



US 20110195436A1

(19) **United States**(12) **Patent Application Publication**
Moreau et al.(10) **Pub. No.: US 2011/0195436 A1**(43) **Pub. Date: Aug. 11, 2011**(54) **METHODS FOR THE CLASSIFICATION AND
DIAGNOSIS OF SCOLIOSIS THROUGH THE
USE OF G_i PROTEIN RECEPTOR****Publication Classification**(51) **Int. Cl.**
G01N 33/567 (2006.01)(52) **U.S. Cl.** **435/7.24; 435/7.21**(76) **Inventors:** **Alain Moreau**, Montreal (CA);
Marie-Yvonne Akoume Ndong,
Montreal (CA)(21) **Appl. No.:** **13/123,146**(22) **PCT Filed:** **Oct. 13, 2009**(86) **PCT No.:** **PCT/CA2009/001453**

§ 371 (c)(1),

(2), (4) **Date:** **Apr. 7, 2011****Related U.S. Application Data**(60) Provisional application No. 61/104,442, filed on Oct.
10, 2008, provisional application No. 61/228,769,
filed on Jul. 27, 2009.(57) **ABSTRACT**

Methods for diagnosing a predisposition to developing a scoliosis (e.g., adolescent idiopathic scoliosis (AIS)) and identifying compounds for treating scoliosis based on the modulation of G_i protein-coupled receptor activity are described. Specific embodiments of the methods involve measuring a change in impedance signals of cells expressing a receptor coupled to a G_i protein with a ligand. To identify compounds useful in treatment, the cell is contacted with a test compound and a ligand. A higher impedance in the presence relative to the absence of said test compound is indicative that the test compound is useful for treating scoliosis.

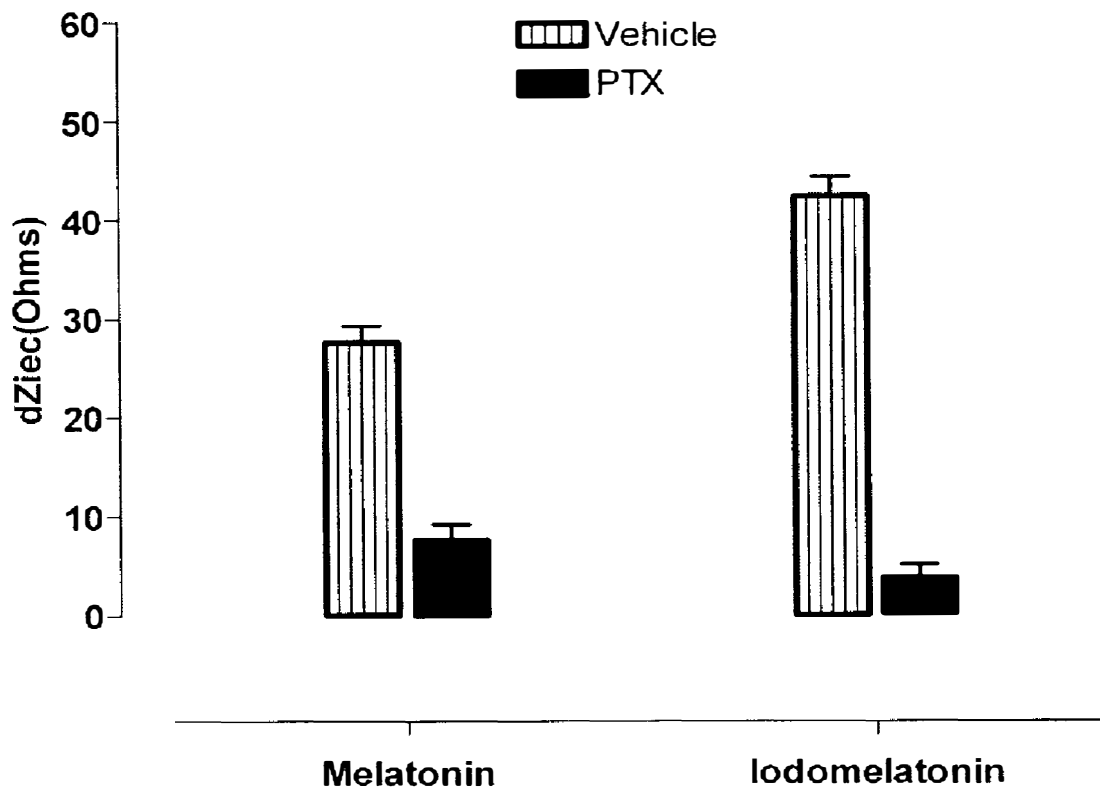
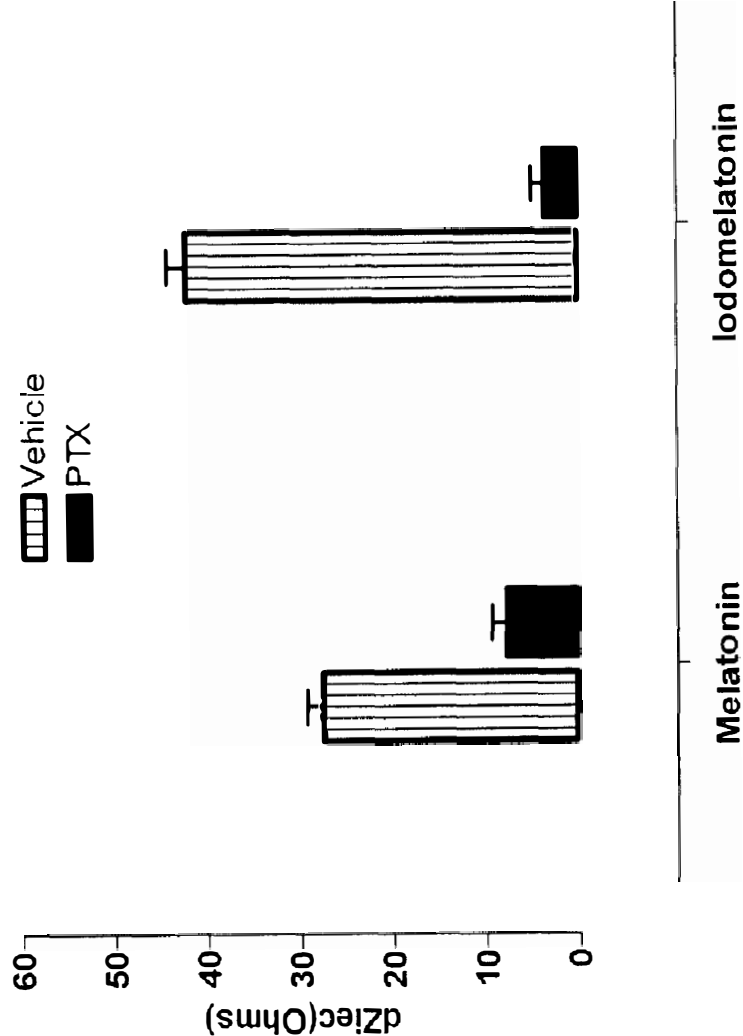


Fig. 1



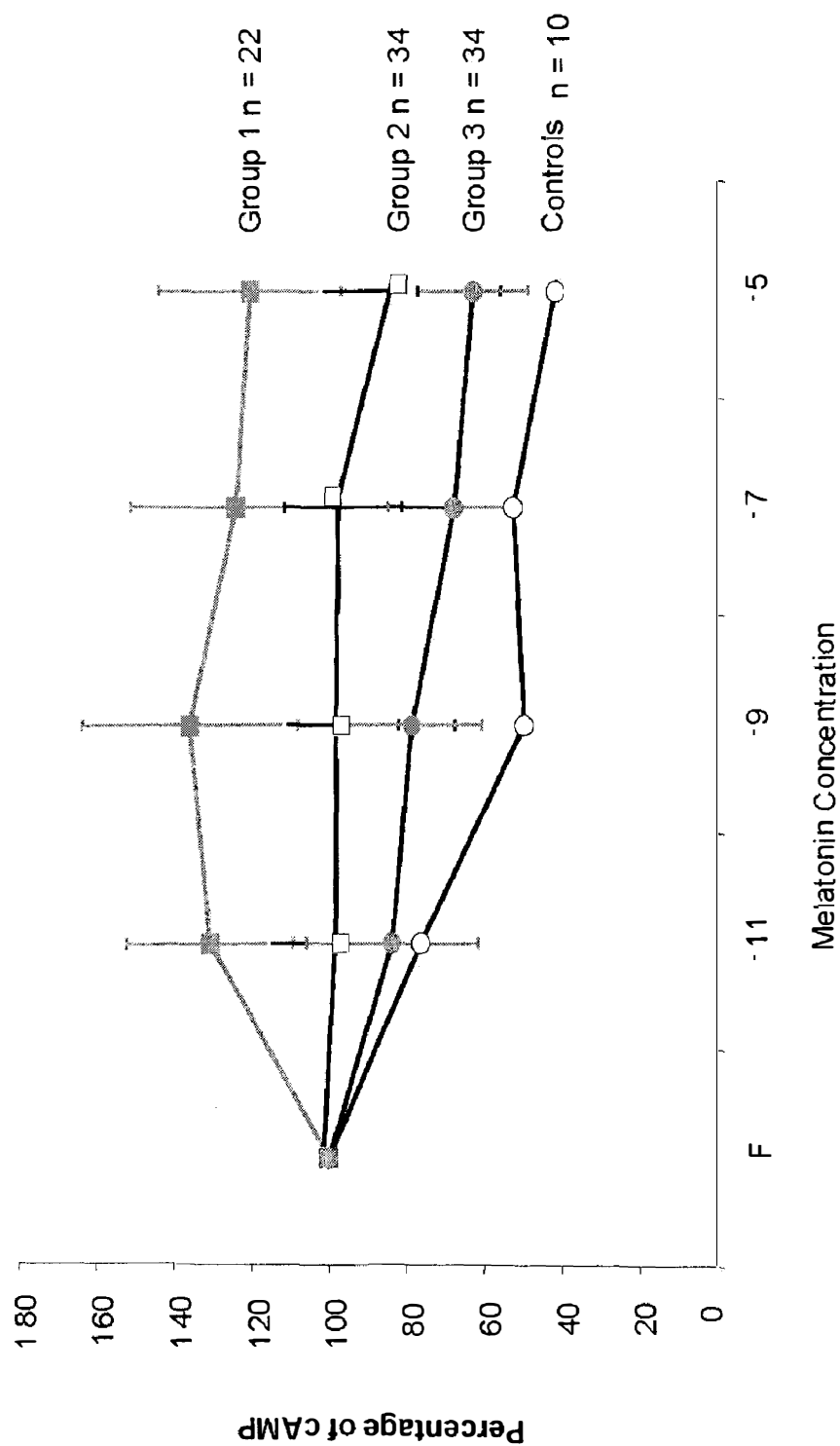


Fig. 2

Fig. 3

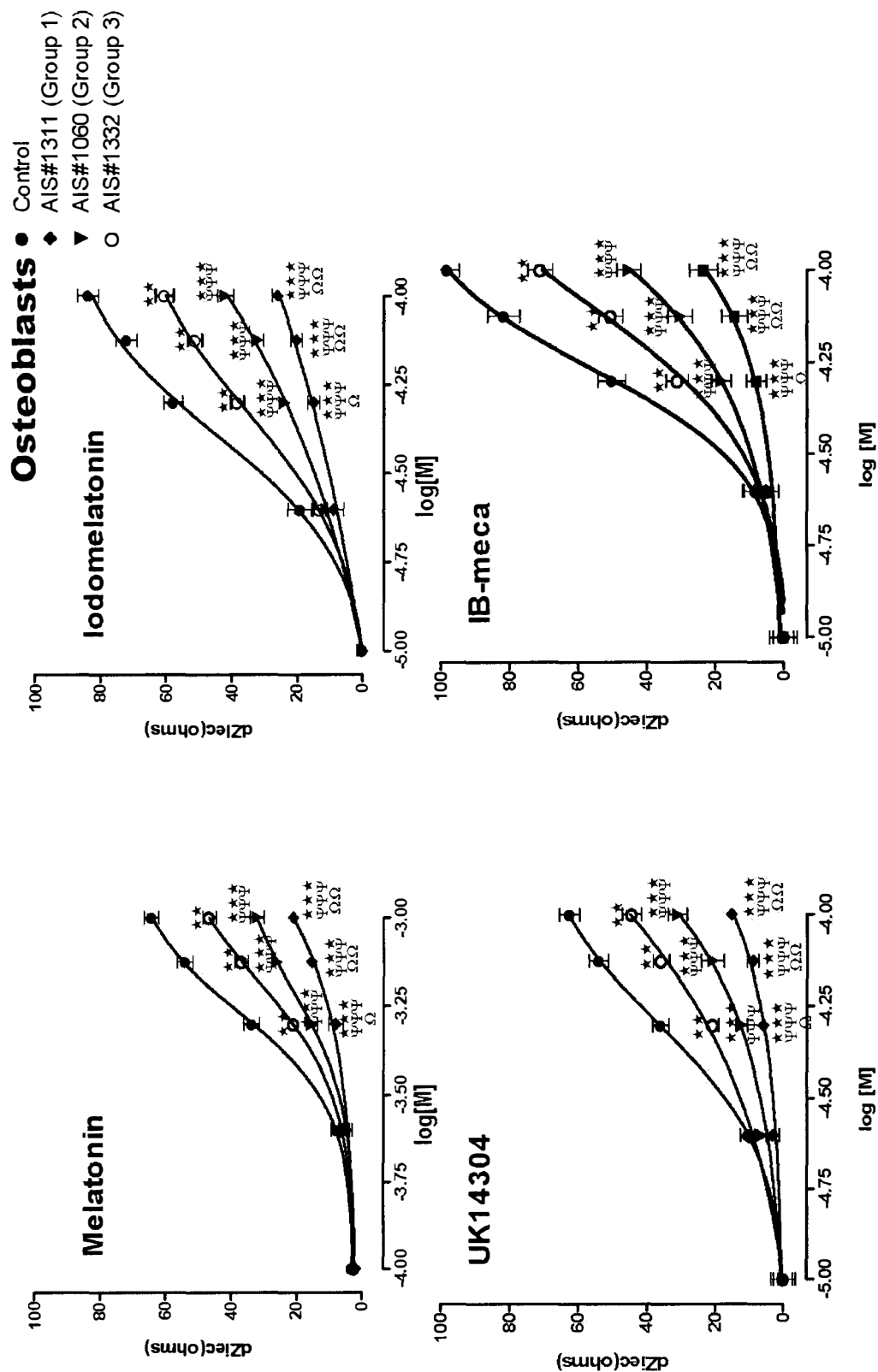
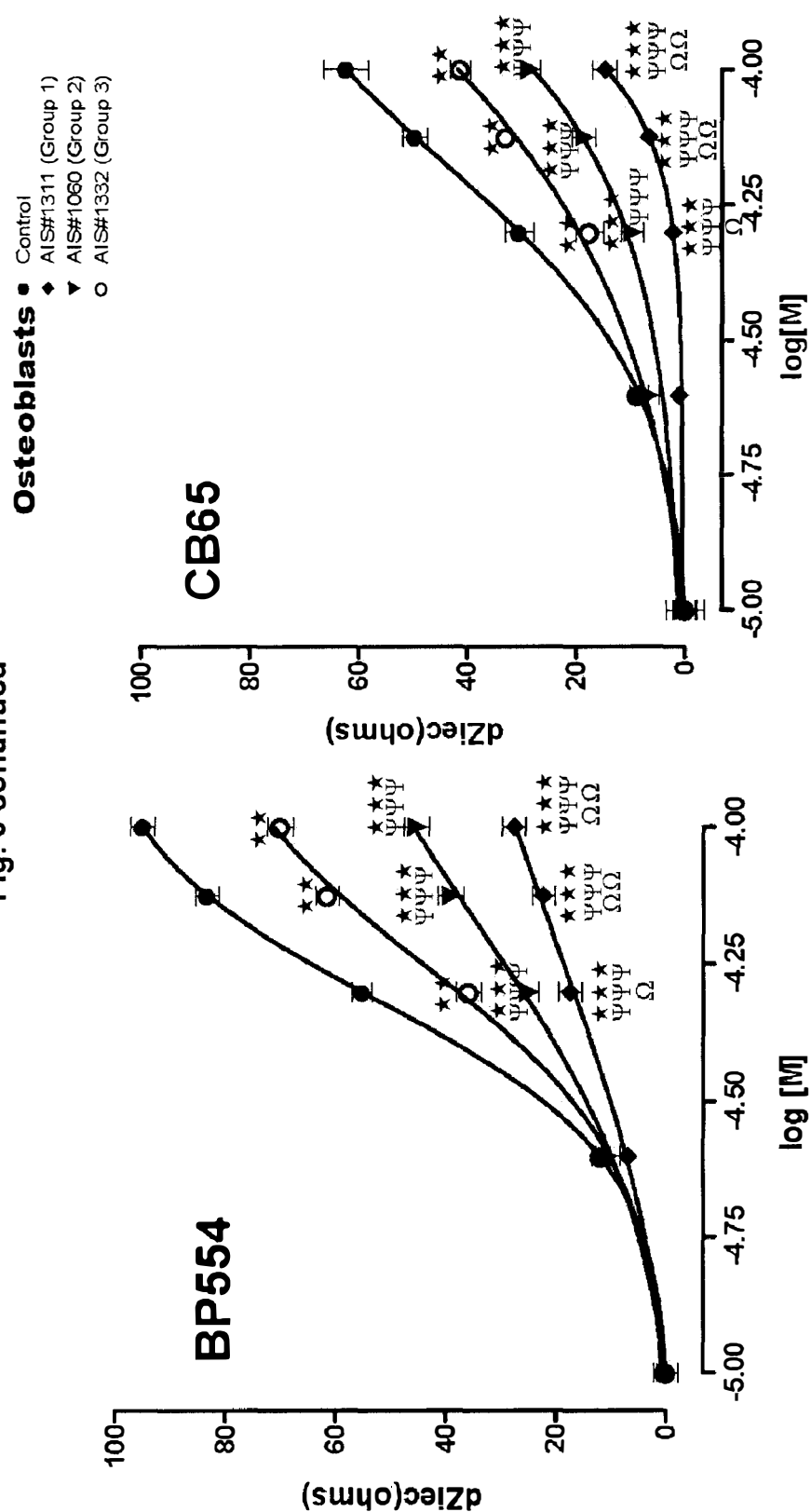


