ 和 R₃ 与 X₂ 所连接的 X₁ 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环; 或者 X₂ 和 X₅ 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环, 且 A 不存在;

X₃ 是 C₁₋₄ 亚烷基、NH、N、CH(OH)、键; 或者 X₁ 和 X₃ 与 X₁ 和 X₃ 所连接的 X₂ 和 X₅ 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环; 或者 X₃ 和 X₅ 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环, 且 A 不存在; 或者 X₃ 和 X₄ 一起形成双键或一起形成饱和的或不饱和的、取代的或未取代的 3-6 元环;

X₄ 是 O、S、SO、C(O)、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键; 或者 X₄ 和 X₅ 与 X₄ 和 X₅ 所连接的 X₃ 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环, 且 A 不存在; 或者 X₃ 和 X₄ 一起形成双键或形成饱和的或不饱和的、取代的或未取代的 3-6 元环;

X_5 是碳或 X_5 和 X_1 与 X_1 和 X_5 所连接的 X_2 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环, 且 A 不存在; 或者 X_5 和 X_2 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环, 且 A 不存在; 或者 X_5 和 X_3 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环, 且 A 不存在; 或 X_5 和 X_4 与 X_5 和 X_4 所连接的 X_3 一起形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环, 且 A 不存在;

A 不存在, 或是 H、OH、SH、NH₂、C₁₋₄ 烷基、CHF₂、CH₂F、CF₃、CN、CH₂OH、CH₂O(C₁₋₄ 烷基)、CH₂O(C₁₋₄ 酰基)、CH₂CN、CH₂N₃、CH₂NCS、CHO、C(O)O(C₁₋₄ 烷基)、CONHR'、C(O)N(R')₂、C(O)O(C₁₋₄ 烷基)、C(O)R'、CH₂CF₃、CF₂CF₃、CH₂OR' CH₂C(O)CH=CH₂ 或 C(O)CH=CH₂; 或者 A 和 R 形成式 III 所表示的双键:



Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br;

Y 是 H、C₁₋₄ 烷基、CH₃、NO₂、CN、F、Cl、I 或 Br;

Q 是 H、CN、OH、NO₂、卤化物、CF₃、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NHSO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂];

R 是 H、OH、C₁₋₄ 烷基、CF₃、CH₂OH、O(C₁₋₄ 烷基)或 O(C₁₋₄ 酰基);

R₁ 是 H、OH、NH₂、F、Cl、Br 或 I;

R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基;

R₃ 是 H、C₁₋₄ 烷基、卤化物、CN、NO₂; 或 R₃ 和 X₂ 以及与 R₃ 相连的苯环和与 X₂ 相连的 X₁ 形成饱和的或不饱和的、取代的或未取代的 5-或 6-元环;

R' 是 NH₂、OH 或 CH₃;

R₄ 和 R₅ 独立地是 H、CH₃、卤化物、OH、C₁₋₄ 烷基、C₁₋₆ 环烷基、卤代(C₁₋₄ 烷基)、苯基、芳基、C₄₋₈ 杂环烷基或羟基(C₁₋₄ 烷基);

R_6 是 CN、 NO_2 、 $NHC(O)CH_3$ 或卤化物；

其中

如果 P 是式 II，X 是 C， X_1 是 NH， X_2 是 C(O)， X_3 是 CH_2 ，R 是 OH 并且 A 是烷基或卤代烷基，那么 X_4 是 $S(O)[=CHCH_2N(CH_3)_2]$ 、 $C(OH)[CH_2CH_2N(CH_3)_2]$ 、 $C[=CHCH_2N(CH_3)_2]$ 、 $N[CH_2CH_2N(CH_3)_2]$ 、 $P(O)(C_{1-4} \text{ 烷基})$ 或 $N(O)[CH_2CH_2N(CH_3)_2]$ ；