Fig. 3 continued



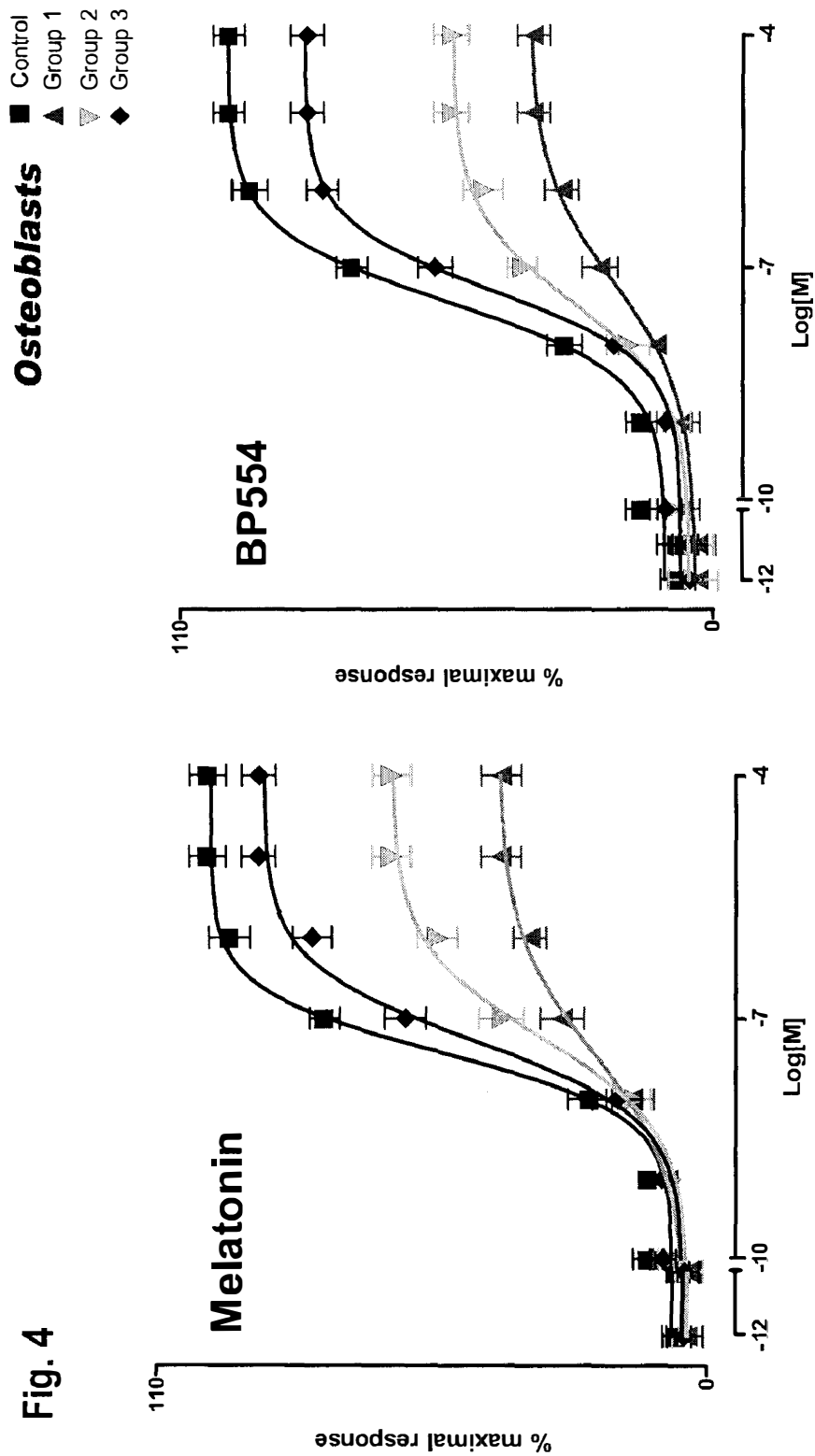


Fig. 4

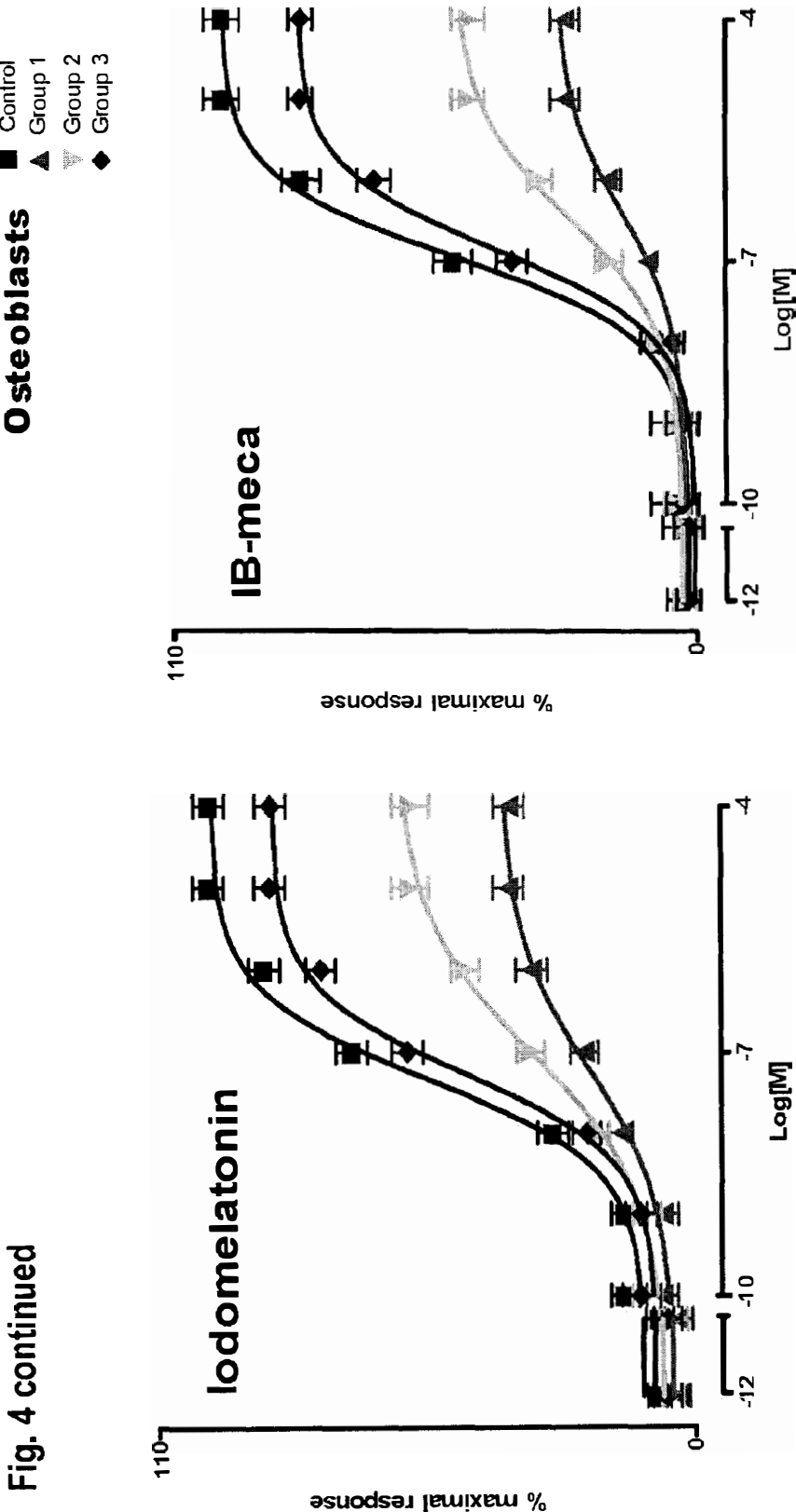


Fig. 4 continued

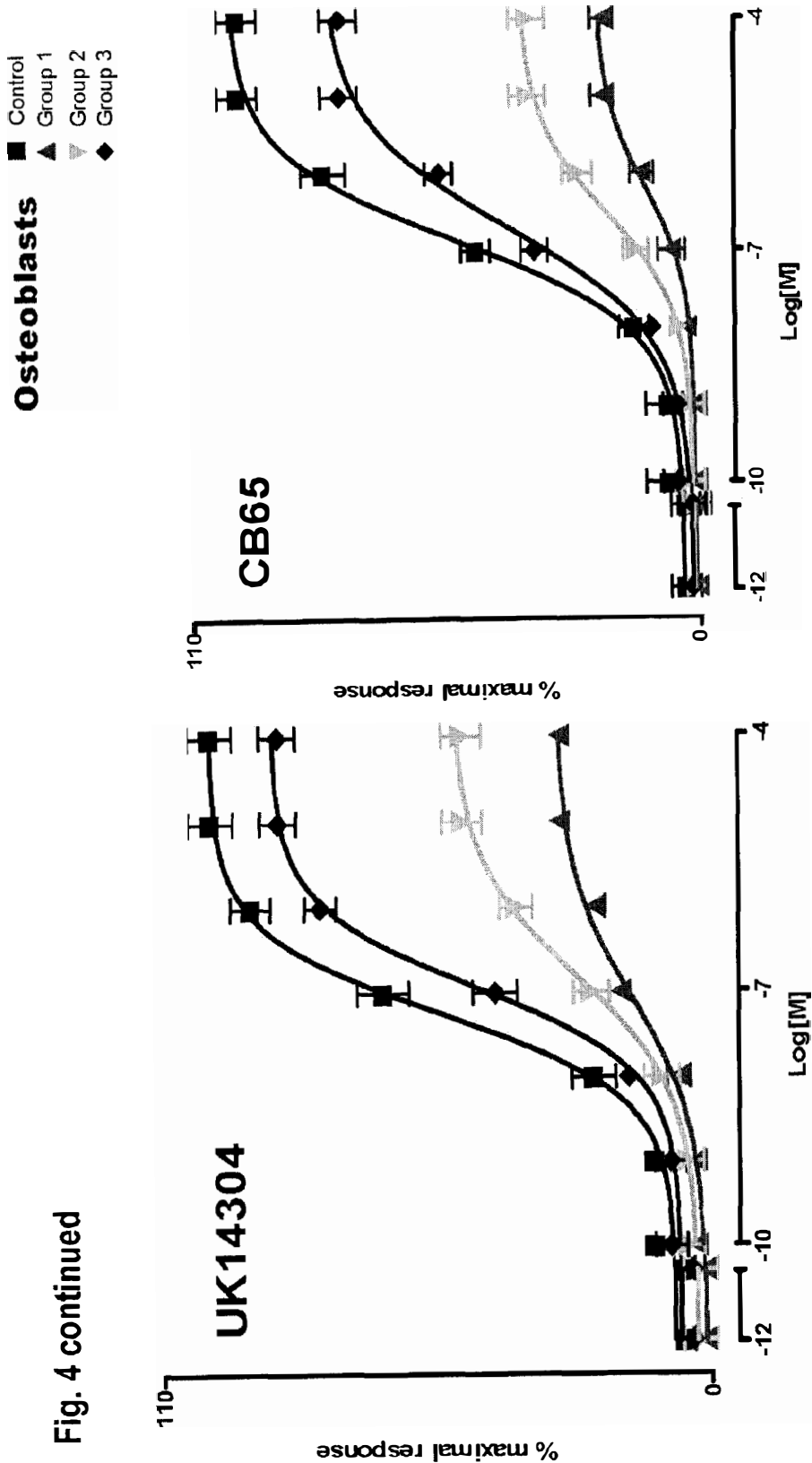


Fig. 4 continued

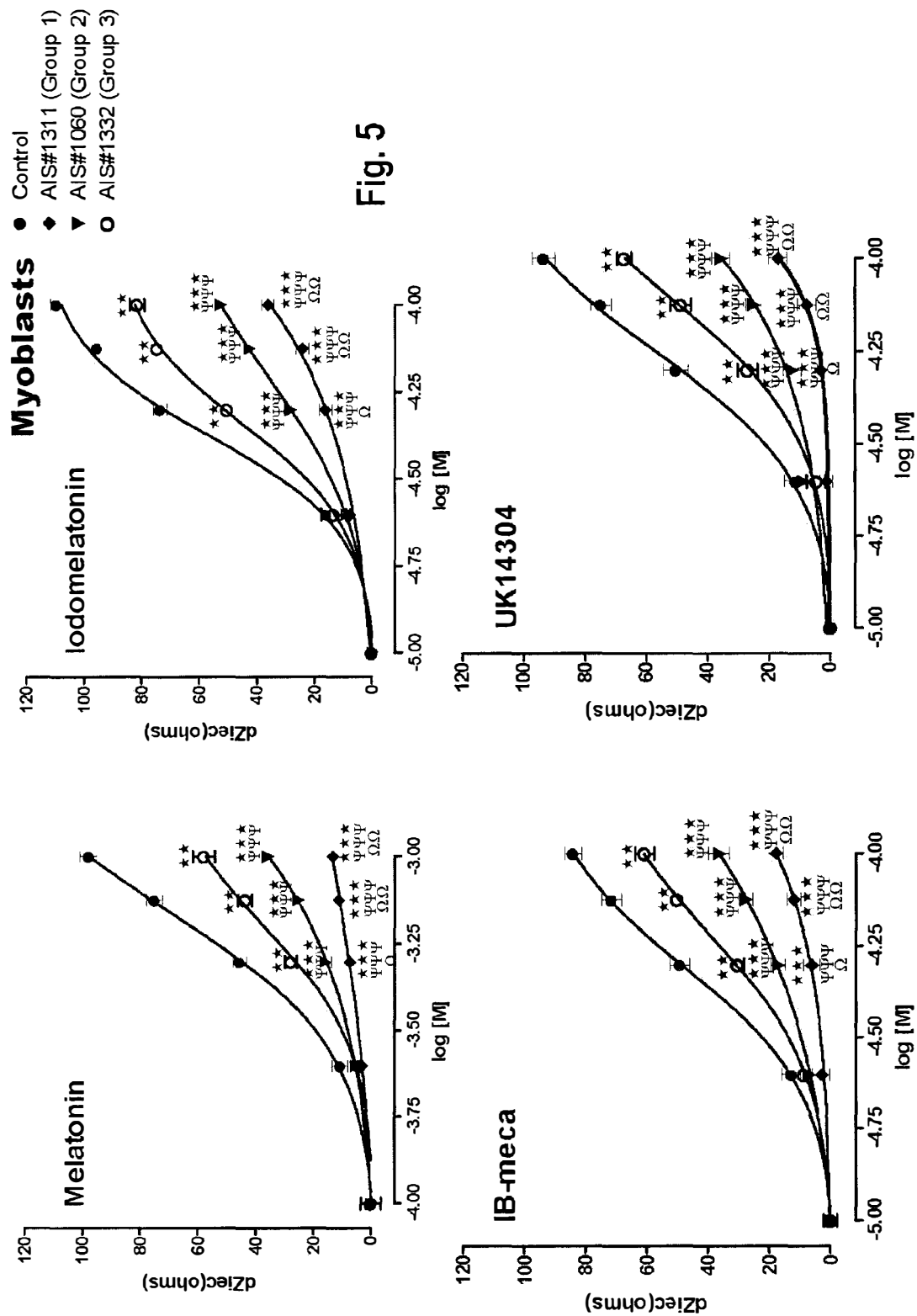
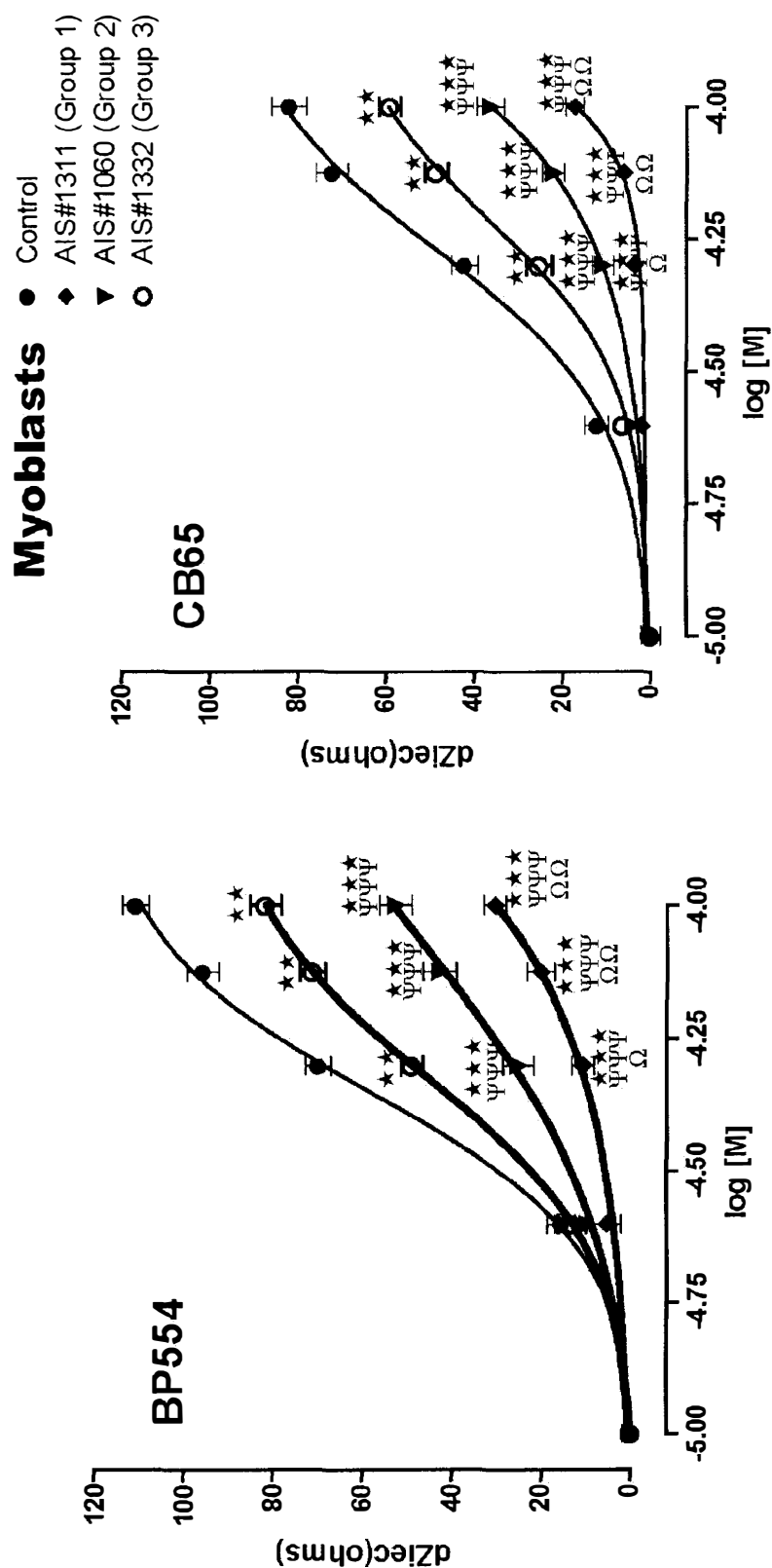


Fig. 5 continued



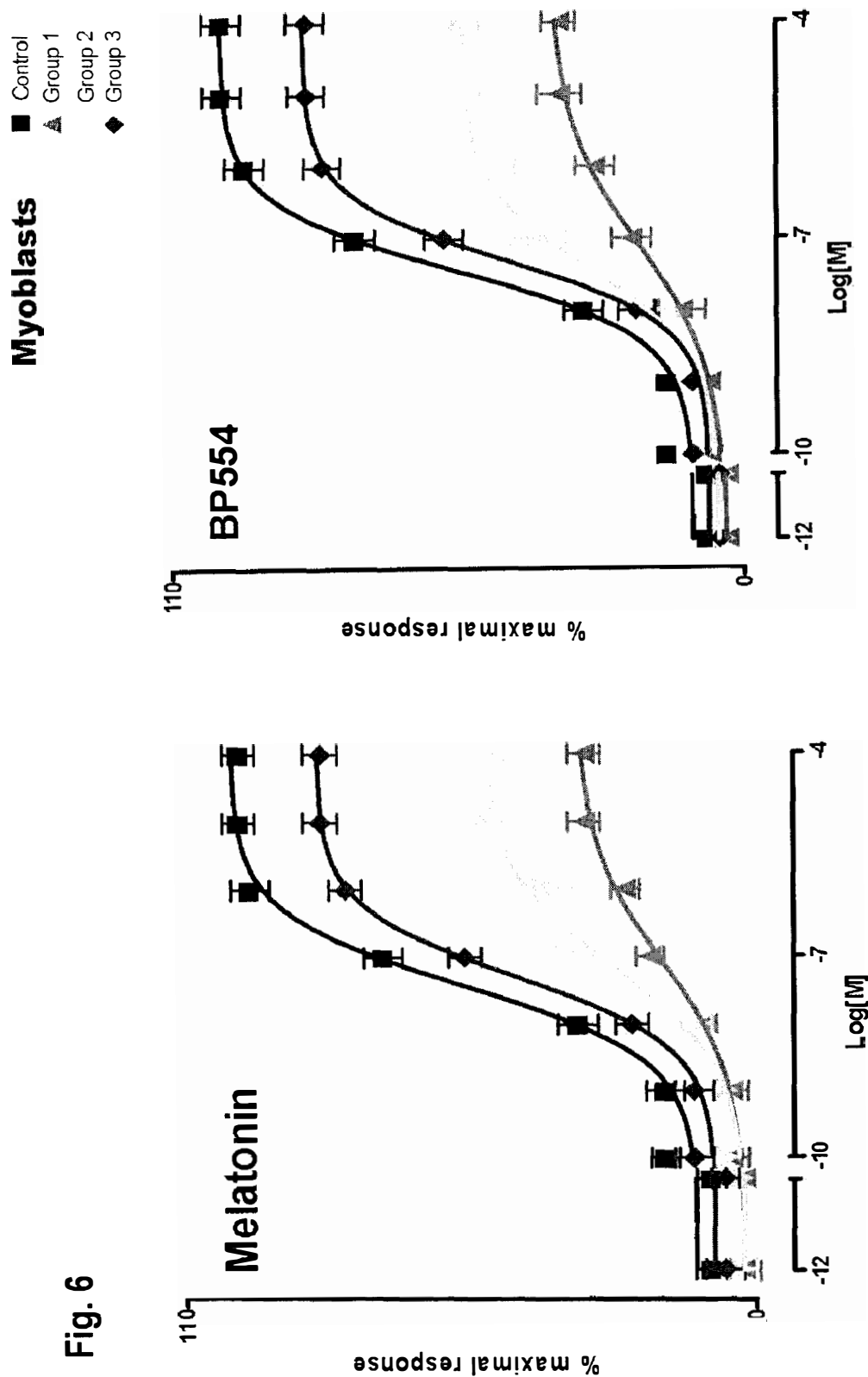


Fig. 6

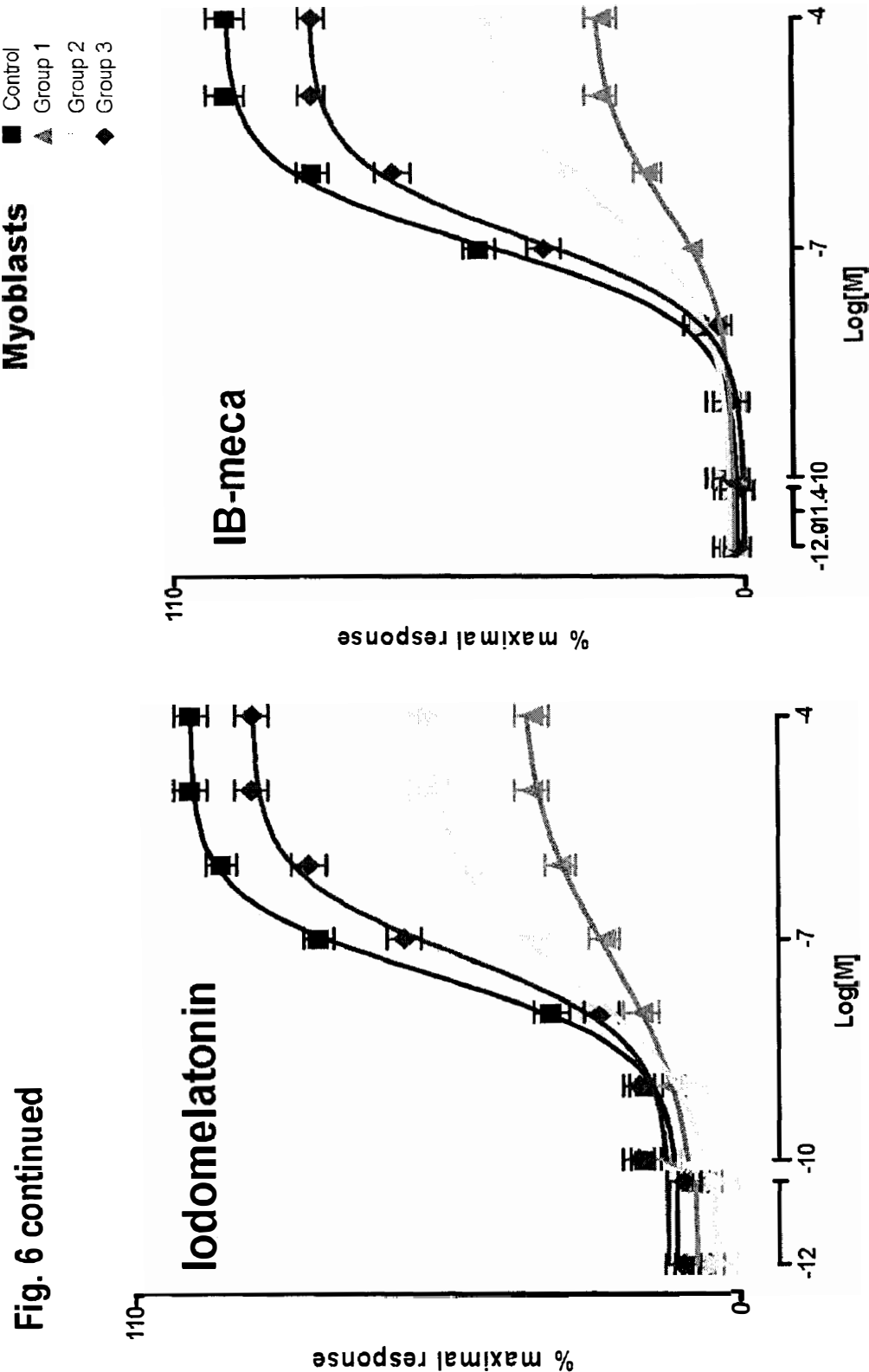


Fig. 6 continued

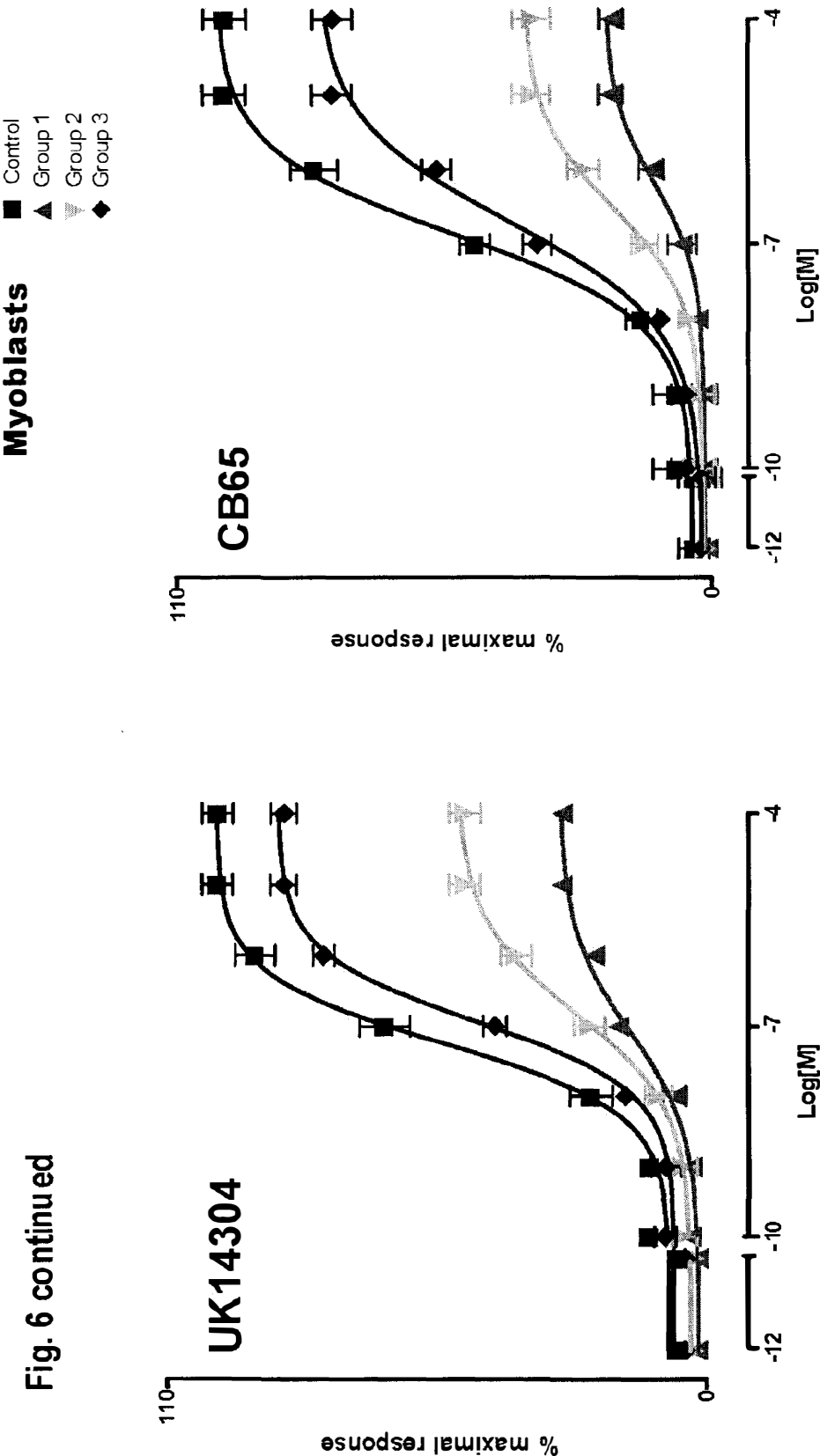


Fig. 6 continued

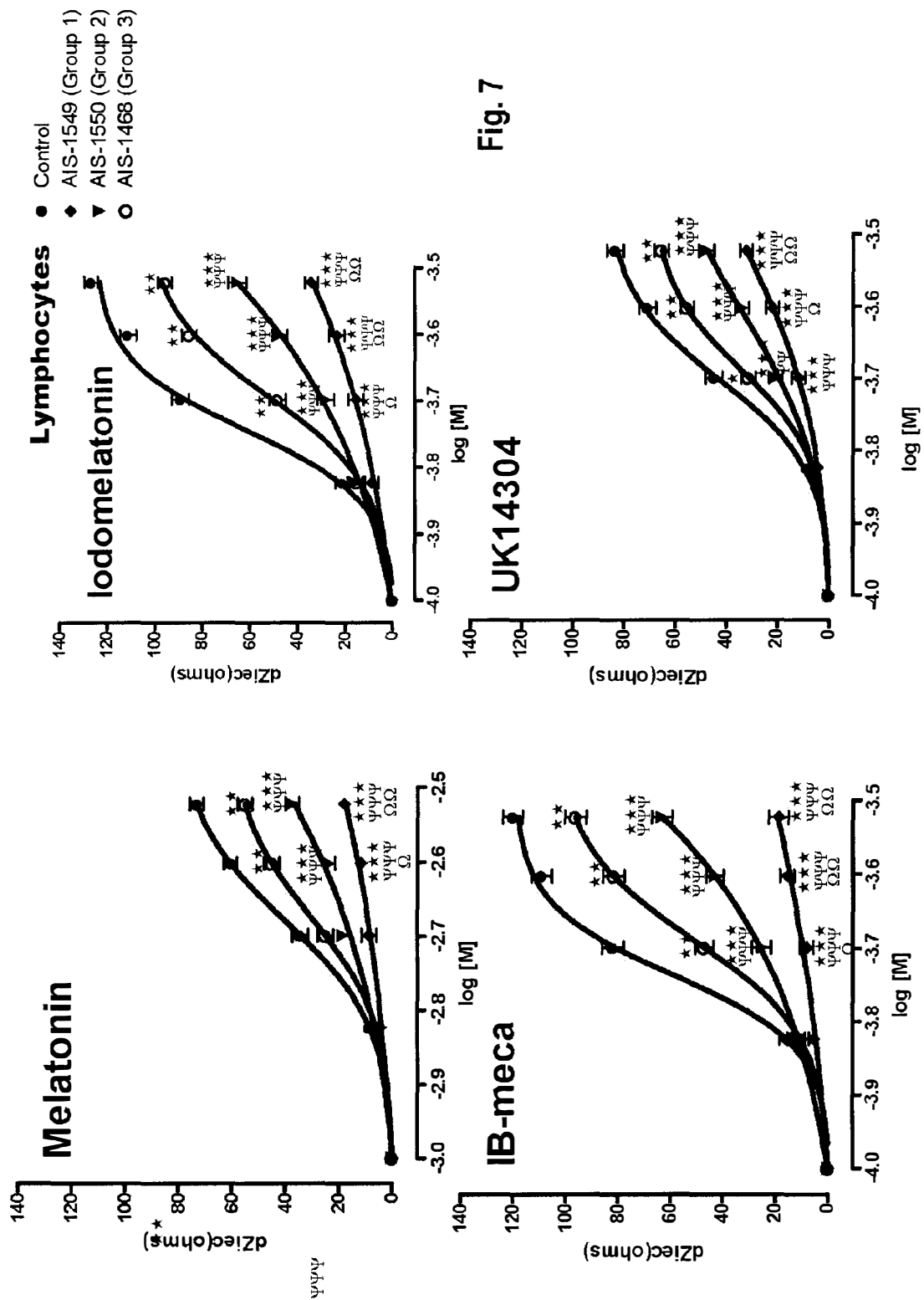
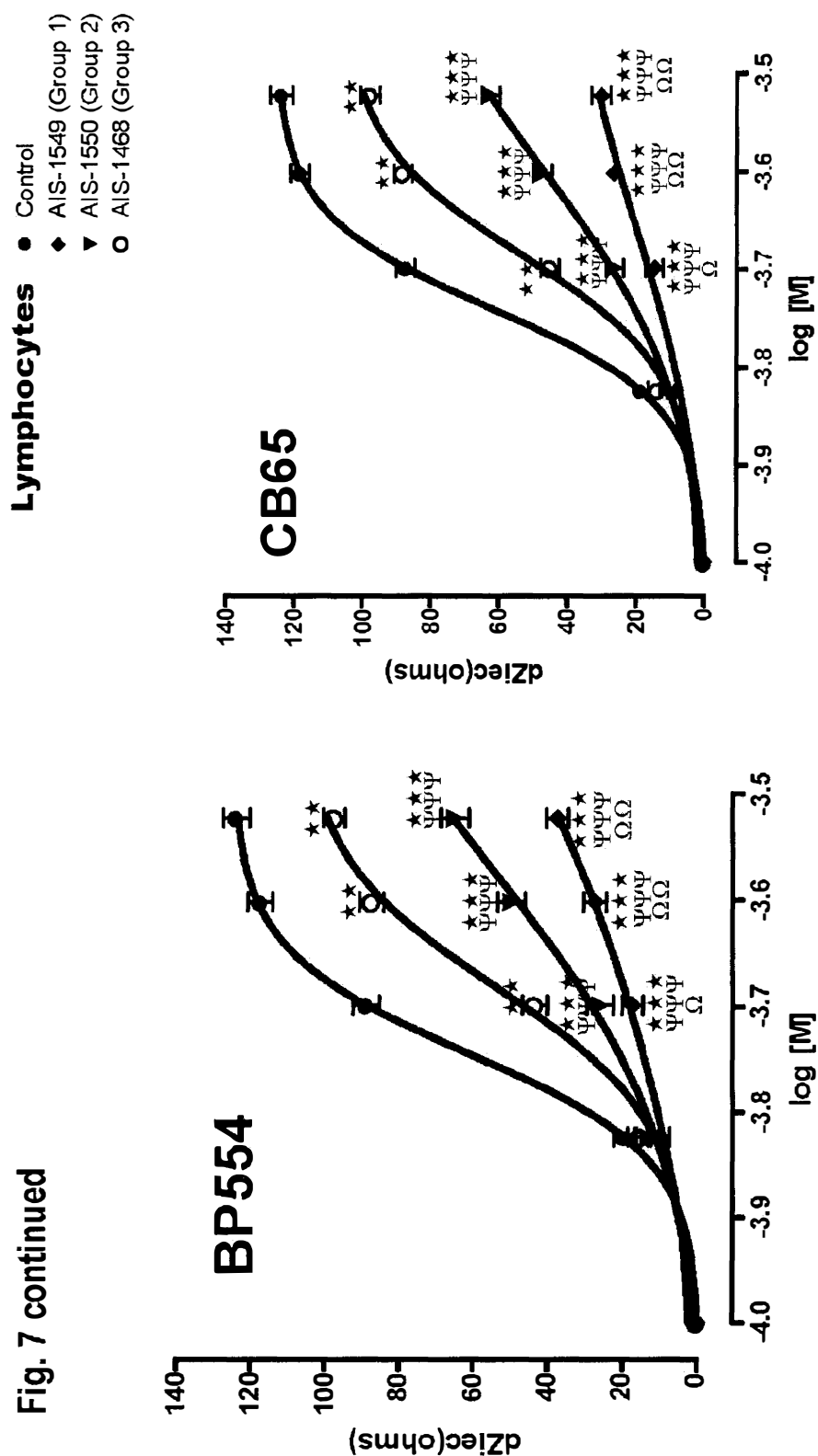
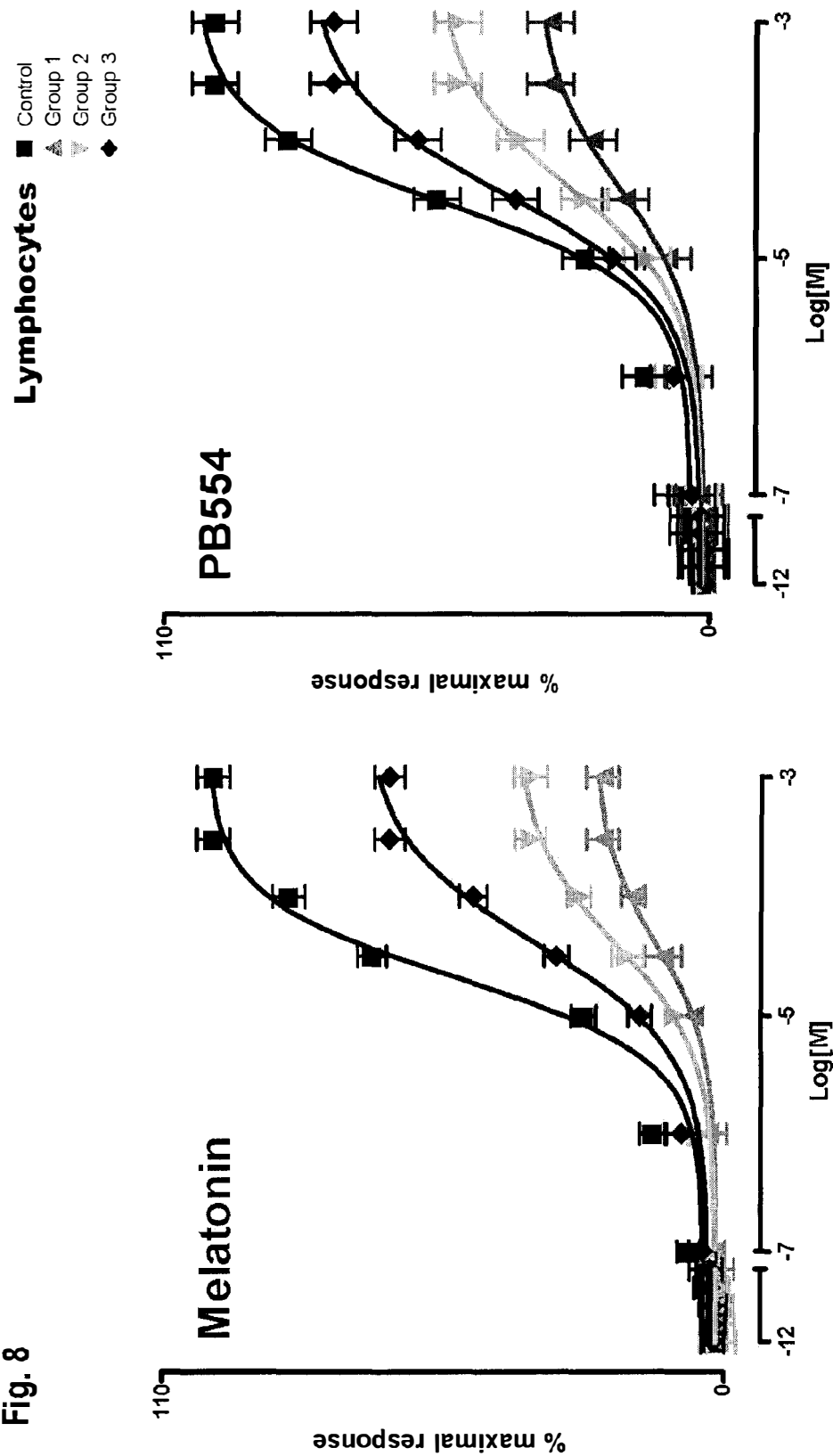


Fig. 7





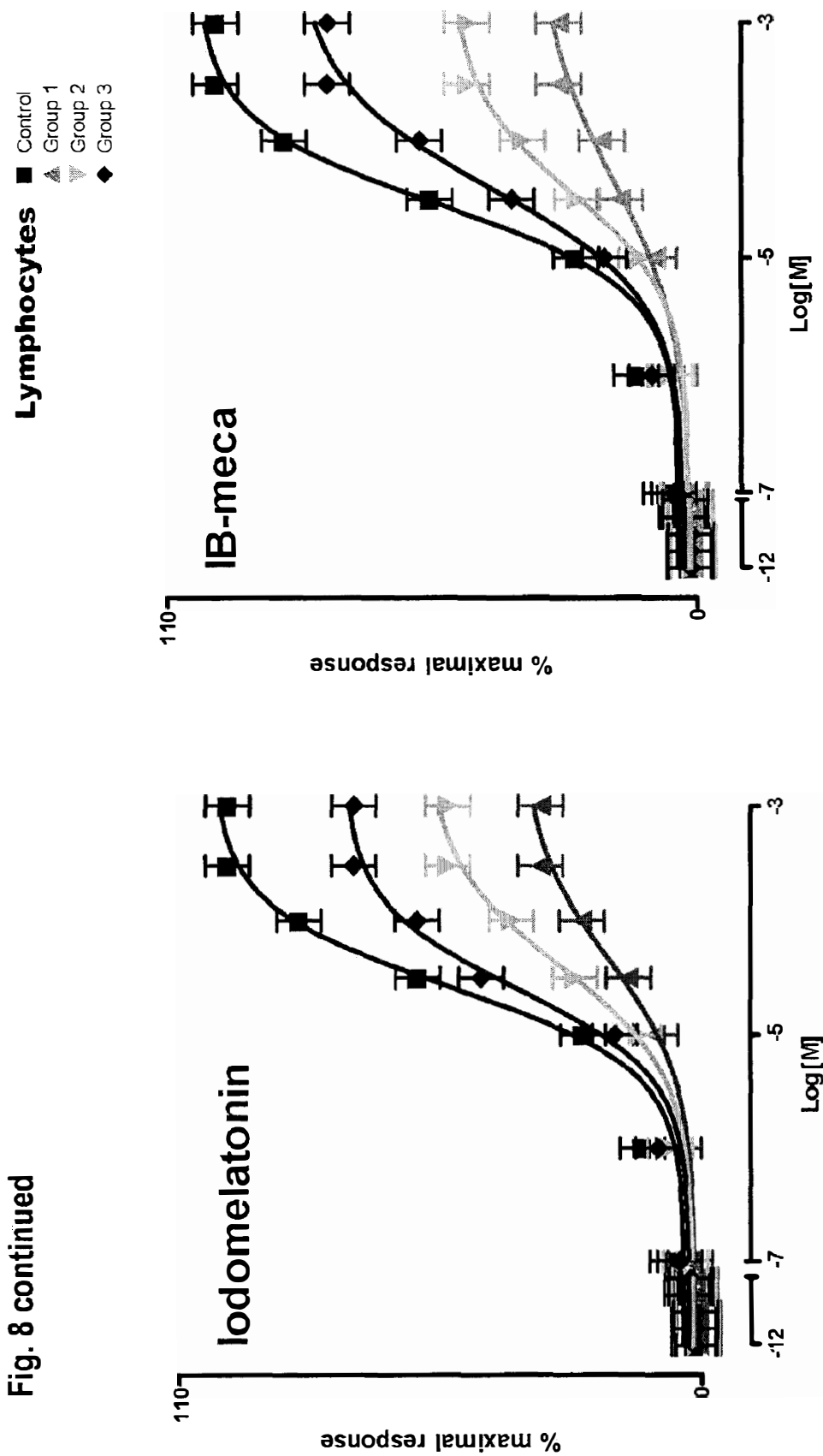
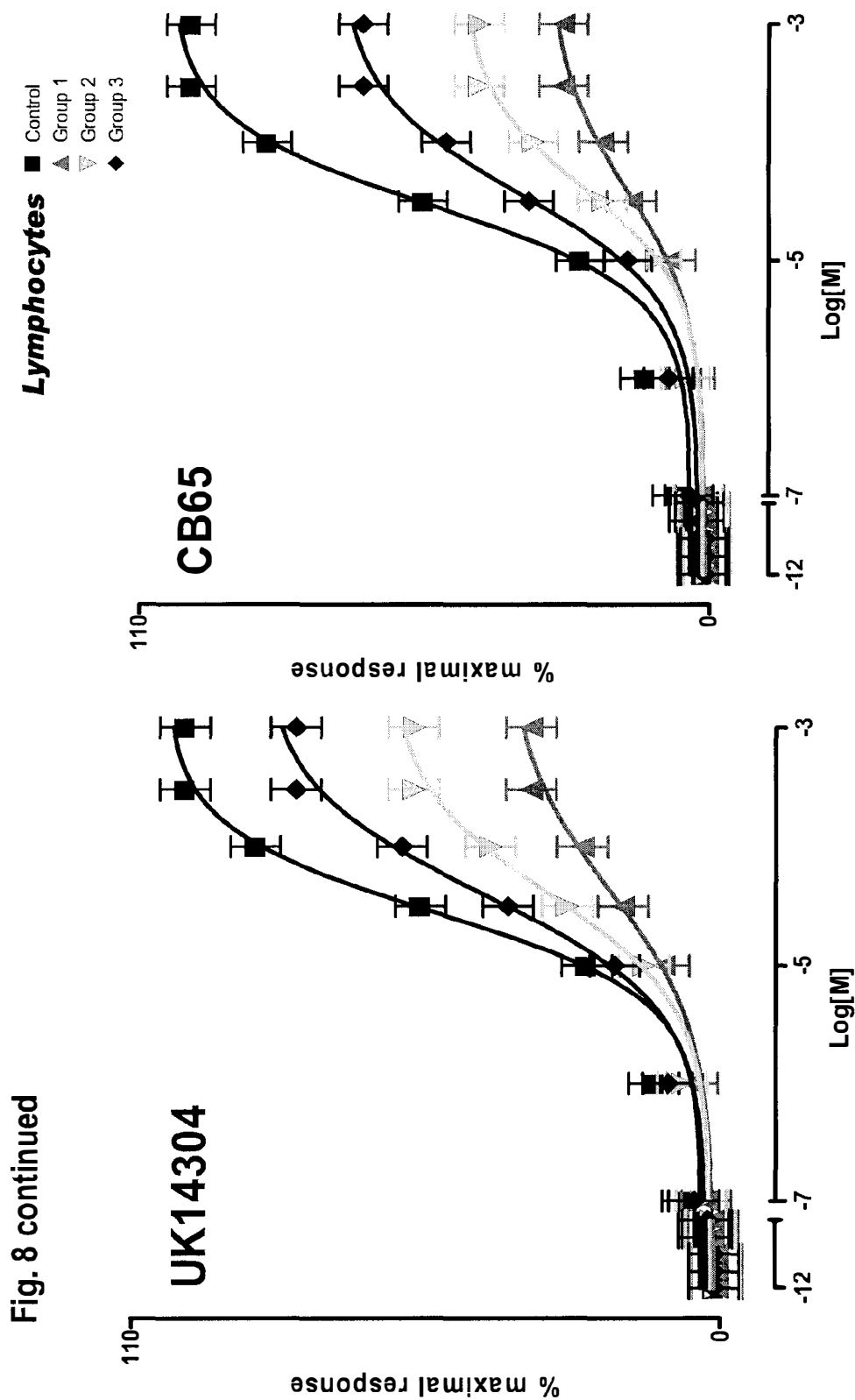