或者

如果 P 是式 II，X 是 C， X_1 是 NH， X_2 是 C(O)， X_3 是 CH_2 ，R 是 OH 或 OAlk 并且 X_4 是 O、S、NH、S、SO、 SO_2 或亚烷基，那么 A 是 CN、 CH_2OH 、 $CH_2O(C_{1-4} \text{ 烷基})$ 、 $CH_2O(C_{1-4} \text{ 酰基})$ 、 CH_2CN 、 CH_2N_3 、 CH_2NCS 、CHO、 $C(O)O(C_{1-4} \text{ 烷基})$ 、CONHR'、 $C(O)N(R')_2$ 、 $C(O)O(C_{1-4} \text{ 烷基})$ 、 $C(O)R'$ 、 $CH_2OR'CH_2C(O)CH=CH_2$ 或 $C(O)CH=CH_2$ ；

或者

如果 P 是苯基，X 是 C， X_1 是 NH， X_2 是 C(O)，R 是 OH 并且 A 是 COOH， X_3 是键， X_4 是 CH_2 ， R_1 、 R_2 是 H，那么 Q 不是 H。

或者

如果 P 是式 II，X 是 C， X_3 是 CH_2 ， X_4 是 O 或 NH， R_1 、 R_2 和 R_3 是 H，并且 X_5 和 X_1 形成 5 元环噁唑烷-2-酮，那么 Q 不是卤素或 H；

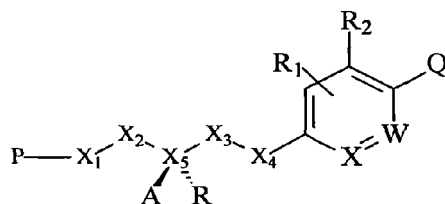
或者

如果 P 是 H，X 是 C， X_1 是 NH， X_2 是 C(O)，R 是 OH，A 是烷基， X_3 是键或 CH_2 ，并且 X_4 是键或 CH_2 ， R_1 和 R_2 是 H，那么 Q 不是 H 或卤素

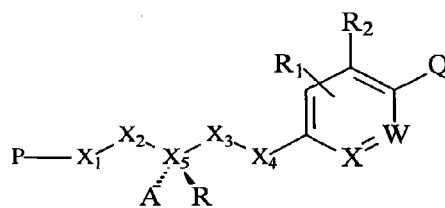
或者

如果 P 是式 II， R_3 是 H，X 和 W 是 C， X_1 和 X_5 与 X_1 和 X_5 所连接的 X_2 一起形成噁唑烷二酮，R 是烷基， X_3 是 CH_2 ，并且 X_4 是 O、NH、S、 SO_2 或 CH_2 ，那么 Q 不是卤化物。

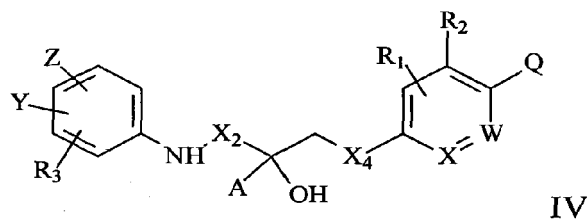
在另一个实施方案中，式 I 的化合物由以下结构表示：



在另一个实施方案中，式 I 的化合物由以下结构表示：



在一个实施方案中，本发明提供由式 IV 的结构表示的化合物：



其中

X 是 C 或 N；W 是 C 或 N；

X₂ 是 C₁₋₄ 亚烷基、SO₂、CH-[CH₂-(C₄₋₈ 环)]、CH(C₁₋₄ 烷基)、CH(NH₂)、CH(OH)、CH(C₁₋₄ 卤代烷基)、键；

X₄ 是 O、S、SO、C(O)、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键；

A 是 H、OH、SH、NH₂、C₁₋₄ 烷基、CHF₂、CH₂F、CF₃、CN、CH₂OH、CH₂O(C₁₋₄ 烷基)、CH₂O(C₁₋₄ 酰基)、CH₂CN、CH₂N₃、CH₂NCS、CHO、C(O)O(C₁₋₄ 烷基)、CONHR'、C(O)N(R')₂、C(O)O(C₁₋₄ 烷基)、C(O)R'、CH₂CF₃、CF₂CF₃、CH₂OR'CH₂C(O)CH=CH₂ 或 C(O)CH=CH₂；