Fig. 8 continued



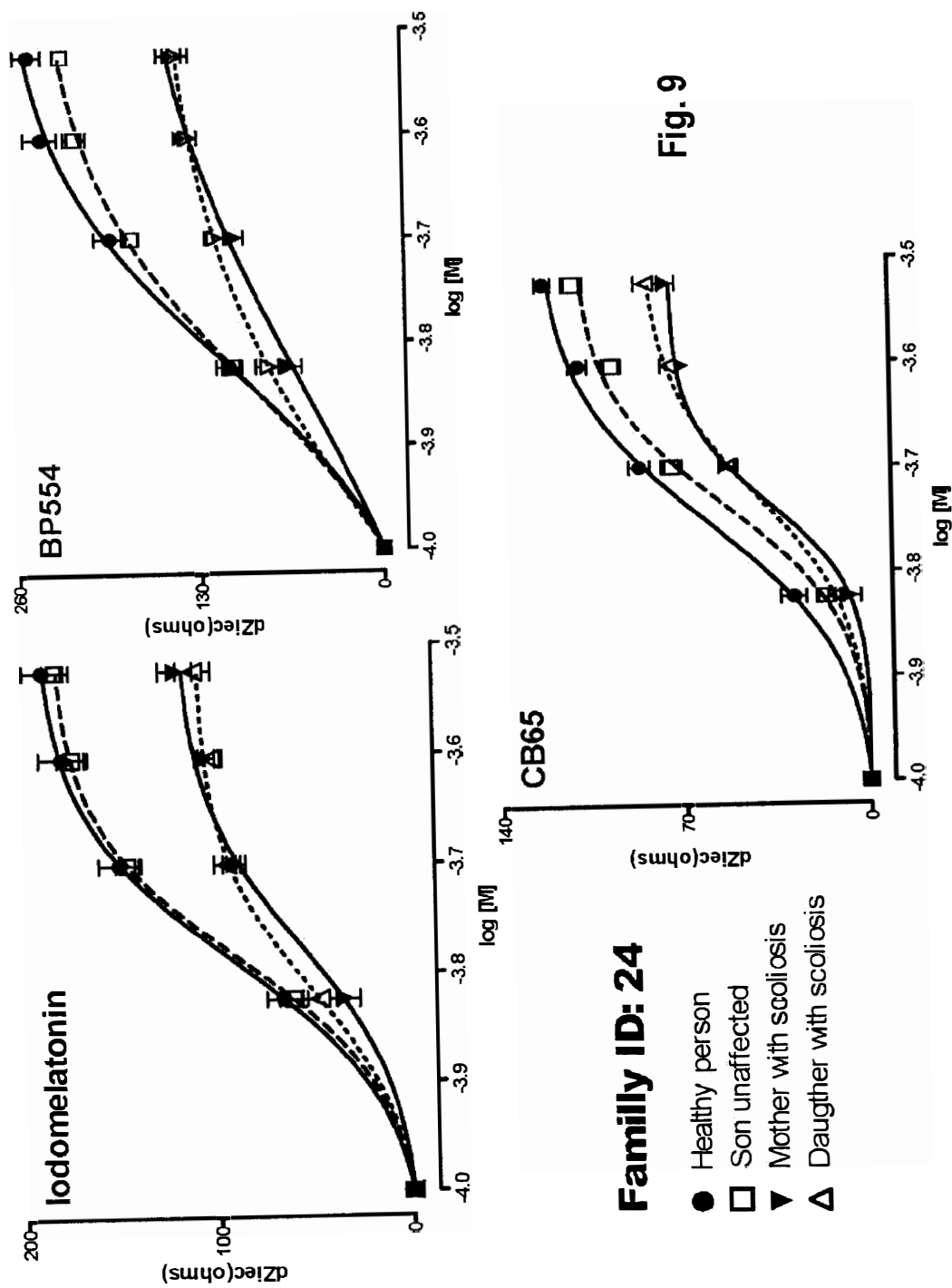


Fig. 9

Fig. 10

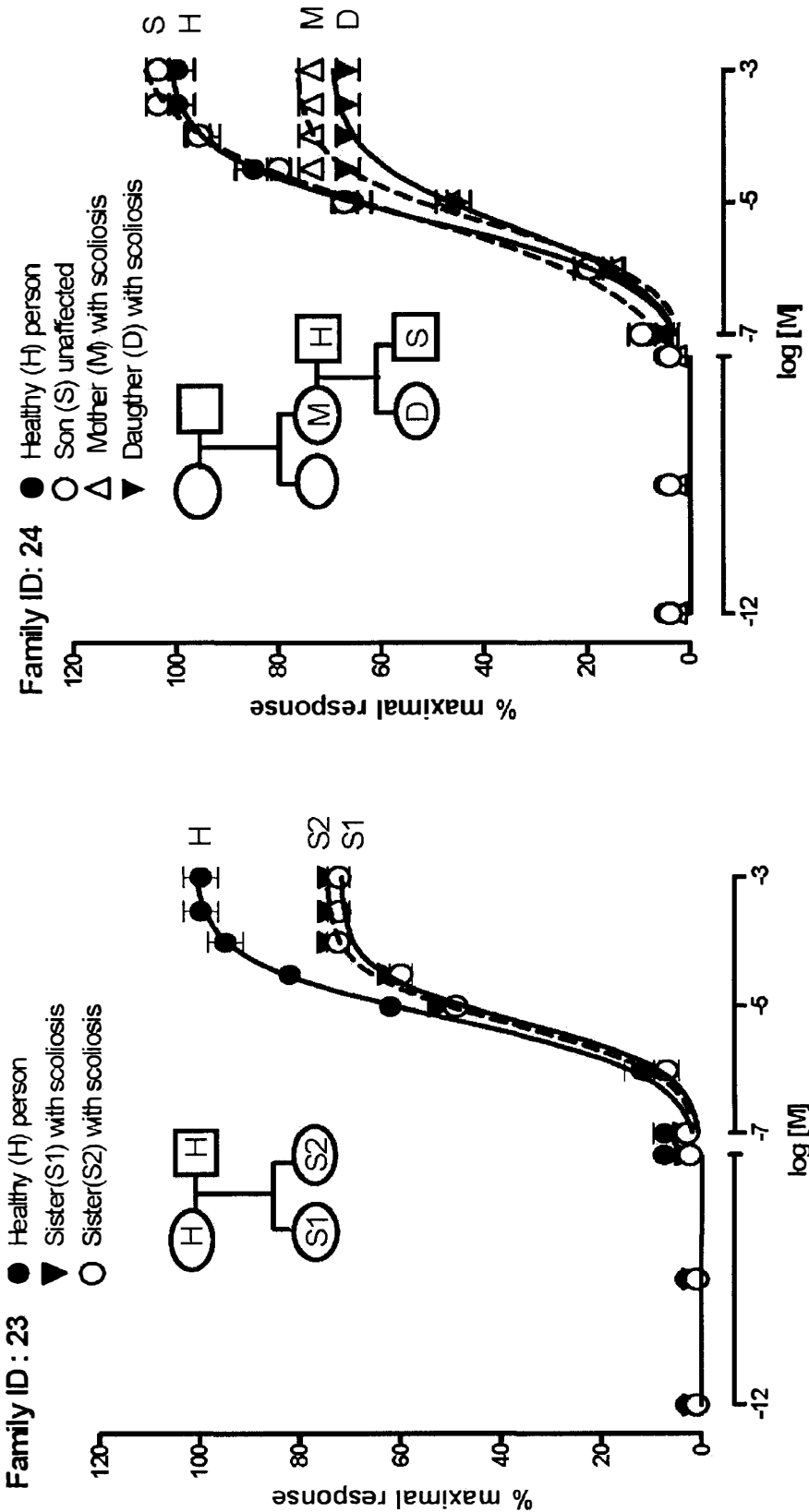


Fig. 10 continued

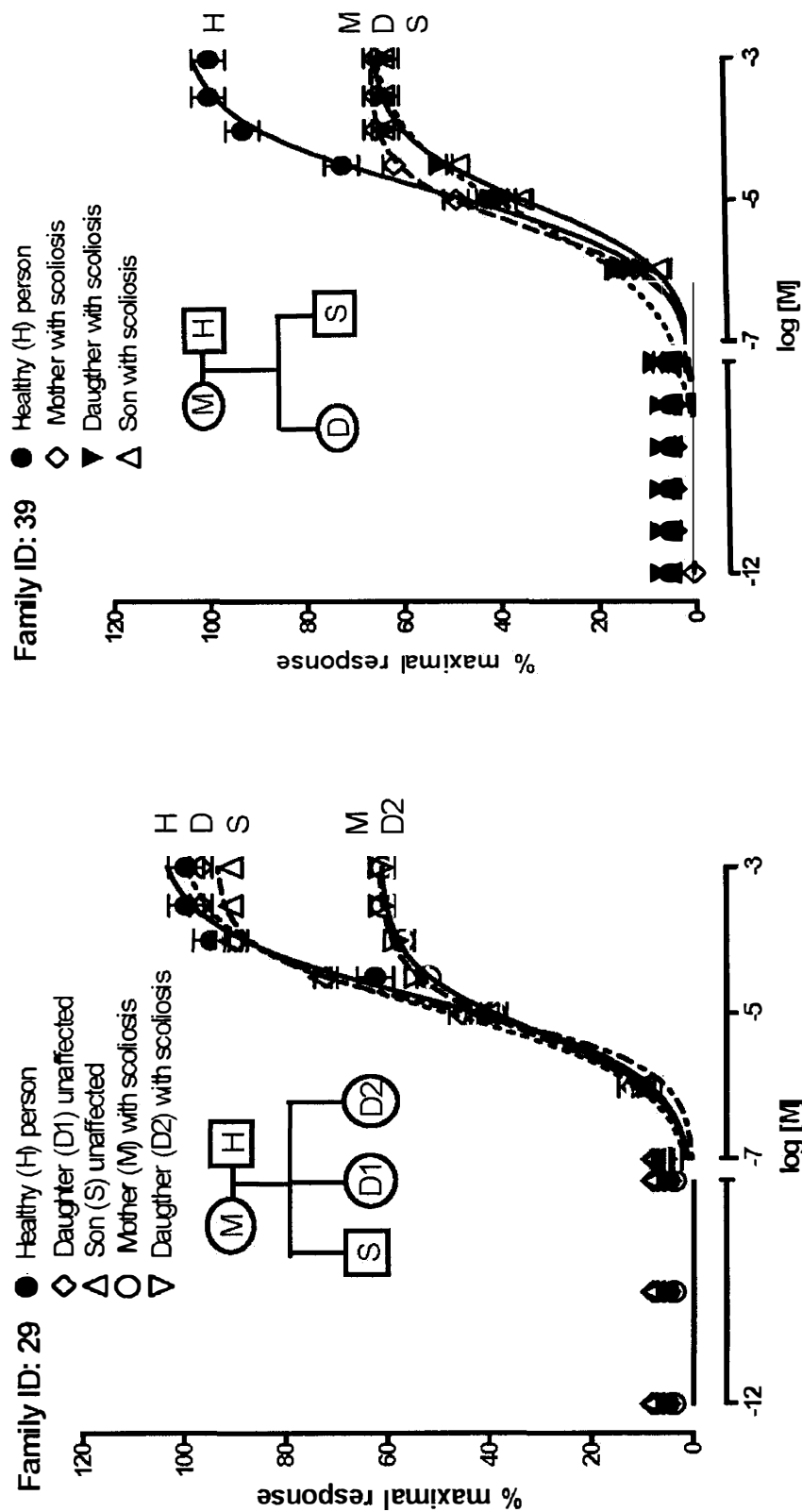


Fig. 11

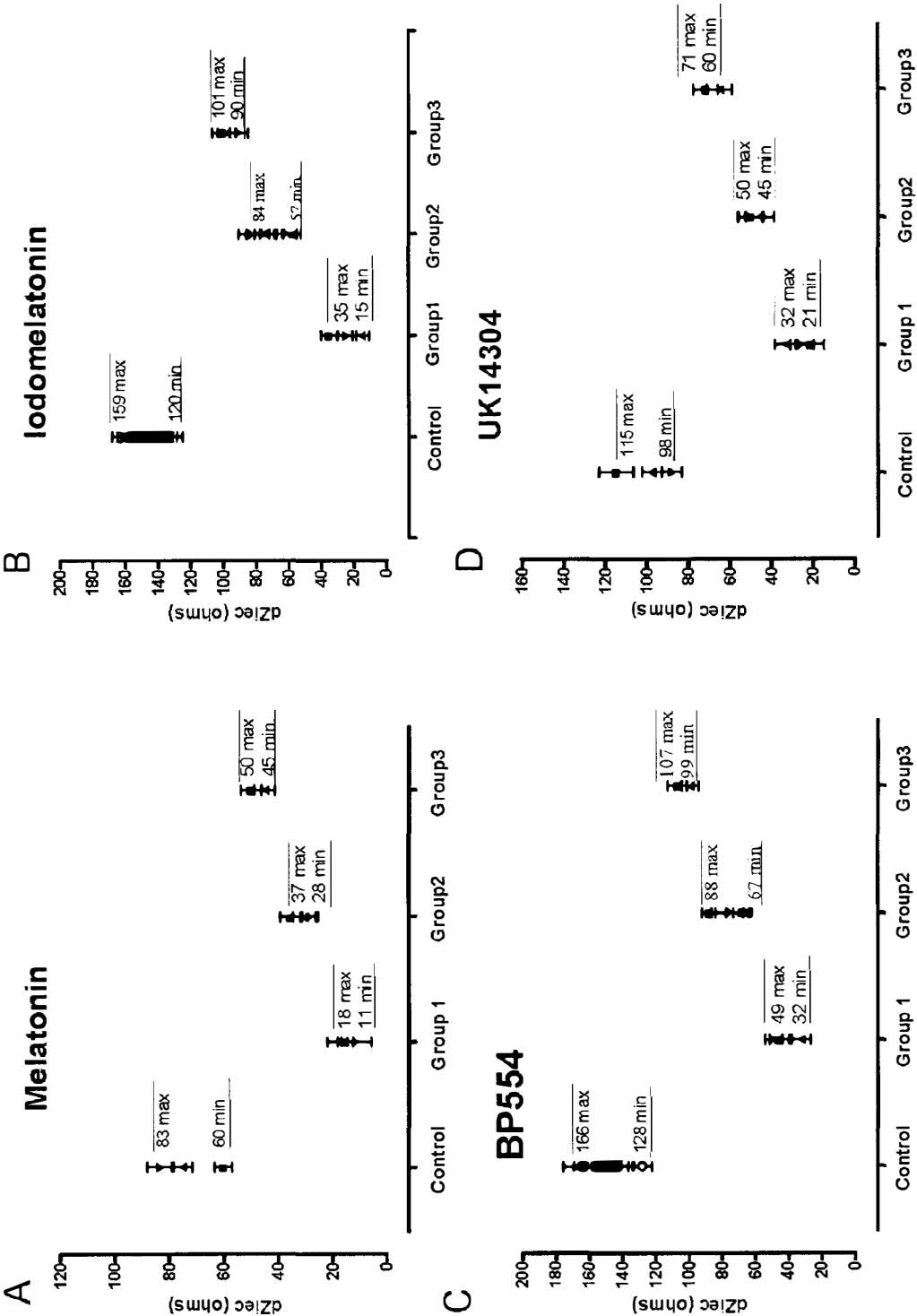
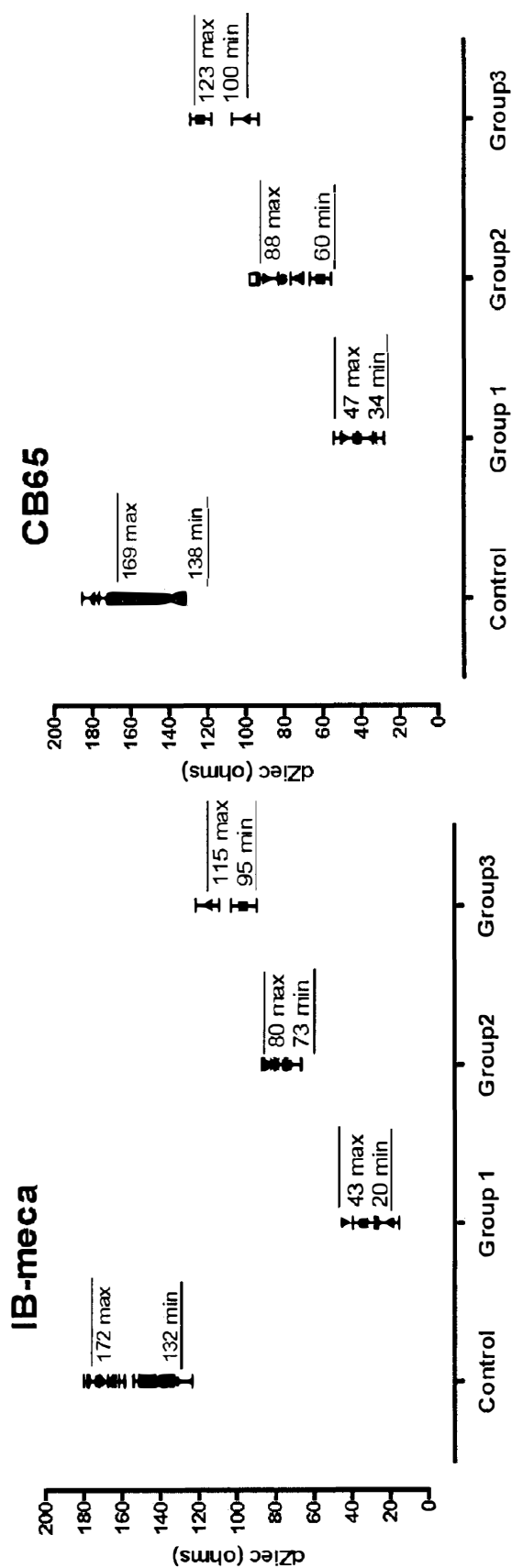
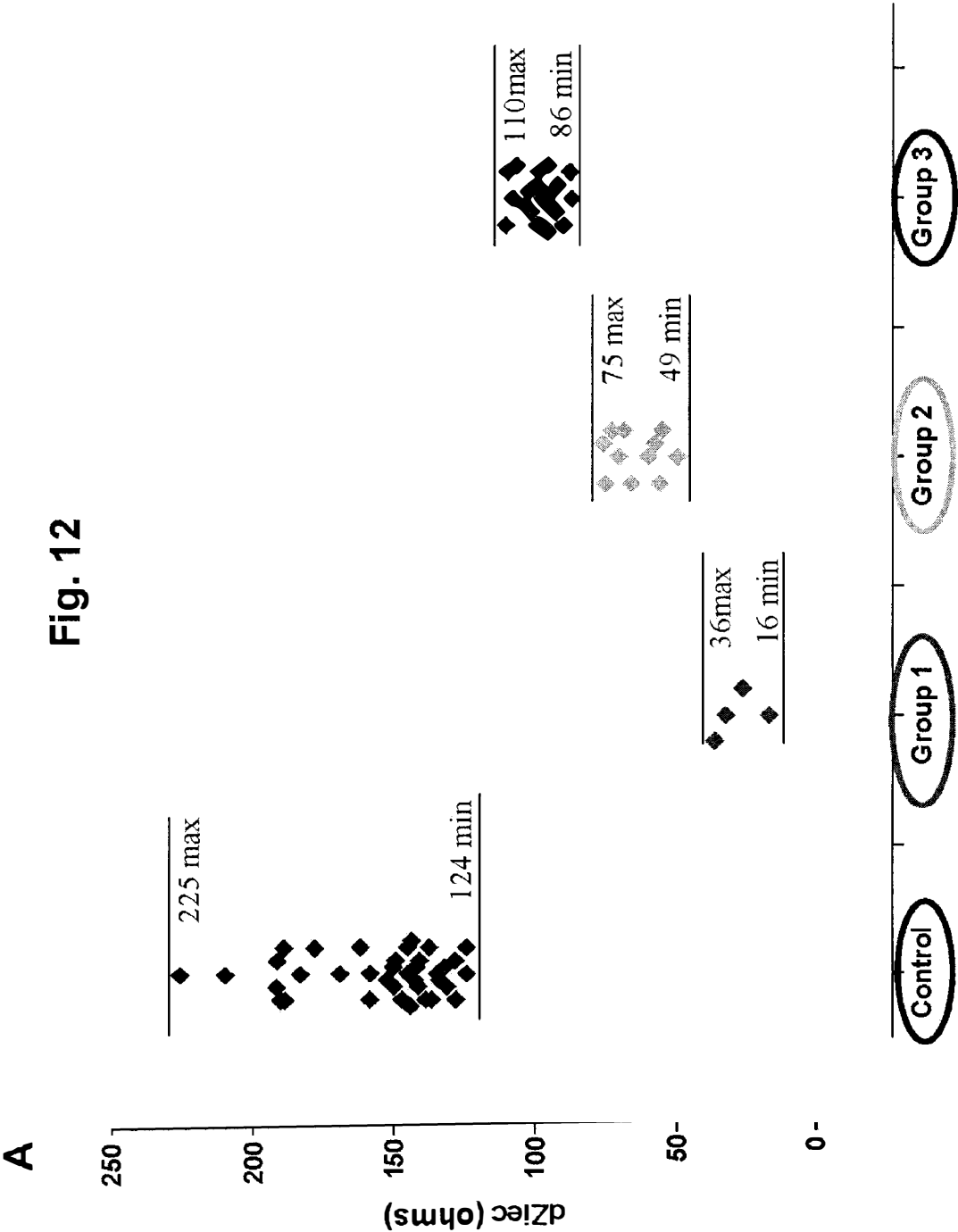
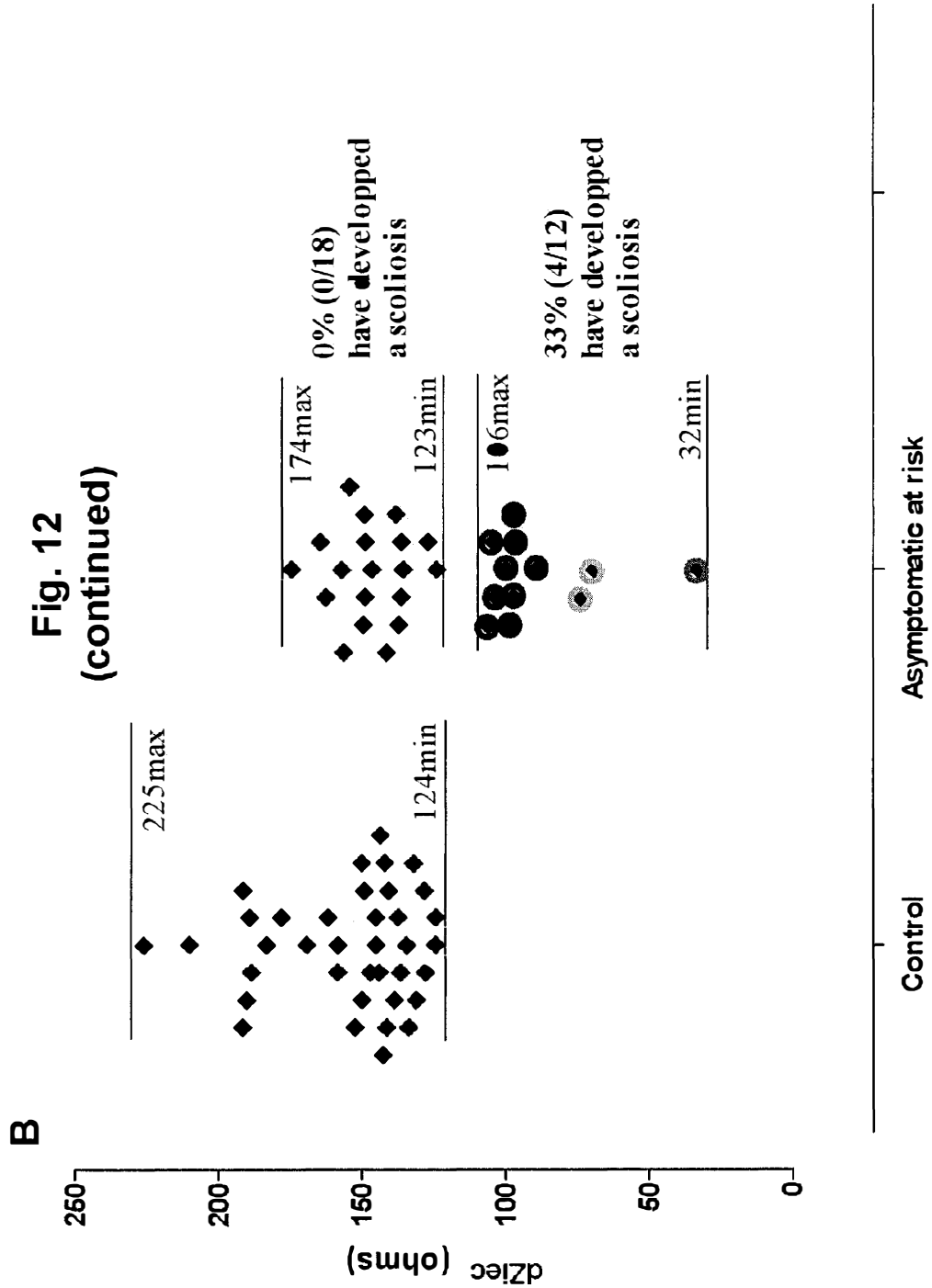
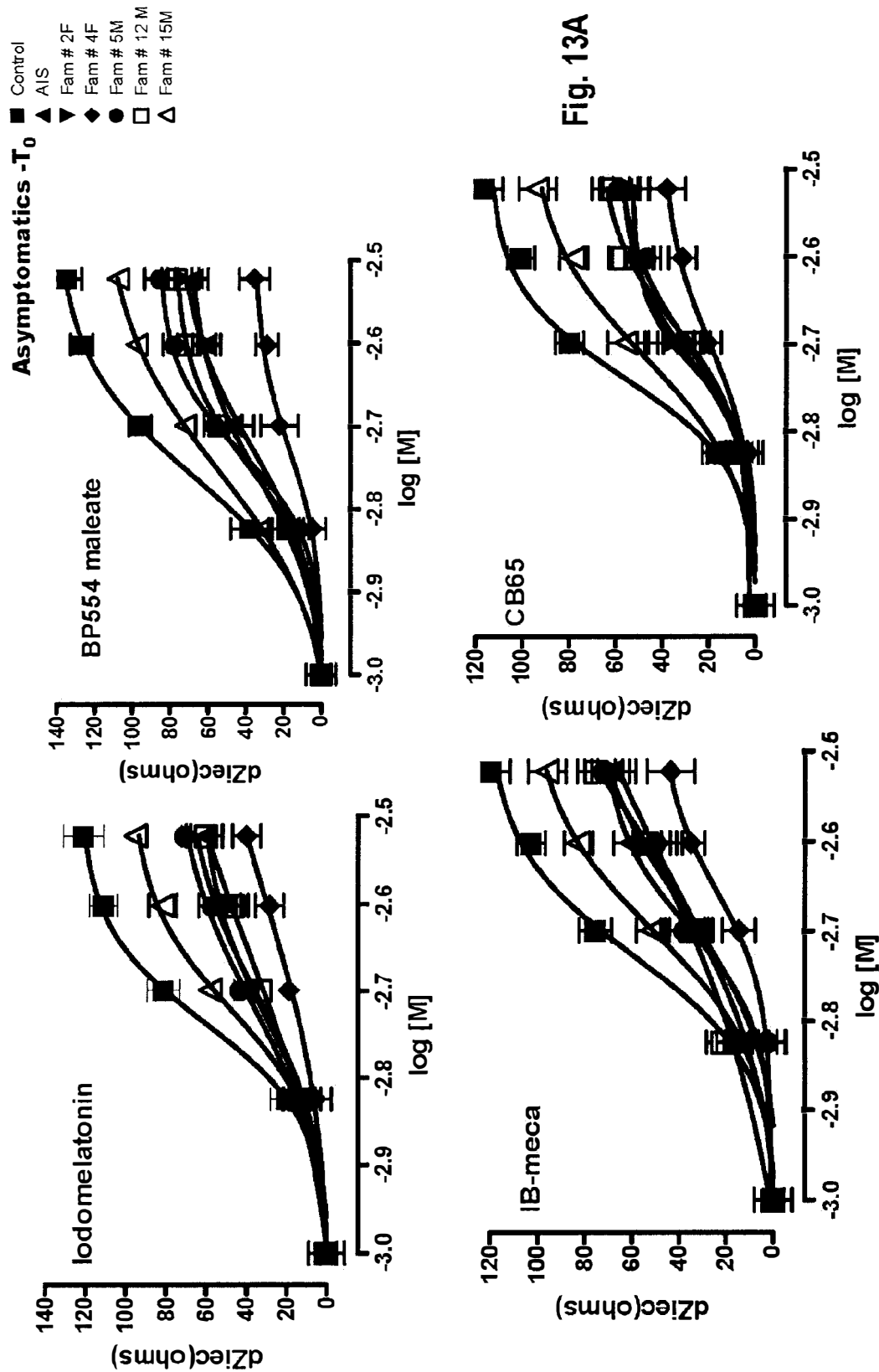


Fig. 11 continued









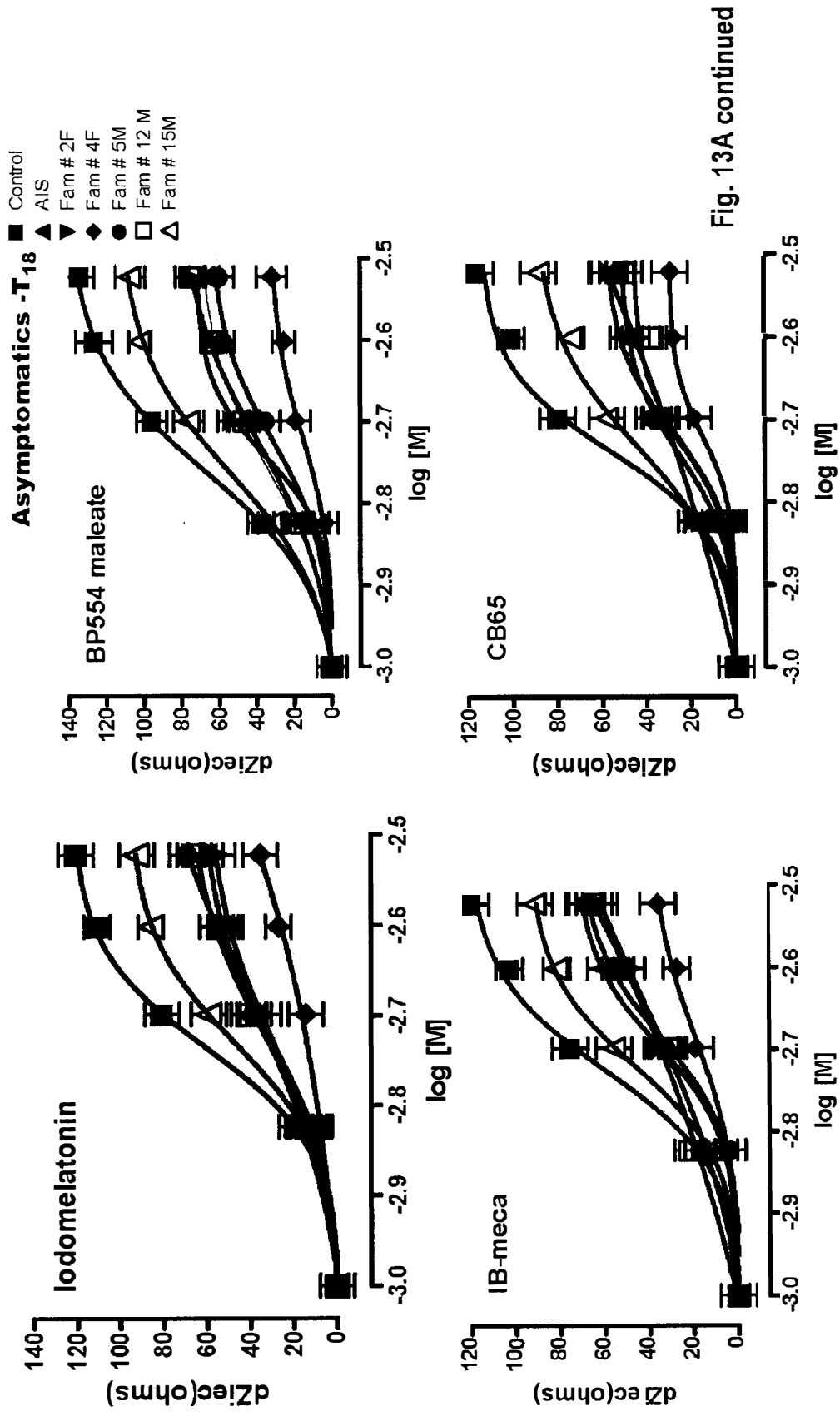


Fig. 13A continued

B

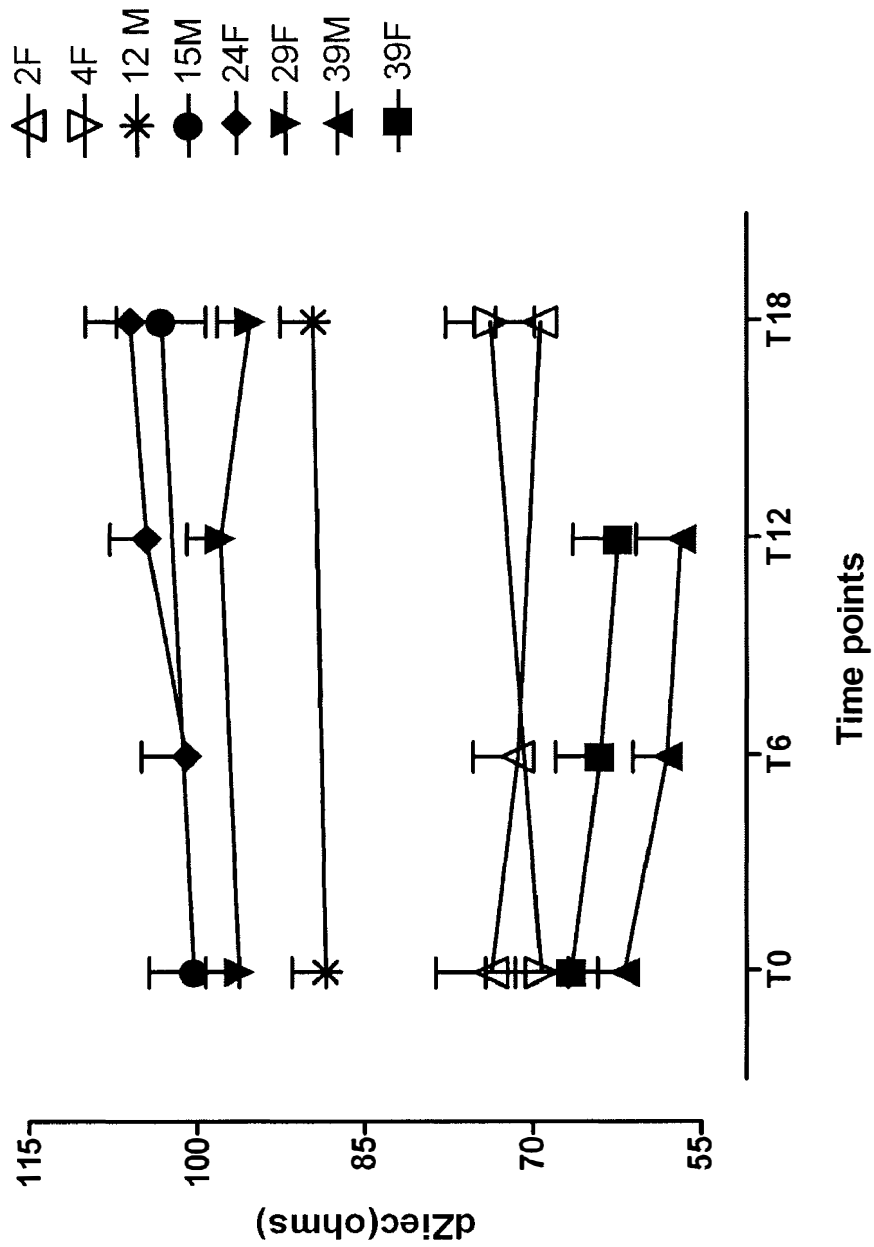


Fig. 13B

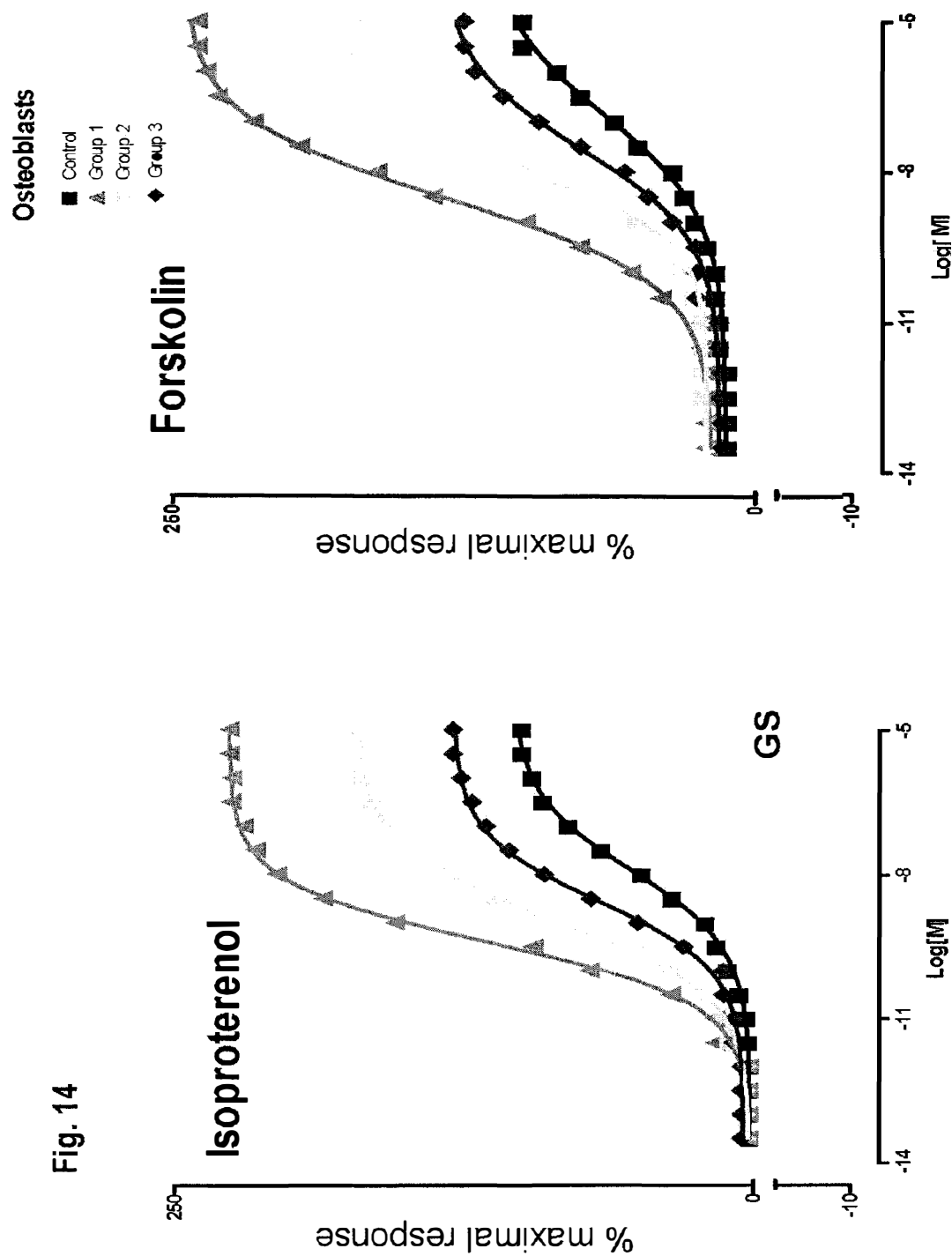


Fig. 14

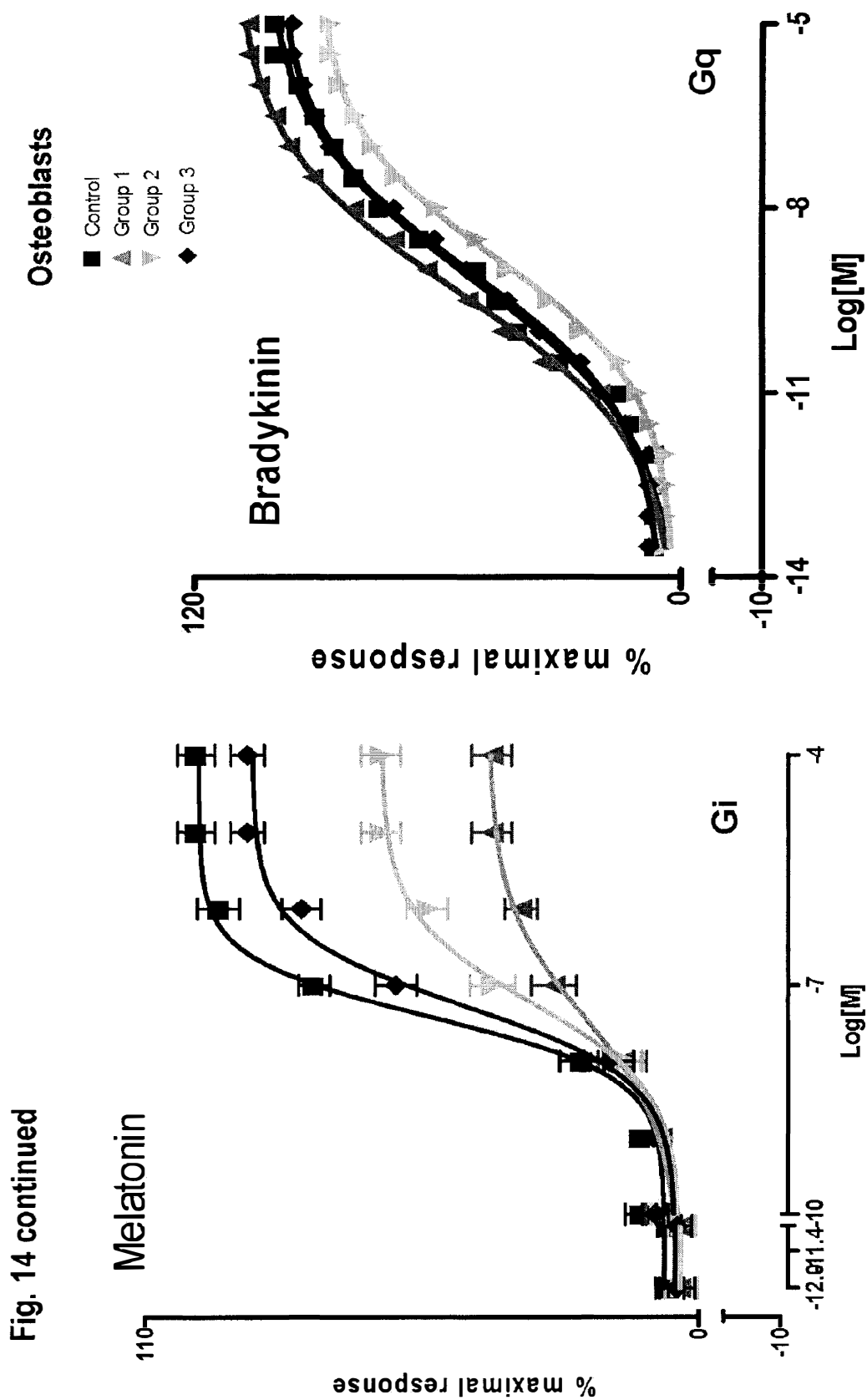


Fig. 14 continued

Adenosine receptors		
Subtype	Coupling	Reference
A1	Gi	Fredholm <i>et al.</i> , 2001
A3	Gi	
Adrenoreceptors		
Subtype	Coupling	Reference
Alpha2	Gi	
Angiotensin receptors		
Subtype	Coupling	Reference
At1	Gi / Gq	Gasparo <i>et al.</i> , 2000 in Pharmacol. Rev. 52 (3)
At2	Gi	
Apelin receptors		
Subtype	Coupling	Reference
APJ	Gi	Iturrios et al., 2007 In: Arch Mal Coeur Vaiss.100 (8)

Bradykinin receptors		
Subtype	Coupling	Reference
B2	Gi / Gq	Leeb-Lundberg <i>et al.</i> , 2005 in Pharmacol. Rev. 54 (1)

Fig. 15

Cannabinoid receptors		
Subtype	Coupling	References
CB2	Gi	Pacher <i>et al.</i> , 2006 In Pharmacol. Rev. 58 (3)
Calcium-sensing receptors		
Subtype	Coupling	References
CaS	Gi /Gq	Chang <i>et al.</i> ,1998 In: J Bone Miner Res., 13 ,
GPRC ₆		
Chemokine receptors		
Subtype	Coupling	Reference
CCR	Gi / Gq	Murphy <i>et al.</i> , 2000 In Pharmacol. Rev. 52 (1):
CXCR		

Dopamine receptors		
Subtype	Coupling	References
D2	Gi	
D3	Gi	
D4	Gi	
Frizzled receptors		
Subtype	Coupling	References
FZD2	Gi /Gq	Ma and Wang 2007 In: J Biol Chem, 282
SMO	Gi	Riobo et a., 2006 In: Proc Natl Acad Sci. 103

Fig. 15 (continued)

Free fatty acid receptors		
Subtype	Coupling	References
FFA2	Gi / Gq	Le Poul <i>et al.</i> , 2003
FFA3		In: J Biol Chem, 278 ,
Galanin receptors		
Subtype	Coupling	References
GAL1	Gi	Lang <i>et al.</i> , 2007
GAL2		In: Pharmacology and
GAL3		therapeutic 115 (2)
GABAB receptors		
Subtype	Coupling	References
GABA	Gi	Barnard et 1998 In:Pharmacol. Rev.50 (2)

Histamine receptors		
Subtype	Coupling	Reference
H3	Gi	Hill et al., 1997
H4		In pharmacol. Rev. 49 (3)
<i>Serotonin receptors</i>		
Subtype	Coupling	Reference
5HT1	Gi	<i>Hoyer et 1994</i>
5HT5	Gi	<i>In Pharmacol. Rev. 46 (2)</i>

Fig. 15 (continued)

Leukotriene receptors		
Subtype	Coupling	Reference
LTB	Gi / Gq	<i>Nabe et al., 1994</i> <i>Prostaglandins Leukot. Essent. Fatty Acids</i>
OXE	Gi	
Lysophospholipid receptors		
Subtype	Coupling	Reference
LPA	Gi / Gq	
SP	Gi	

Neuropeptide W/neuropeptide B receptors		
Subtype	Coupling	References
NPBW1	Gi	<i>Fujii et al., 2002</i> In: J. Biol. Chem. 277
NPBW2	Gi	<i>Brezillon et al., 2003</i> In: J. Biol. Chem. 278

Fig. 15 (continued)

Metabotropic glutamate receptors		
Subtype	Coupling	Reference
mGLu2	Gi	Tanabe et al., 1992 In: Neuron 8
mGLu3	Gi	Kingston et al., 1998 In: Neuropharmacology 37
mGLu4	Gi/Gq	<i>Tanabe 1993</i> <i>In: J Neurosci., 13</i> ; Brabet et al., 1998 In: Neuropharmacology 37
mGLU6	Gi	Laurie et al., 1997 In: Neuropharmacology 36
mGLu7	Gi/Gq	Kingston et al., 1998 In: Neuropharmacology 37; <i>Abe et al., 1992</i> In: J Biol. Chem. 267
mGLu8	Gi	Wu et al., 1998 In: Brain Res. Mol. Brain Res., 53,

Fig. 15 (continued)

Neuropeptide Y receptors		
Subtype	Coupling	References
Y1	Gi/Gq	Krause <i>et al.</i> , 1992 In: Mol. Pharmacol. 41; Herzog <i>et al.</i> , 1992 In: Proc. Natl. Acad. Sci. 89
Y2	Gi/Gq	Shigeri and Fujimoto 1994 In: J. Biol. Chem. 269; Lynch <i>et al.</i> , 1994 In: J. Biol. Chem. 269
Y4	Gi/Gq	Bard <i>et al.</i> , 1995 In: J. Biol. Chem. 270
Y5	Gi	Gerald <i>et al.</i> , 1996 In: Nature, 382
Neuropeptide FF/AF receptors		
Subtype	Coupling	References
NPFF1	Gi	Hinuma <i>et al.</i> , 2000 In: Nat Cell Biol 2
NPFF2	Gi	Elshourbagy <i>et al.</i> , 2000 In: J. Biol. Chem. 275
Nicotinic acid receptor family		
GPR81	Gi	Wise <i>et al.</i> , 2003 In: J. Biol. Chem. 278
GPR109A	Gi	Soga <i>et al.</i> , 23003 Biochem Biophys Res Commun, 303
GPR109B	Gi	Jung <i>et al.</i> , 2007. In: J. Med Chem. 50

Fig. 15 (continued)

Opioid receptors		
Subtype	Coupling	References
δ	Gi	Sharma <i>et al.</i> , 1977 In: Proc Natl Acad Sci 74
κ	Gi	Lawrence and Bidlack 1993 J Pharmacol Exp Ther, 266,
μ	Gi	Yu <i>et al.</i> , 1990 J Neurochem, 55
Orexin receptors		
Subtype	Coupling	References
OX ₁	Gi / Gq	Holmqvist <i>et al.</i> , 2005 In: J Biol Chem, 280
P2Y receptors		
Subtype	Coupling	References
P2Y ₁₂	Gi	Hollopeter <i>et al.</i> , 2001 Nature, 409
P2Y ₁₃	Gi	Marteau <i>et al.</i> , 2003. In: Mol Pharmacol, 64,
P2Y ₁₄	Gi	Scrivens and Dickenson 2005 Br J Pharmacol, 146
Prostanoid receptors		
Subtype	Coupling	References
DP2	Gi	Sawyer <i>et al.</i> , 2002 In: Br J Pharmacol, 137
Somatostatin receptors		
Subtype	Coupling	References
SST1	Gi	Rivier <i>et al.</i> , 2001., J Med Chem, 44,
SST2		Nunn <i>et al.</i> , 2003, Eur J Pharmacol, 465
SST3		Poitout <i>et al.</i> , 2001 J Med Chem, 44
SST4		Liu <i>et al.</i> , 1998, J Med Chem, 41

Fig. 15 (continued)

Ligands of Receptors Coupled to Gi Protein

Adenosine A1 receptor		
Agonists	References	Antagonists
(R,S)-PHPNECA	Volpini et al., 2002 <i>J Med Chem</i> , 45	AS100; AS70;
(R)-PIA	Rivkees et al., 1999, <i>J. Biol. Chem.</i> , 274	caffeine
(S)-PIA	Jockers, et al., 1994, <i>J Biol Chem</i> , 269,	CGS 15943
CCPA	Obiefuna et al 2005 <i>J Pharmacol Exp Ther</i> , 315,	CPT
CGS 21680	Gao et al., 2004 <i>Biochem Pharmacol</i> , 68	DPCPX,
2-chloroadenosine	Rivkees et al., 1999 <i>J. Biol. Chem.</i> , 274	Flavanone
CV-510	Peterman and Sanoski, () <i>Cardiol Rev</i> , 13	FR194921
cyclopentyladenosine	Rivkees et al., 1999 <i>J. Biol. Chem.</i> , 274	galangin
2-hexynyl-NECA	Volpini et al., 2002 <i>J Med Chem</i> , 45	IBMX
LUF5831	Heitman, et al., 2006 <i>Br J Pharmacol</i> , 147	L-97-1
NECA	Rivkees et al., 1999 <i>J. Biol. Chem.</i> , 274	morin
PENECA	Volpini et al., 2002 <i>J Med Chem</i> , 45	MRE 2029F20
		MRS1041, sakuranetin
		SCH 58261
		Theophylline, XAC
		ZM 241385
		References
		Varani, K., et al., 2005 <i>Biochem Pharmacol</i> , 70
		Deckert, ET AL., 1993 <i>Neurosci Lett</i> , 150
		Ongini, ET AL., 1999, <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 359,
		Dalpiatz, ET AL., 1998, <i>Biochem Pharmacol</i> , 56
		Rivkees, ET AL., 1999, <i>J. Biol. Chem.</i> , 274
		Karlon, et AL., 1996 <i>J Med Chem</i> , 39
		Maemoto, et al., 2004 <i>J Pharmacol Sci</i> , 96,
		Karlon, et AL., 1996 <i>J Med Chem</i> , 39
		Jockers et al., 1994, <i>J Biol Chem</i> , 269
		Obiefuna, et al., 2005, <i>J Pharmacol Exp Ther</i> , 315
		Karlon, et AL., 1996 <i>J Med Chem</i> , 39
		Varani, et al., 2005, <i>Biochem Pharmacol</i> , 70
		Karlon, et AL., 1996 <i>J Med Chem</i> , 39
		Ongini, et al., 1999, <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 359
		Jockers, et al., 1994, <i>J Biol Chem</i> , 269
		Ongini, et al., 1999, <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 359