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br；

Y 是 H、C₁₋₄ 烷基、CH₃、CN、NO₂、F、Cl、I 或 Br；

Q 是 H、CN、OH、NO₂、CF₃、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、

$C(O)NH(C_{1-4} \text{ 烷基})$ 、 $C(O)N(C_{1-4} \text{ 烷基})_2$ 、 $C(O)NH_2$ 、 $NH(C_{1-4} \text{ 烷基})$ 、 $NHC(O)NH(C_{1-4} \text{ 烷基})$ 、 $CH_2C(O)NH_2$ 、 $NHC(O)NH_2$ 、 $NHCO(C_{1-4} \text{ 烷基})$ 、 $NHSO_2(C_{1-4} \text{ 烷基})$ 、 $O-[CH_2CH_2CH(CH_3)_2]$ 、 $O-[CH_2CH_2(C_{4-8} \text{ 环烷基})]$ 、 $O-[CH_2CH_2(C_{4-8} \text{ 杂环烷基})]$ 或 $O-[CH_2CH_2N(CH_3)_2]$;

R 是 H、OH、 C_{1-4} 烷基、 CF_3 、 CH_2OH 、 $O(C_{1-4} \text{ 烷基})$ 或 $O(C_{1-4} \text{ 酰基})$;

R_1 是 H、OH、 NH_2 、F、Cl、Br 或 I;

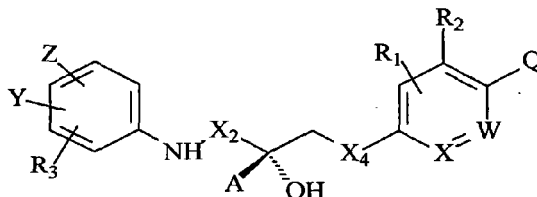
R_2 是 H、由 R_6 取代的苄基、 $(CH_2)_2$ -O-对氰基苯基、 CH_2 -O-对氰基苯基或 $CH=CH$ -O-对氰基苯基;

R_3 是 H、 C_{1-4} 烷基、卤化物、CN 或 NO_2 ;

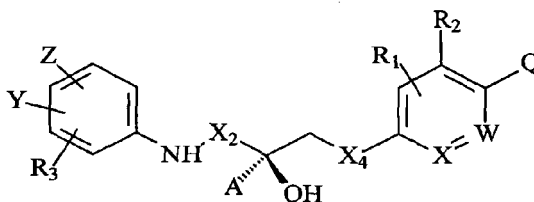
R' 是 NH_2 、OH 或 CH_3 ; 并且

R_6 是 CN、 NO_2 、 $NHC(O)CH_3$ 或卤化物。

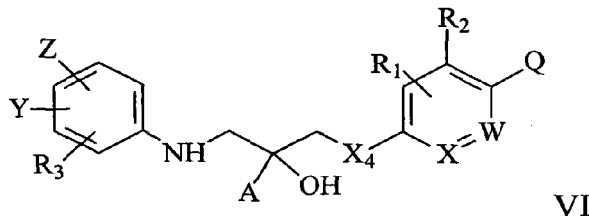
在另一个实施方案中, 式 IV 的化合物由以下结构表示:



在另一个实施方案中, 式 IV 的化合物由以下结构表示:



在一个实施方案中, 本发明提供由式 VI 的结构表示的化合物:



其中

X 是 C 或 N; W 是 C 或 N;

X_4 是 O、S、SO、C(O)、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键；

A 是 H、OH、SH、NH₂、C₁₋₄ 烷基、CHF₂、CH₂F、CF₃、CN、CH₂OH、CH₂O(C₁₋₄ 烷基)、CH₂O(C₁₋₄ 酰基)、CH₂CN、CH₂N₃、CH₂NCS、CHO、C(O)O(C₁₋₄ 烷基)、CONHR'、C(O)N(R')₂、C(O)O(C₁₋₄ 烷基)、C(O)R'、CH₂CF₃、CF₂CF₃、CH₂OR'CH₂C(O)CH=CH₂ 或 C(O)CH=CH₂；