Fig. 16 (continued)

Adenosine A3 receptor			
Agonists	References	Antagonists	References
(R,S)-PHPNECA	Volpiniet al., 2002 <i>J Med Chem</i> , 45	(R)-niguldipine	van Rhee et al., 1996 <i>J Med Chem</i> , 39
(R)-PIA	Olah et al., 1994 <i>J Biol Chem</i> , 269	(R,S)-nicardipin	
(S)-PIA		BW-A1433	Salvatoreet al.,1993 <i>Proc. Natl. Acad. Sci.</i> 90
AB-MECA	Arani et al., 2000 <i>Mol Pharmacol</i> , 57	CGS 15943	Varani et al., 2000 <i>Mol Pharmacol</i> , 57
[¹²⁵ I]AB-MECA		DPCPX	Olah et al., 1994 <i>J Biol Chem</i> , 269
[³ H]AB-MECA	Olah et al., 1994 <i>J Biol Chem</i> , 269	Flavone, Flavanone,	Karton et al., 1996 <i>J Med Chem</i> , 39
[³ H]APNEA	Zhou et al., 1992 <i>Proc. Natl. Acad. Sci.</i> 89	galagnin	
CCPA	Klotz et al., 1998 <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 357	I-ABOPX	Salvatoreet al.,1993 <i>Proc. Natl. Acad. Sci.</i> 90
CGS 21680		MRE 3008F20,	Varani et al., 2000 <i>Mol Pharmacol</i> , 57
CHIB-MECA	Jacobson et al., 1997 <i>Neuropharmacology</i> , 36	[³ H]MRE 3008F20	
cyclopentyladenosine	Salvatoreet al., 1993 <i>Proc. Natl. Acad. Sci.</i> 90	MRE 3010F20,	Karton et al.,1996, <i>J Med Chem</i> , 39
2-hexynyl-NECA	Volpiniet al., 2002 <i>J Med Chem</i> , 45	RS1041,	Jacobson et al., 1997 <i>Neuropharmacology</i> , 36
I-ABA	Salvatoreet al., 1993 <i>Proc. Natl. Acad. Sci.</i> 90	MRS1042 MRS1067,	van Rhee et al., 1996, <i>J Med Chem</i> , 39
		MRS1088, MRS1093,	Kim et al.,1996 <i>J Med Chem</i> , 39,
		MRS1097, MRS1177	
		MRS1186, MRS1191	
		MRS1191, MRS1220	
		MRS1476, MRS1486	
		MRS1505, MRS1523	
		MRS1523, MRS928	

Fig. 16 (continued)

Adenosine A3 receptor			
Agonists	References	Antagonists	References
IAB-MECA, IB-MECA	Klotz et al., 1998 <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 357	sakuranetin	Karton et al., 1996, <i>J Med Chem</i> , 39
MPC-MECA	Varani et al., 2000 <i>Mol Pharmacol</i> , 57	theophylline	Klotz et al., 1998 <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 357
NECA	Jacobson et al., 1997 <i>Neuropharmacology</i> , 36	visnagin	Karton et al., 1996, <i>J Med Chem</i> , 39
[³ H]NECA	Feoktistov et al., 2001 <i>Biochem Pharmacol</i> , 62	VUF8504	van et al., 1998 <i>J. Med. Chem.</i> , 41
PENECA	Volpini et al., 2002 <i>J Med Chem</i> , 45	VUF8507	
		XAC	Olah et al., 1994 <i>J Biol Chem</i> , 269

Fig. 16 (continued)

Adrenoreceptor Alpha2A		
Agonists	References	Antagonists
adrenaline		ARC-239
apomorphine		BRL 44408
brimondine		bromocriptine
clonidine	Asper et al., 1998 <i>Biochem Pharmacol.</i> , 55	cabergoline
dexmedetomidine		chlorpromazine
guanfacine		lisuride
noradrenaline		[³ H]MK-912
oxymetazoline		phenotolamine
pergolide	Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303	piribedil
xylazine	Asper et al., 1998 <i>Biochem Pharmacol.</i> , 55	prazosin
		rauwolscine
		[³ H]rauwolscine
		RX821002
		spiroxatrine
		terguride
		WB 4101
		yohimbine
		Uhlén et al., 1994 <i>J Pharmacol Exp Ther</i> , 271
		Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303
		Devedjian et al., 1994 <i>Eur J Pharmacol.</i> , 252,
		Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303
		Uhlén et al., 1994 <i>J Pharmacol Exp Ther</i> , 271
		Devedjian et al., 1994 <i>Eur J Pharmacol.</i> , 252
		Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303
		Devedjian et al., 1994 <i>Eur J Pharmacol.</i> , 252
		Uhlén et al., 1994 <i>J Pharmacol Exp Ther</i> , 27
		Bylund et al., 1992 <i>Mol Pharmacol.</i> , 42
		Uhlén et al., 1994 <i>J Pharmacol Exp Ther</i> , 271
		Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303
		Uhlén et al., 1994 <i>J Pharmacol Exp Ther</i> , 271

Fig. 16 (continued)

Adrenoreceptor Alpha2B		
Agonists	References	Antagonists
adrenaline		apomorphine
brimonidine		ARC-239
clonidine		BRL 44408
dexmedetomidine	Jasper et al., 1998 <i>Biochem Pharmacol.</i> , 55	bromocriptine
guanfacine		cabergoline
noradrenaline		chlorpromazine
oxymetazoline		lisuride
pergolide	Millan, et al., 2002 <i>J Pharmacol Exp Ther</i> , 303	[³ H]MK-912
xylazine	Jasper et al., 1998 <i>Biochem Pharmacol.</i> , 55	phenotolamine
		prazosin
		rauwolscine
		roxindole
		RX821002
		spiroxatrine
		terguride
		WB 4101
		yohimbine
		Uhlen et al., 1994 <i>J Pharmacol Exp Ther</i> , 271
		Millan, et al., 2002 <i>J Pharmacol Exp Ther</i> , 30
		Uhlen et al., 1994 <i>J Pharmacol Exp Ther</i> , 271
		Millan, et al., 2002 <i>J Pharmacol Exp Ther</i> , 30
		Bylund, et al., 1992 <i>Mol Pharmacol.</i> , 42,

Fig. 16 (continued)

Adrenoreceptor Alpha2C		
Agonists	Antagonists	References
adrenaline	apomorphine	Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303
brimonidine	ARC-239	Bylund et al., 1992, <i>Mol Pharmacol.</i> , 42,
clonidine	BRL 44408	Uhlén et al., 1994, <i>J Pharmacol Exp Ther</i> , 271
dexmedetomidine	bromocriptine	Millan, et al., 2002
guanfacine	cabergoline	<i>J Pharmacol Exp Ther</i> , 303
noradrenaline	chlorpromazine	Bylund et al., 1992, <i>Mol Pharmacol.</i> , 42,
Oxymetazoline	lisuride	Millan, et al., 2002
Pergolide	[³ H]MK-912	<i>J Pharmacol Exp Ther</i> , 303
xylazine	phentolamine	Uhlén et al., 1994, <i>J Pharmacol Exp Ther</i> , 271
	Piribedil, prazosin	Bylund et al., 1992, <i>Mol Pharmacol.</i> , 42,
	rauwolscine	Millan, et al., 2002
	roxindole	<i>J Pharmacol Exp Ther</i> , 303
	RX821002	Uhlén et al., 1994, <i>J Pharmacol Exp Ther</i> , 271
	spiroxatrine	Millan, et al., 2002
	Terguride, WB 4101	<i>J Pharmacol Exp Ther</i> , 303
	yohimbine	Uhlén et al., 1994, <i>J Pharmacol Exp Ther</i> , 271

Fig. 16 (continued)

Angiotensin AT2 receptors			
Agonists		References	
ang I		Whitebread et al, 1989 <i>Biochem. Biophys. Res. Commun.</i> , 163	
ang II		Speth and Kim 1990 <i>Biochem. Biophys. Res. Commun.</i> , 169	
[p-aminoPhe6] ang II		Whitebread et al, 1989 <i>Biochem. Biophys. Res. Commun.</i> , 163	
ang III			
CGP42112			
Antagonists		References	
PD123177		Chiu et al., 1989 <i>Biochem. Biophys. Res. Commun.</i> , 165	
PD123319		Wiest et al., 1991 <i>J. Cardiovasc. Pharmacol.</i> , 17	
Saralasin		Chiu et al., 1989 <i>Biochem. Biophys. Res. Commun.</i> , 165	

Apelin receptor			
Agonists		References	
[³ H](Pyr ¹)[Met(0)1]-apelin-13		Medhurst et al., 2003 <i>J Neurochem.</i> , 84,	
[¹²⁵ I](Pyr ¹)[Nle ⁷⁵ , Tyr ⁷⁷]-apelin-13		Hosoya et al., 2000 <i>J Biol Chem.</i> , 275	
[¹²⁵ I](Pyr ¹)apelin-13		Katugampola et al., 2001 <i>Br J Pharmacol.</i> , 132	
{ ¹²⁵ I}[Nle ⁷⁵ , Tyr ⁷⁷]apelin-36		Medhurst et al., 2003 <i>J Neurochem.</i> , 84,	
apelin-13		Medhurst et al., 2003 <i>J Neurochem.</i> , 84,	
apelin-36			
Pyr ¹ -apelin-13			
Antagonists		References	
ALX40-4C		Zhou et al., 2003 <i>Virology.</i> , 307	

Fig. 16 (continued)

Cannabinoid CB2 receptors		
Agonists	References	Antagonists
Δ^9 -THC	Felder et al., 1995 <i>Mol. Pharmacol.</i> , 48	
arachidonoyl ethanolamide		
2-arachidonoylglycerol	Mechoulam et al., 1995 <i>Biochem. Pharmacol.</i> , 50	
CP55940		
[^{3} H]CP55940	Felder et al., 1995 <i>Mol. Pharmacol.</i> , 48	
HU-210		
HU-308	Hanus et al., 1999 <i>Proc. Natl. Acad. Sci.</i> 96	
JWH-133	Huffman et al., 1999, <i>Bioorg. Med. Chem.</i> , 7	
L-759,633	Ross et al., 1999 <i>Br. J. Pharmacol.</i> , 126	
WIN55212-2	Felder et al., 1995 <i>Mol. Pharmacol.</i> , 48	
[^{3} H]WIN55212-2	Munro et al., 1993 <i>Nature</i> , 365	
		AM630
		Ross et al., 1999 <i>Br. J. Pharmacol.</i> , 126
		SR144528

Fig. 16 (continued)

Calcium-sensing CaS receptors			
Agonists	References	Antagonists	References
Ca ²⁺		1-aryl-methylpyrrolidin-2-yl ethanol amines	Gavai et al., 2005 <i>Bioorg Med Chem Lett</i> , 15
Mg ²⁺		2-benzylpyrrolidine-substituted aryloxypropanols	Yang et al., 2005 <i>Bioorg Med Chem Lett</i> , 15
Neomycin and other aminoglycosides	Riccardi et al., 1995 <i>Proc Natl Acad Sci</i> 92	Calhex 231	Petrel et al., 2003 <i>J Biol Chem</i> , 278
spermine	Quinn et al., 1997 <i>Am J Physiol</i> , 273	2-methyl-3-phenethyl-3H-pyrimidin-4-ones	Shcherbakova et al., 2005 <i>Bioorg Med Chem Lett</i> , 15
		N(1)-Arylsulfonyl-N(2)-(1-(1-naphthyl)ethyl)-1,2-diaminocyclohexane	Kessler et al., 2004, <i>Chembiotechnol</i> , 5
		NPS 2143	Nemeth et al., 2001 <i>J Pharmacol Exp Ther</i> , 299

Fig. 16 (continued)

Chemokine CCR1 receptors		
Agonists	references	
BP-CCL3	Zoffmann et al., 2001, <i>J Med Chem</i> , 44	
CCL14	Chou et al., 2002, <i>Br J Pharmacol</i> , 137	
CCL15	Coulin et al., 1997, <i>Eur J Biochem</i> , 248	
[¹²⁵ I]CCL2	Sarau et al., 1997, <i>J Pharmacol Exp Ther</i> , 283	
CCL23		
CCL3	Chou et al., 2002, <i>Br J Pharmacol</i> , 137	
CCL4		
CCL5	Combadiere et al., 1995, <i>J Biol Chem</i> , 270	
CCL7	Chou et al., 2002, <i>Br J Pharmacol</i> , 137	
[¹²⁵ I]CCL8	Gong et al., 1997, <i>J Biol Chem</i> , 272	
Flu-CCL3	Zoffmann et al., 2001, <i>J Med Chem</i> , 44	
MIP-1δ	Chou et al., 2002, <i>Br J Pharmacol</i> , 137	

Antagonists	References
BX 471	Liang et al., 2000, <i>J Biol Chem</i> , 275
vMIP-II	Kledal et al., 1997, <i>Science</i> , 277

Fig. 16 (continued)

Dopamine D2 receptor			
Agonists	References	Antagonists	References
6,7-ADTN	Lajiness et al., 1993, <i>J. Pharmacol. Exp. Ther.</i> , 267	(+)-butaclamol	Eorge et al., 1985, <i>Endocrinology</i> , 117
apomorphine		chlorpromazine	
bromocriptine		clozapine	
Dopamine	Sautel et al., 1995, <i>Neuroreport</i> , 6	domperidone	
lisuride		fluphenazine	
(-)-N-propylorapomorphine	Eorge et al., 1985, <i>Endocrinology</i> , 117	haloperidol	
7-OHDPAT		nemonapride	
pergolide		spiperone	
PD128907		S-sulpiride	
quinelorane			
quinpirole			

Fig. 16 (continued)

Dopamine D3 receptor		
Agonists	References	Antagonists
apomorphine	Sautel et al., 1995 <i>Neuroreport</i> , 6 Chio et al., 1994 <i>J. Biol. Chem.</i> , 269	U99194A
BP 897		
bromocriptine		
Dopamine		
7-OHDPAT		nafadootide
PD128907		
pramipexole		
quinelorane		
quinpirole		
		References
		Waters et al., 1993 <i>J. Neural Transm. Gen. Sect.</i> , 94
		Sautel et al., 1995 <i>J. Pharmacol. Exp. Ther.</i> , 275

Fig. 16 (continued)

Dopamine D4 receptor		
Agonists	References	Antagonists
(-)-apomorphine	Chio et al., 1994 <i>J. Biol. Chem.</i> , 269,	CP293019
bromocriptine		
di-propyl-ADTN		L745870
dopamine		
lisuride		NGD941
pergoline	Newman-Tancredi et al., 1997 <i>J. Pharmacol. Exp. Ther.</i> , 282	U101387
peribedil		
quinelorane		YM50001
quinpirole		clozapine

Fig. 16 (continued)

Frizzled FZD2 receptor		
Agonists	references	
WNT-5A	Ma and Wang, 2007 <i>J Biol Chem</i> , 282	

Frizzled SMO receptor		
Agonists	references	
Oxysterols	Corcoran and Scott 2006 <i>Proc Natl Acad Sci U S A</i> , 103	

Free fatty acid FFA2 receptor		
Agonists	References	
acetate	Nilsson et al., 2003 <i>Biochem Biophys Res Commun</i> , 303	
butyrate	Le Poul et al., 2003 <i>J Biol Chem</i> , 278	
isobutyrate	Brown et al., 2003 <i>J Biol Chem</i> , 278	
pentanoate	Le Poul et al., 2003 <i>J Biol Chem</i> , 278	
propionate	Xiong et al., 2004 <i>Proc Natl Acad Sci</i> , 101	

Free fatty acid FFA3 receptor		
Agonists	References	
acetate	Brown et al., 2003 <i>J Biol Chem</i> , 278	
butyrate	Xiong et al., 2004 <i>Proc Natl Acad Sci</i> , 101	
isobutyrate		
pentanoate		
propionate		

NB: Currently no antagonists are known for FZD2, SMO, FFA2 or FFA3

Fig. 16 (continued)

GABAB receptor		
Agonists	References	Antagonists
(-)-baclofen	Kaupmann et al., 1997 <i>Nature</i> , 386	CGP 35348
APPA		CGP 54626A
CGP 47656		CGP 56999A
GABA		CGP 62349
	Kaupmann et al., 1997 <i>Nature</i> , 386	CGP 64213
		[¹²⁵ I]-CGP 64213
		CGP 71872
		[¹²⁵ I]-CGP 71872
	Bolser et al., 1995 <i>J Pharmacol Exp Ther.</i> , 274	2-OH-saclofen
		aclofen
		SCH 50911

Fig. 16 (continued)

Histamine H3 receptor			
Agonists	References	Antagonists	References
(R)- α -methylhistamine	Lovenberg et al., 2000 <i>J Pharmacol Exp Ther</i> , 293	4-(3-piperidin-1-yl-propoxy)benzonitrile	Liu et al., 2001 <i>Mol Pharmacol</i> , 59,
(S)- α -methylhistamine	Wulff et al., 2002 <i>Eur J Pharmacol</i> , 453	1-[4-(3-piperidin-1-ylpropoxy)benzyl]piperidine	Apodaca et al., 2003 <i>J Med Chem</i> , 46
N-[³ H] α -methylhistamine	Chen et al., 2003 <i>Eur J Pharmacol</i> , 467	A-304121, A-317920, ABT-239	Esbenshade et al., 2004 <i>Biochem Pharmacol</i> , 68
N- α -methylhistamine	Liu et al., 2001 <i>Mol Pharmacol</i> , 59,	Burimamide,	Liu et al., 2001 <i>Mol Pharmacol</i> , 59,
dimaprit		ciproxifan	Rouleau et al., 2004 <i>J Neurochem</i> , 90
GR 175737, Perceptin®, proxyfan	Wulff et al., 2002 <i>Eur J Pharmacol</i> , 453	Clobenpropit, clozapine	Lovenberg et al., 2000 <i>J Pharmacol Exp Ther</i> , 293
histamine	Chen et al., 2003 <i>Eur J Pharmacol</i> , 467	FUB 349, FUB 465, GT 2394	Ligneau et al., 2000 <i>Br J Pharmacol</i> , 131
Imbutamine, iodoproxyfan	Kitbunnadaj et al., 2003 <i>J Med Chem</i> , 46,	Impentamine, iodophenpropit	Wieland et al., 2001 <i>J Pharmacol Exp Ther</i> , 299
imetit	Rouleau et al., 2004 <i>J Neurochem</i> , 90	[³]iodoproxyfan, proxyfan	Ligneau et al., 2000 <i>Br J Pharmacol</i> , 131
imnepip	Liu et al., 2001 <i>Mol Pharmacol</i> , 59,	JB 98064	Wulff et al., 2002 <i>Eur J Pharmacol</i> , 453
Impentamine, methimepip	Kitbunnadaj et al., 2003 <i>J Med Chem</i> , 46,	thioperamide	Wieland et al., 2001 <i>J Pharmacol Exp Ther</i> , 299
imprimidine	Liu et al., 2001 <i>Mol Pharmacol</i> , 59,	VUF 4904	
VUF 5207	Wieland et al., 2001 <i>J Pharmacol Exp Ther</i> , 299		

Fig. 16 (continued)

Histamine H4 receptor			
Agonists	References	Agonists	References
2-(3-bromophenyl)histamine (R)- α -methylhistamine (S)- α -methylhistamine N- α -methylhistamine CCL16 dimaprit Histamine, imetit HTMT im nepip improgan impromidine methimepip methylhistamine VUF 8430	Lim et al., 2005 <i>J Pharmacol Exp Ther</i> , 314	burinamide	Liu et al., 2001 <i>J Pharmacol Exp Ther</i> , 299
	Liu et al., 2001 <i>J Pharmacol Exp Ther</i> , 299	5-chloroindole-2-piperazinecarboximide	Terzioglu et al., 2004 <i>Bioorg Med Chem Lett</i> , 14
	Morse et al., 2001 <i>J Pharmacol Exp Ther</i> , 296	ciproxifan	Esbenshade et al., 2004 <i>Biochem Pharmacol</i> , 68,
	Nakayama et al., 2004 <i>J Immunol</i> , 173,	clobenpropit	Liu et al., 2001 <i>J Pharmacol Exp Ther</i> , 299
	Liu et al., 2001 <i>Mol Pharmacol</i> , 59	iodophenpropit	Zhu et al., 2001 <i>Mol Pharmacol</i> , 59
	Liu et al., 2001 <i>J Pharmacol Exp Ther</i> , 299	JNJ 7777120	Thurmond et al., 2004 <i>J Pharmacol Exp Ther</i> , 309
	Zhu et al., 2001 <i>Mol Pharmacol</i> , 59	[³ H]pyrilamine	Nguyen et al., 2001 <i>Mol Pharmacol</i> , 59
	Lim et al 2005 <i>J Pharmacol Exp Ther</i> , 314	thiopetamide	Liu et al., 2001 <i>J Pharmacol Exp Ther</i> , 299
	Kitbunnadaj et al., 2005 <i>J Med Chem</i> , 48,		
	Lim et al 2005 <i>J Pharmacol Exp Ther</i> , 314		
Lim et al., 2006 <i>J Med Chem</i> , 49			

Fig. 16 (continued)

Leukotriene BLT1 receptor		
Agonists	References	Antagonists
20-COOH-LTB ₄	Devchand et al., 1996 <i>Nature</i> , 384	U75302
12R-HETE		
12-keto-LTB ₄		
20-OH-LTB ₄		
LTB ₄		
		Devchand et al., 1996 <i>Nature</i> , 384

Fig. 16 (continued)

Melatonin MT1 receptor		
Agonists	References	Antagonists
2-[¹²⁵ I]MLT	Audinot et al., 2003 <i>Naunyn Schmiedeberg's Arch Pharmacol</i> , 367	4P-PDOT
agomelatine		
6-CI-MLT		
GR 128107	Teh and Sugden 1999, <i>Br J Pharmacol</i> , 126	K185
GR 196429	Browning et al., 2000 <i>Br. J. Pharmacol.</i> , 129	
5-HEAT	Nonno et al., 2000 <i>J Pineal Res.</i> , 29, 23	luzindole
2-I-MLT	Audinot et al., 2003 <i>Naunyn Schmiedeberg's Arch Pharmacol</i> , 367	
LIK7	Faust et al., 2000 <i>J Med Chem</i> , 43	
LY 156735	Mulchahey et al., 2004 <i>Life Sci</i> , 75	S20928
[³ H]MLT		
6-OH-MLT	Browning et al., 2000 <i>Br. J. Pharmacol.</i> , 129	S22153
S24014		
S24773	Audinot et al., 2003 <i>Naunyn Schmiedeberg's Arch Pharmacol</i> , 367	
S26284		
TAK375	Kato et al., 2005 <i>Neuropharmacology</i> , 48	S26131
		Audinot et al., 2003 <i>Naunyn Schmiedeberg's Arch Pharmacol</i> , 367
		Faust et al., 2000 <i>J Med Chem</i> , 43
		Audinot et al., 2003 <i>Naunyn Schmiedeberg's Arch Pharmacol</i> , 367

Fig. 16 (continued)

Metabotropic glutamate mGlu2 receptor			
Agonists	References	Antagonists	References
(1S,3R)-ACPD	Johnson et al., 1999 <i>Neuropharmacology</i> , 38	eGlu	Schweitzer et al., 2000 <i>Neuropharmacology</i> , 39
(2R,3R)-APDC		LY341495	Johnson et al., 1999 <i>Neuropharmacology</i> , 38
(S)-4C3HPG	Cartmell et al., 1998 <i>Br J Pharmacol</i> , 123	[³ H]LY341495	
DCG-IV		(+)-MCPG	Cartmell et al., 1998 <i>Br J Pharmacol</i> , 123
L-CCG-I	Johnson et al., 1999 <i>Neuropharmacology</i> , 38	MGS0039	Chaki et al., 2004 <i>Neuropharmacology</i> , 46
L-glutamate		MSOP	Cartmell et al., 1998 <i>Br J Pharmacol</i> , 123
LY354740			
[³ H]LY35740	Schweitzer et al., 2000 <i>Neuropharmacology</i> , 39,		
LY379268	Monn et al., 1999 <i>J Med Chem</i> , 42,		
MGS0028	Nakazato et al., 2000 <i>J Med Chem</i> , 43		

Fig. 16 (continued)

Metabotropic glutamate mGlu3 receptor		
Agonists	References	Antagonists
(1S,3R)-ACPD (2R,4R)-APDC	Johnson et al., 1999 <i>Neuropharmacology</i> , 38	eGlu
DCG-IV	Cartmell et al., 1998 <i>Br J Pharmacol.</i> , 123	LY341495
L-CCG-I	Johnson et al., 1999 <i>Neuropharmacology</i> , 38	[³ H]LY341495
L-glutamate		
[³ H]LY341495 LY354740	Schweitzer et al., 2000 <i>Neuropharmacology</i> , 39,	Cartmell et al., 1998 <i>Br J Pharmacol.</i> , 123
LY379268	Monn et al., 1999 <i>J Med Chem.</i> , 42,	(+)-MCPG
NAAG	Schweitzer et al., 2000 <i>Neuropharmacology</i> , 39	MGS0039
		Chaki et al., 2004 <i>Neuropharmacology</i> , 46

Fig. 16 (continued)

Metabotropic glutamate mGlu4 receptor		
Agonists	References	Agonists
(R,S)-4-PPG	Gasparini et al., 1999 <i>J. Pharmacol. Exp. Ther.</i> , 289	CPPG
(S)-3,4-DCPG	Thomas et al., 2001 <i>Neuropharmacology</i> , 40	
ACPT-I	Acher et al., 1997 <i>J Med Chem</i> , 40,	LY341495
[³ H]AP4	Han and Hampson 1999 <i>J Biol Chem.</i> , 274,	
FP0429	Muto et al., 2007 <i>Proc Natl Acad Sci U S A</i> , 104	MAP4
L-AP4	Acher et al., 1997 <i>J Med Chem</i> , 40	
L-CCG-I	Hayashi et al., 1992 <i>Br J Pharmacol.</i> , 107	MPPG
L-glutamate	Han and Hampson 1999 <i>J Biol Chem.</i> , 274	
L-SOP		

Fig. 16 (continued)

Metabotropic glutamate mGlu6 receptor		
Agonists	References	Antagonists
(1S,3R)-ACPD	Tuckmantel et al., 1997 <i>Bioorg Med Chem Lett.</i> , 7	Brabet et al., 1998 <i>Neuropharmacology.</i> , 37
(1S,3R)-ACPD	Laurie et al., 1997 <i>Neuropharmacology.</i> , 36	
(2S,1S,2S)-L-CCG-I		
(R,S)-4-PPG	Thomas et al., 2001 <i>Neuropharmacology.</i> , 40	[³ H]LY341495
(S)-3,4-DCPG		
ACPT-I	Uckmantel et al., 1997 <i>Bioorg Med Chem Lett.</i> , 7,	Pin and Acher 2002 <i>Curr Drug Targets CNS Neurol Disord.</i> , 1
1-benzyl-APDC		
L-AP4	Laurie et al., 1997 <i>Neuropharmacology.</i> , 36	Ma et al., 1997 <i>Bioorg Med Chem Lett.</i> , 7,
L-glutamate		
L-SOP		
LY354740	Monn et al., 1999 <i>J Med Chem.</i> , 42	Pin and Acher 2002 <i>Curr Drug Targets CNS Neurol Disord.</i> , 1
LY379268		

Fig. 16 (continued)

Metabotropic glutamate mGlu7 receptor		
Agonists	References	Antagonists
(1S,3R)-ACPD	Wright et al., 2000 <i>Naunyn Schmiedebergs Arch Pharmacol.</i> , 362	DCG-IV LY341495
(R,S)-4-PPG	Gasparini et al., 1999 J. <i>Pharmacol. Exp. Ther.</i> , 289	[³ H]LY341495
L-AP4		MAP4
L-CCG-I		MCCG
L-glutamate		(+)-MCPG
L-SOP	Wright et al., 2000 <i>Naunyn Schmiedebergs Arch Pharmacol.</i> , 362	MPPG
PPG		MSOP
		MSOPPE

Fig. 16 (continued)

Metabotropic glutamate mGlu8 receptor		
Agonists	References	Antagonists
(1S,3R)-1-ACPD	Wu et al., 1998 <i>Brain Res. Mol. Brain Res.</i> , 53,	
(R,S)-4-PPG	Thomas et al., 2001 <i>Neuropharmacology</i> , 40	CPPG <i>Brain Res Mol Brain Res</i> , 76
(S)-3,4-DCPG	de Colle et al., 2000 <i>Eur J Pharmacol</i> , 394	
ACPT-I	Malherbe et al., 1999 <i>Brain Res. Mol. Brain Res.</i> , 67	DCG-IV <i>Brain Res. Mol. Brain Res.</i> , 67
[³ H]AP4	Wu et al., 1998 <i>Brain Res. Mol. Brain Res.</i> , 53	LY341495 <i>Neuropharmacology</i> , 37
D-AP4		
L-AP4		MAP4 <i>Brain Res Mol Brain Res</i> , 76
L-CCG-I	Malherbe et al., 1999 <i>Brain Res. Mol. Brain Res.</i> , 67	
L-CCG-I		
L-glutamate		Pin and Acher 2002 <i>Curr Drug Targets CNS Neural Disord.</i> , 1
L-SOP		
LY354740		

Fig. 16 (continued)

Neuropeptide W/neuropeptide B receptors			
NPBW1		NPBW2	
Agonists	References	Agonists	Antagonists
[¹²⁵ I]Tyr ¹¹]des-Br-NPB-23		NPB-23	Brezillon et al., 2003 <i>J Biol Chem.</i> , 278
Br-NPB-29	Fujii et al., 2002 <i>J Biol Chem.</i> , 277	NPB-29	
des-Br-NPB-23			
des-Br-NPB-29		[¹²⁵ I]NPW	Shimomura et al., 2002 <i>J Biol Chem.</i> , 277
NPW-23			
[¹²⁵ I]NPW-23	Shimomura et al., 2002 <i>J Biol Chem.</i> , 277	NPW-23	Brezillon et al., 2003 <i>J Biol Chem.</i> , 278
NPW-30		NPW-30	

NB: No antagonists are currently available neither for NPBW1 nor for NPBW2

Fig. 16 (continued)

Neuropeptide Y2 receptors			
Agonists	References	Antagonists References	
N- α -Ac-PYY ₂₅₋₃₆	Goumain, et al., 2001 <i>Mol Pharmacol</i> , 60	BIE0246	Goumain et al., 2001 <i>Mol Pharmacol</i> , 60
C2-NPY	Gerald et al., 1995 <i>J Biol Chem</i> , 270		
[¹²⁵ I]NPY	Rose et al., 1995 <i>J Biol Chem</i> , 270, Gehlert et al., 1996 <i>Mol Pharmacol</i> , 49		
[D-Trp ³²]-NPY			
NPY (human)	Gerald et al., 1995 <i>J Biol Chem</i> , 270		
[Leu ³¹ ,Pro ³⁴]-NPY			
[Ala ³¹ ,Alb ³²]-NPY	Cabrele, et al., 2002 <i>Biochemistry</i> , 41		
NPY ₁₃₋₃₆ (human)			
NPY ₁₆₋₃₆ (porcine)			
NPY ₃₋₃₆ (porcine)			
[¹²⁵ I]PYY			
PYY (human)	Gerald et al., 1995 <i>J Biol Chem</i> , 270		
[Leu ³¹ ,Pro ³⁴]-PYY (human)			
[Pro ³⁴]-PYY (human)			
PYY (porcine)			
PYY ₁₃₋₃₆ (porcine)			