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br；

Y 是 H、C₁₋₄ 烷基、CH₃、CN、NO₂、F、Cl、I 或 Br；

Q 是 H、CN、OH、NO₂、CF₃、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NHCO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂]；

R 是 H、OH、C₁₋₄ 烷基、CF₃、CH₂OH、O(C₁₋₄ 烷基)或 O(C₁₋₄ 酰基)；

R₁ 是 H、OH、F、Cl、Br 或 I；

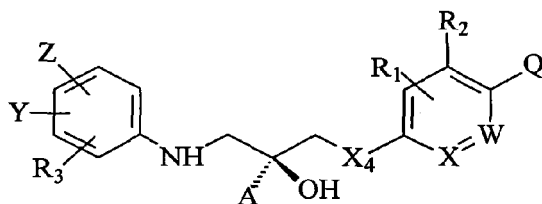
R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基；

R₃ 是 H、C₁₋₄ 烷基、卤化物、CN 或 NO₂；

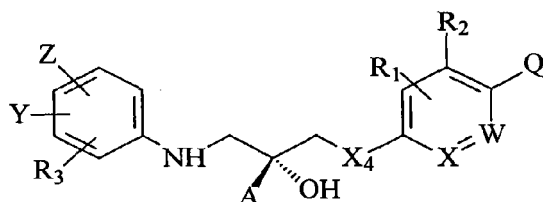
R' 是 NH₂、OH 或 CH₃；并且

R₆ 是 CN、NO₂、NHC(O)CH₃ 或卤化物。

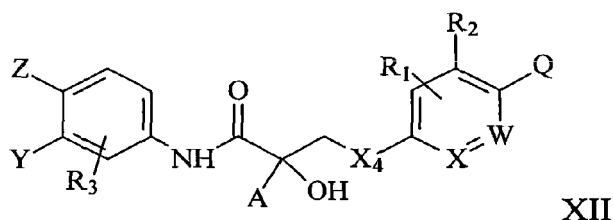
在另一个实施方案中，式 VI 的化合物由以下结构表示：



在一个实施方案中，式 VI 的化合物由以下结构表示：



在一个实施方案中，本发明提供由式 XII 的结构表示的化合物：



其中

X 是 C 或 N；W 是 C 或 N；

X₄ 是 O、S、SO、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、C₁₋₄ 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C₁₋₄ 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键；

A 是 CN、CH₂OH、CH₂O(C₁₋₄ 烷基)、CH₂O(C₁₋₄ 酰基)、CH₂CN、CH₂N₃、CH₂NCS、CHO、C(O)O(C₁₋₄ 烷基)、CONHR'、C(O)N(R')₂、C(O)O(C₁₋₄ 烷基)、C(O)R'、CH₂OR'CH₂C(O)CH=CH₂ 或 C(O)CH=CH₂；

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br；

Y 是 H、C₁₋₄ 烷基、CF₃、CN、NO₂、F、Cl、I 或 Br；

Q 是 H、CN、OH、NO₂、CF₃、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NH₂SO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂]；

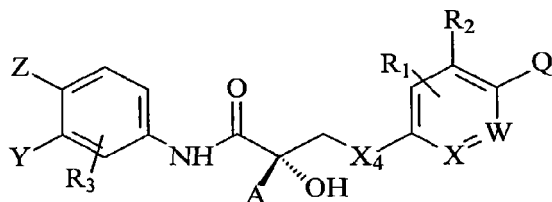
R₁ 是 H、OH、F、Cl、Br 或 I；

R₂ 是 H、由 R₆ 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基；

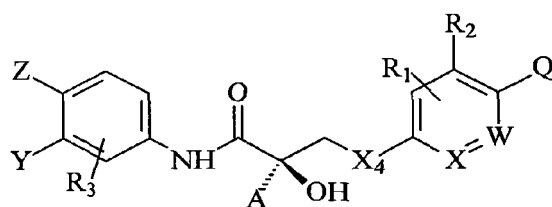
R₃ 是 H、C₁₋₄ 烷基、卤化物、CN 或 NO₂；

R' 是 NH₂、OH 或 CH₃；并且

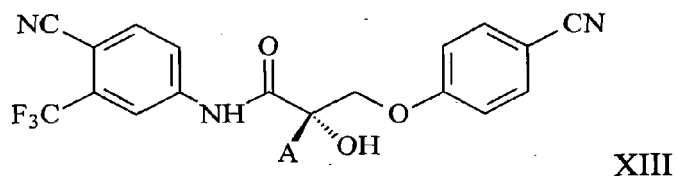
R_6 是 CN 、 NO_2 、 $NHC(O)CH_3$ 或卤化物。在另一个实施方案中，式 XII 的化合物由以下结构表示：



在另一个实施方案中，式 XII 的化合物由以下结构表示：

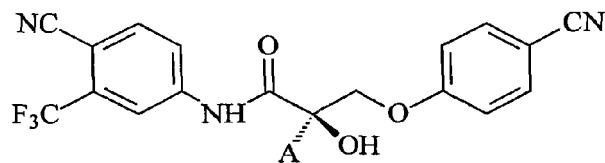


在一个实施方案中，本发明提供由式 XIII 的结构表示的化合物：

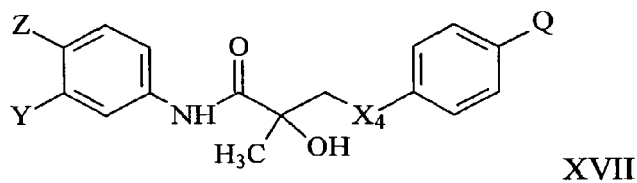


其中 A 如以上在化合物 XII 中所述。

在一个实施方案中，本发明提供由式 XIII 的结构表示的化合物：



在一个实施方案中，本发明提供由式 XVII 的结构表示的化合物：



其中

X_4 是 $S(O)[=CHCH_2N(CH_3)_2]$ 、 $C(OH)[CH_2CH_2N(CH_3)_2]$ 、

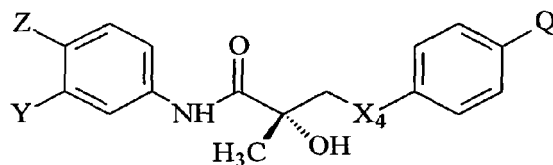
$C[=CHCH_2N(CH_3)_2]$ 、 $PO(C_{1-4} \text{ 烷基})$ 、 $N[CH_2CH_2N(CH_3)_2]$ 、 $NO[CH_2CH_2N(CH_3)_2]$;

Q 是 H、CN、OH、NO₂、CF₃、卤化物、C₂₋₆ 烯基、C₂₋₆ 炔基、C(O)CH₂NH₂、C(O)NH(C₁₋₄ 烷基)、C(O)N(C₁₋₄ 烷基)₂、C(O)NH₂、NH(C₁₋₄ 烷基)、NHC(O)NH(C₁₋₄ 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C₁₋₄ 烷基)、NHSO₂(C₁₋₄ 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C₄₋₈ 环烷基)]、O-[CH₂CH₂(C₄₋₈ 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂]CHF₂;

Z 是 H、CN、NO₂、NHC(O)CH₃、C₁₋₄ 烷基、F、Cl、I 或 Br; 并且

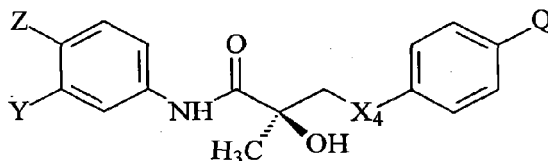
Y 是 H、C₁₋₄ 烷基、CF₃、CN、NO₂、F、Cl、I 或 Br。

在另一个实施方案中, 式 XVII 的化合物由以下结构表示:

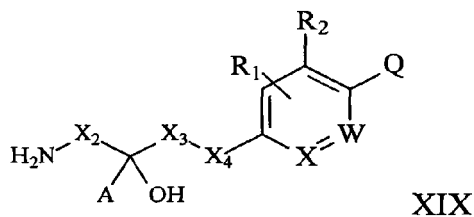


XVII

在另一个实施方案中, 式 XVII 的化合物由以下结构表示:



在一个实施方案中, 本发明提供由式 XIX 的结构表示的化合物:



XIX

其中

X 是 C 或 N; W 是 C 或 N;

X₂ 是 C₁₋₄ 亚烷基、SO₂、C(O)、CH-[CH₂-(C₄₋₈ 环)]、CH(C₁₋₄ 烷基)、CH(NH₂)、CH(OH)、CH(C₁₋₄ 卤代烷基)、键;

X_3 是 C_{1-4} 亚烷基、NH、N、CH(OH)或键；

X_4 是 O、S、SO、C(O)、S(O)[=CHCH₂N(CH₃)₂]、SO₂、NH、NHR'、NO、 C_{1-4} 亚烷基、C(OH)[CH₂CH₂N(CH₃)₂]、C[=CHCH₂N(CH₃)₂]、PO(C_{1-4} 烷基)、N[CH₂CH₂N(CH₃)₂]、NO[CH₂CH₂N(CH₃)₂]或键；

A 是 H、OH、SH、NH₂、 C_{1-4} 烷基、CHF₂、CH₂F、CF₃、CN、CH₂OH、CH₂O(C_{1-4} 烷基)、CH₂O(C_{1-4} 酰基)、CH₂CN、CH₂N₃、CH₂NCS、CHO、C(O)O(C_{1-4} 烷基)、CONHR'、C(O)N(R')₂、C(O)O(C_{1-4} 烷基)、C(O)R'、CH₂CF₃、CF₂CF₃、CH₂OR'CH₂C(O)CH=CH₂ 或 C(O)CH=CH₂；

Z 是 H、CN、NO₂、NHC(O)CH₃、 C_{1-4} 烷基、F、Cl、I 或 Br；

Y 是 H、 C_{1-4} 烷基、CF₃、NO₂、CN、F、Cl、I 或 Br；

Q 是 H、CN、OH、NO₂、CF₃、卤化物、 C_{2-6} 烯基、 C_{2-6} 炔基、C(O)CH₂NH₂、C(O)NH(C_{1-4} 烷基)、C(O)N(C_{1-4} 烷基)₂、C(O)NH₂、NH(C_{1-4} 烷基)、NHC(O)NH(C_{1-4} 烷基)、CH₂C(O)NH₂、NHC(O)NH₂、NHCO(C_{1-4} 烷基)、NH₂SO₂(C_{1-4} 烷基)、O-[CH₂CH₂CH(CH₃)₂]、O-[CH₂CH₂(C_{4-8} 环烷基)]、O-[CH₂CH₂(C_{4-8} 杂环烷基)]或 O-[CH₂CH₂N(CH₃)₂]；

R_1 是 H、OH、F、Cl、Br 或 I；

R_2 是 H、由 R_6 取代的苄基、(CH₂)₂-O-对氰基苯基、CH₂-O-对氰基苯基或 CH=CH-O-对氰基苯基；

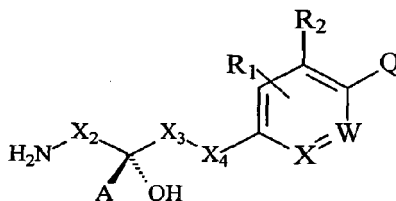
R' 是 NH₂、OH 或 CH₃；并且

R_6 是 CN、NO₂、NHC(O)CH₃ 或卤化物；

其中

如果 X 是 C， X_1 是 NH， X_2 是 C(O)，R 是 OH，A 是烷基， X_3 是键或 CH₂，并且 X_4 是键或 CH₂， R_1 和 R_2 是 H，那么 Q 不是 H 或卤素。

在另一个实施方案中，式 XIX 的化合物由以下结构表示：



在另一个实施方案中，式 XIX 的化合物由以下结构表示：