Fig. 16 (continued)

Neuropeptide Y4 receptors		
Agonists	References	Antagonists
GR231118	Tough et al., 2006, <i>J Pharmacol Exp Ther</i> , 319	1229U91
NPY, [Leu ³¹ ,Pro ³⁴]-NPY	Gregor et al., 1996, <i>FEBS Lett</i> , 381	
[Ala ³¹ ,Alb ³²]-NPY	Cabrele et al., 2002 <i>Biochemistry</i> , 41	Gehlert et al., 1997 <i>Peptides</i> , 18
NPY ₁₃₋₃₆	Lundell et al., 1995 <i>J Biol Chem</i> , 270	
NPY ₂₋₃₆		
PP		
[Ile ³¹ ,Gln ³⁴]-PP	Walker et al., 1997 <i>Peptides</i> , 18	
PP ₂₀₋₃₆		
PYY	Gregor et al., 1996, <i>FEBS Lett</i> , 381	
[Leu ³¹ ,Pro ³⁴]-PYY	Dumont and Quirion 2000 <i>Br J Pharmacol</i> , 129	
[Leu ³¹ ,Pro ³⁴]-PYY	Gehlert et al., 1997 <i>Peptides</i> , 18	
[Pro ³⁴]-PYY	Tough et al., 2006 <i>J Pharmacol Exp Ther</i> , 319	
[¹²⁵ I]PYY	Lundell et al., 1995 <i>J Biol Chem</i> , 270,	
PYY ₃₋₃₆ (human)	Walker et al., 1997 <i>Peptides</i> , 18	

Fig. 16 (continued)

Neuropeptide Y5 receptors		
Agonists	References	Antagonists
[¹²⁵ I][PP ₁₋₁₇ ,Ala ³¹ ,Aib ³²]-NPY	Dumont et al., 2003 <i>Br J Pharmacol</i> , 139	CGP 71683A Dumont et al., 2003 <i>Br J Pharmacol</i> , 139
GR231118		
[Ala ³¹ ,Aib ³²]-NPY		FMS586 Kakui et al., 2006 <i>J Pharmacol Exp Ther</i> , 317
[D-Trp ³²]-NPY		
[PP ₁₋₁₇ ,Ala ³¹ ,Aib ³²]-NPY	Hu et al., 1996 <i>J Biol Chem</i> , 271	JCF 109 Dumont et al., 2003 <i>Br J Pharmacol</i> , 139
NPY		
[Ala ³¹ ,Aib ³²]-NPY	Cabrele et al., 2002 <i>Biochemistry</i> , 41	
[Leu ³¹ ,Pro ³⁴]-NPY		L-152,804 Kanatani et al., 2000 <i>Biochem Biophys Res Commun</i> , 272
NPY ₂₋₃₆		
PP	Hu et al., 1996 <i>J Biol Chem</i> , 271	
PYY		
PYY ₃₋₃₆		L-152,804

Fig. 16 (continued)

Neuropeptide FF/neuropeptide AF receptors		
NPFF1		
Agonists	References	Antagonists References
1DMe		
[¹²⁵ I]1DMe		
EFWSLAAPQRF-NH ₂	Bonini et al., 2000 <i>J Biol Chem</i> , 275	Mollereau et al., 2002 <i>Eur J Pharmacol</i> , 451
NPFF	Gouardères et al., 2002 <i>Neuroscience</i> , 115,	
PP (frog)		
RFRP-3 (human)	Mollereau et al., 2002 <i>Eur J Pharmacol</i> , 451	
Y-RFRP-3		RF9 Simonin et al., 2006, <i>Proc Natl Acad Sci U S A</i> , 103
[¹²⁵ I]Y-RFRP-3		

Fig. 16 (continued)

Neuropeptide FF/neuropeptide AF receptors		
NPFF2		
Agonists	References	Antagonists
1DMe	Gouardères et al., 2002 <i>Neuroscience</i> , 115,	
dNPA	Quelven et al., 2005 <i>Eur J Pharmacol</i> , 508	
EFWSLAAPQRF-NH ₂	Mollereau et al., 2002 <i>Eur J Pharmacol</i> , 451	BIBP3226
NPFF	Mollereau et al., 2001 <i>Br J Pharmacol</i> , 133	
PP (frog)		
RFRP-3 (human)	Mollereau et al., 2002 <i>Eur J Pharmacol</i> , 451	
YVPNLPQRF-NH ₂		RF9
		Simonin et al., 2006, <i>Proc Natl Acad Sci U S A</i> , 103

Fig. 16 (continued)

Nicotinic acid receptor family			
GPR81		GPR109A	
Agonists	references	Agonists	References
Nicotinic acid	Wise et al., 2003 <i>J Biol Chem</i> , 278	(+)-5-(5-bromothiophen-3-yl)-5-methyl-4-oxo-4,5-dihydro-furan-2-carboxylic acid	Taggart et al., 2005 <i>J Biol Chem</i> , 280
GPR109B		D-β-hydroxybutyrate	
		acifran	
		acipimox	Wise et al., 2003 <i>J Biol Chem</i> , 278
		5-butyl-1H-pyrazole-3-carboxylic acid	van Herk et al., 2003 <i>J Med Chem</i> , 46
		5-methyl nicotinic acid	
		nicotinic acid	Wise et al., 2003 <i>J Biol Chem</i> , 278
acifran	Jung et al., 2007 <i>J Med Chem</i> , 50	[³ H]nicotinic acid	
5-ethyl-4-oxo-5-phenyl-4,5-dihydro-furan-2-carboxylic acid	Sample et al., 2006 <i>J Med Chem</i> , 49	3-pyridine-acetic acid	
1-isopropylbenzotriazole-5-carboxylic acid	Jung et al., 2007 <i>J Med Chem</i> , 50		
5-methyl-5-(5-methyl-triophen-3-yl)-4-oxo-4,5-dihydro-furan-2-carboxylic acid	Wise et al., 2003 <i>J Biol Chem</i> , 278		
nicotinic acid			

NB: Currently no antagonists are known for GPR81, GPR109A or GPR109B

Fig. 16 (continued)

Opioid δ Receptor		
Agonists	References	Antagonists
β endorphin, (-)-bremazocine (-)-cyclazocine (-)-EKC (-)-pentazocine DADLE deltorphin II dihydromorphine diprenorphine [³ H]diprenorphine DPDPE DSLET dynorphin 1-11 dynorphin 1-13 dynorphin 1-17 dynorphin 1-8 dynorphin B EKC endomorphin-1 [Leu]-enkephalin [Met]-enkephalin etonitazene etorphine fentanyl morphine nalmeferone nalorphine α -neoendorphin normorphine SNC80	Raynor et al., 1994 <i>Mol Pharmacol</i> , 45 Toll et al., 1998 <i>NIDA Res Monogr</i> , 178 Yasuda et al., 1993 <i>Proc. Natl. Acad. Sci.</i> , 90 Neilan et al., 1999 <i>Br J Pharmacol</i> , 128, Raynor et al., 1994 <i>Mol Pharmacol</i> , 45 Quock et al., 1997 <i>Eur J Pharmacol</i> , 326	(-)-naloxone (-)-quadazocine BNTX CTAP β -FNA ICI 174,864 naloxone naltrexone naltrexone naltrindole nor-binaltorphimine TIPP ψ

Fig. 16 (continued)

Opioid K receptor		
Agonists	References	Antagonists
β endorphin (human) ($\pm$)-EKC (-)-bremazocine (-)-cyclazocine (-)-EKC (-)-methadone (-)-pentazocine bremazocine CI 977 DAMGO dihydromorphine dynorphin 1-11 dynorphin 1-13 dynorphin 1-13 [D-Ala ² , F, Phe ⁴]-dynorphin 1-13-NH ₂ dynorphin 1-17 [Met ⁵]-dynorphin 1-17 dynorphin 1-17-NH ₂ [D-Ala ² , F, Phe ⁴]-dynorphin 1-17-NH ₂ dynorphin 1-8 dynorphin B E2078 EKC enadoline [Leu]-enkephalin [Met]-enkephalin etonitazene etorphine fentanyl GR 89696	Toll et al., 1998 <i>NIDA Res Monogr</i> , 178 Toll et al., 1998 <i>NIDA Res Monogr</i> , 178 Zhu et al., 1997 J <i>Pharmacol Exp Ther</i> , 282 Yasuda et al., 1993 <i>Proc. Natl. Acad. Sci.</i> , 90 Hjorth et al., 1996 <i>Mol Pharmacol</i> , 50 Meng et al., 1993 <i>Proc Natl Acad Sci</i> 90 Neumeier et al., 2003 <i>J Med Chem</i> , 46	(-)-naloxone (-)-quadazocine BNTX buprenorphine diprenorphine [³ H]diprenorphine β -FNA GNTI nalmeferene (+)-naloxone naltrexone naltriben naltrindole nor- binaltorphimine
		Toll et al., 1998 <i>NIDA Res Monogr</i> , 178 Yasuda et al., 1993 <i>Proc. Natl. Acad. Sci.</i> , 90 Simonin et al., 1995 <i>Proc Natl Acad Sci</i> 92 Jones and Portoghese 2000 <i>Eur J Pharmacol</i> , 396 Chen et al., 1993 <i>Biochem J</i> , 295

Fig. 16 (continued)

Opioid K receptor		
Agonists	References	
ICI 204448		
morphine		
nalorphine		
naloxone benzoylhydrazone	Toll et al., 1998 <i>NIDA Res Monogr</i> , 178	
α -neoendorphin	Zhu et al., 1997 <i>J Pharmacol Exp Ther</i> , 282	
β -neoendorphin	Yasuda et al., 1993 <i>Proc. Natl. Acad. Sci.</i> , 90	
normorphine		
pentazocine	Hjorth et al., 1996 <i>Mol Pharmacol</i> , 50	
salvinorin A	Meng et al., 1993 <i>Proc Natl Acad Sci</i> 90	
tifluadom	Neumeyer et al., 2003 <i>J Med Chem</i> , 46	
TRK820		
U50488		
U63640		
U69593		
[³ H]U69593		

Fig. 16 (continued)

Opioid μ receptor		
Agonists	References	Antagonists
β endorphin (-)-cyclazocine (-)-EKC (-)-methadone (-)-pentazocine buprenorphine codeine DADLE DAMGO [tyrosyl 3,5- ^{3} H]DAMGO dihydromorphine DSLET dynorphin 1-11 dynorphin 1-13 dynorphin 1-17 dynorphin 1-8 dynorphin B endomorphin-1 [Leu]-enkephalin [Met]-enkephalin etonitazene etorphine fentanyl morphine normorphine PL017	Raynor et al., 1994 <i>Mol Pharmacol</i> , 45 Toll et al., 1998 <i>NIDA Res Monogr</i> , 178 Gong et al., 1998 <i>FEBS Lett</i> , 439	(-)-bremazocine (-)-naloxone (-)-naloxone (-)-quadazocine BNTX CTAP CTOP diprenorphine diprenorphine [15,16- ^{3} H]diprenorphine ^{3} H]diprenorphine β -FNA β -FNA nalmefene nalorphine naloxonazine [N-allyl-2,3- ^{3} H]naloxone naloxone benzoylhydrazone naloxone benzoylhydrazone naltrexone naltriben naltrindole nor- binaltorphimine
	Raynor et al., 1994 <i>Mol Pharmacol</i> , 45	
	Toll et al., 1998 <i>NIDA Res Monogr</i> , 178	
	Gong et al., 1998 <i>FEBS Lett</i> , 439	

Fig. 16 (continued)

Orexin OX1 receptor			
Agonists	references	Antagonists	References
Orexin-A	Langmead et al., 2004 <i>Br J Pharmacol</i> , 141	1-(2,4-dibromo-phenyl)-3-((4S,5S)-2,2-dimethyl-4-phenyl-[1,3]dioxan-5-yl)-urea	McAtee et al., 2004 <i>Bioorg Med Chem Lett</i> , 14
Orexin-A 2-33	Lang et al., 2004 <i>J Med Chem</i> , 47	1-(2-bromo-phenyl)-3-((4S,5S)-2,2-dimethyl-4-phenyl-[1,3]dioxan-5-yl)-urea	Porter et al., 2001 <i>Bioorg Med Chem Lett</i> , 11
Orexin-B	Langmead et al., 2004 <i>Br J Pharmacol</i> , 141	SB-334867	
		SB-408124	
		SB-410220	Langmead et al., 2004 <i>Br J Pharmacol</i> , 141
[Ala ¹¹ , D-Leu ¹¹]Orexin-B	Asahi et al., 2003 <i>Bioorg Med Chem Lett</i> , 13	[³ H]SB-674042	

Fig. 16 (continued)

P2Y ₁₂ receptor		
Agonists	References	Antagonists
2MeSADP	Herbert and Savi 2003 <i>Semin Vasc Med</i> , 3	2MeSAMP
[³ H]2MeSADP	Takasaki et al., 2001 <i>Mol Pharmacol</i> , 60	active metabolite of clopidogrel
2MeSATP	Herbert and Savi 2003 <i>Semin Vasc Med</i> , 3	AR-C69931MX
ADP	Takasaki et al., 2001 <i>Mol Pharmacol</i> , 60	
ADPβS	Herbert and Savi 2003 <i>Semin Vasc Med</i> , 3	pCMPS
ATP		
ATPγS		

Fig. 16 (continued)

P2Y13 receptor			
Agonists	References	Antagonists	References
2MeSADP	Marteau et al., 2003 <i>Mol Pharmacol</i> , 64,	2MeSAMP	Marteau et al., 2003 <i>Mol Pharmacol</i> , 64,
[³³ P]2MeSADP		Ap ₄ A	
2MeSATP		AR-C67085MX	
ADP		AR-C69931MX	
ADPβS		PPADS	
ATP		reactive blue 2	
ATPyS		suramin	

P2Y14 receptor	
Agonists	References
UDP-glucose	Freeman et al., 2001 <i>Genomics</i> , 78
UDP-glucuronic acid	
UDP-N-acetylglucosamine	

NB: No antagonists are known for P2Y14

Fig. 16 (continued)

Prostanoid DP2 receptors		
Agonists	References	Antagonists References
Δ^{12} -PGJ ₂		
13,14-dihydro-15-keto-PGD ₂		
13,14-dihydro-15-keto-PGF _{2a}		
15(R)-15-methyl-PGD ₂	Sawyer et al., 2002 <i>Br J Pharmacol</i> , 137	Hata et al., 2005 <i>J Biol Chem</i> , 280
15(S)-15-methyl-PGD ₂		
15-deoxy- $\Delta^{12,14}$ -PGD ₂	Hata et al., 2003 <i>J Pharmacol Exp Ther</i> , 306	
15-deoxy- $\Delta^{12,14}$ -PGJ ₂		
CAY 10471	Ulven and Kostenis 2005 <i>J Med Chem</i> , 48	
indomethacin		
L-883,595	Gervais et al., 2005 <i>Mol Pharmacol</i> , 67	[³ H]ramatroban
L-888,291		
L-888,607	Shichijo et al., 2003 <i>J Pharmacol Exp Ther</i> , 307	
PGD ₂		
[³ H]PGD ₂		
PGD ₃		
PGE ₂		
PGF _{2a}		
PGJ ₂		
U46619		

Fig. 16 (continued)

Serotonin 5-HT _{1A} receptor		
Agonists	References	Antagonists
(-)-quinpirole		(-)-propranolol
apomorphine		(-)-tertatolol
ariprazole		(R)-flurocarazolol
BMY-14802	Newman-Tancredi et al., 1999 <i>Naunyn Schmiedeberg's Arch Pharmacol</i> , 359	(S)-flurocarazolol
BMY-7378		[³ H]-p-MPPF
BRL-15572	Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303	p-MPPI
bromocriptine		(+)-butaclamol
buspirone	Shapiro et al., 2003 <i>Neuropsychopharmacology</i> , 28	chlorpromazine
cabergoline		cyamemazine
clozapine		fluspirilene
CP 93129	Newman-Tancredi et al., 1998 <i>Eur J Pharmacol</i> , 355	GR 125,743
5-CT		GR 218,231
donitriptan	Khawaja et al., 1997 <i>Life Sci</i> , 60	haloperidol
eletriptan		iloperidone
EMDT	Price et al., 1997 <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 356	iloperidone
FG-5893		ketanserine
(+)-flesinoxan	Newman-Tancredi et al., 1992 <i>Biochem J</i> , 285	methiothepin
fluparoxan		MPDT
GR-127935		NAD 299
5-HT		[³ H]NAD 299
		Newman-Tancredi et al., 1997 <i>Naunyn Schmiedeberg's Arch Pharmacol</i> , 355
		Newman-Tancredi et al., 1998 <i>Eur J Pharmacol</i> , 355
		Roth et al., 2001 <i>Psychopharmacology (Berl)</i> , 157
		Kalipatnapu et al., 2004 <i>Biosci Rep</i> , 24
		Hameg et al., 2003 <i>Biochem Pharmacol</i> , 65,
		Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124
		Millan et al., 2000 <i>J Pharmacol Exp Ther</i> , 293

Fig. 16 (continued)

Serotonin 5-HT _{1A} receptor		
Agonists	References	Antagonists
ipsapirone		NAN 190
L-694,247		9-OH-risperidone
L-772,405		pimozide
lisuride		pindolol
LSD	Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303	pipamperone
LY293284		pizotifen
LY334370	Newman-Tancredi et al., 1998 <i>Eur J Pharmacol</i> , 355	raclopride
LY344864		Rec 15/3079
7-methoxy-1-naphthylpiperazine	Newman-Tancredi et al., 1992 <i>Biochem J</i> , 285	repinotan
nafadotride	John et al., 1999 <i>J Pharmacol Exp Ther</i> , 290	risperidone
1-naphthylpiperazine		risperidone
naratriptan		ritanserlin
ocaperidone	Napier et al., 1999 <i>Eur J Pharmacol</i> , 368	SB 272183
8-OH-DPAT		SB 649915
[³ H]8-OH-DPAT	Millan et al., 2000 <i>Synapse</i> , 35	SB 714786
olanzapine		SDZ-216525
ORG-5222		sertindole
PAPP		spiperone
pergolide		thioridazine
		Newman-Tancredi et al., 1997 <i>Naunyn-Schmiedeberg's Arch Pharmacol</i> , 355
		Kalkman et al., 2001 <i>Neuropsychopharmacology</i> , 25
		Kongsamut et al., 1996 <i>Eur J Pharmacol</i> , 317
		Newman-Tancredi et al., 1992 <i>Biochem J</i> , 285
		Newman-Tancredi et al., 1998 <i>Eur J Pharmacol</i> , 355

Fig. 16 (continued)

Serotonin 5-HT _{1A} receptor		
Agonists	References	Antagonists
piribedil		
quetiapine		
rizatriptan	Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303	tiospirone
roxindole		(+)-UH 301
RU 24969	Newman-Tancredi et al., 1998 <i>Eur J Pharmacol</i> , 355	WAY-100135
S-14506		
S-14671	Newman-Tancredi et al., 1992 <i>Biochem J</i> , 285	
S-15535		
[³ H]S-15535		
S-16924	Russell et al., 1999 <i>J Med Chem</i> , 42	WAY-100635
SB 216641		
SL65.0155	Newman-Tancredi et al., 1997 <i>Naunyn Schmiedeberg's Arch Pharmacol</i> , 355	yohimbine
spiroxatrine		
sumatriptan	Nichols et al., 2002 <i>J Med Chem</i> , 45	
tandospirone		
terguride		
U92012A		
xanomeline	Blair et al., 2000 <i>J Med Chem</i> , 43	
zalospirone		
ziprasidone		
zolmitriptan		

Fig. 16 (continued)

Serotonin 5-HT _{1B} receptor		
Agonists	References	Antagonists
5-(nonyloxy)-tryptamine		(-)-pindolol
alniditan	Granas and Larhammar 1999 <i>Eur J Pharmacol</i> , 380	(R)-flurocarazodolol
[³ H]alniditan		(S)-flurocarazodolol
aripiprazole		cyanopindolol
BMS 181,101	Leysen et al., 1996 <i>Mol Pharmacol</i> , 50	[³ H]GR-125743
BRL-15572		GR-127935
bromocriptine		ketanserin
cabergoline	Lesage et al., 1998 <i>Br J Pharmacol</i> , 123	ketanserin
CGS-12066		metergoline
clozapine		methiothepin
CP-122288	Shapiro et al., 2003 <i>Neuropsychopharmacology</i> , 28	methysergide
5-CT		mianserin
dihydroergotamine	Newman-Tancredi et al., 2000 <i>Mol Pharmacol</i> , 58	ocaperidone
dipropyl-5-CT		5-OH-DPAT
donitriptan		9-OH-risperidone
eletriptan	Price et al., 1997 <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 356	pipamperone
[³ H]eletriptan		rauwolscine
F 11356		risperidone
GR-55562		ritanserin
GR 127935		
5-HT		S33084
		Roth et al., 2001 <i>Psychopharmacology (Berl)</i> , 157
		Maroteaux et al., 1992 <i>Proc Natl Acad Sci U S A</i> , 89
		Granas and Larhammar 1999 <i>Eur J Pharmacol</i> , 380
		Lesage et al., 1998 <i>Br J Pharmacol</i> , 123
		Parker et al., 1996 <i>J Neurochem</i> , 67
		Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124

Fig. 16 (continued)

Serotonin 5-HT _{1B} receptor		
Agonists	References	
[³ H]-5-HT		
L-694,247	Davidson et al., 1997 <i>Br J Pharmacol</i> , 121	
L-772,405		
L-775,606	Selkirk et al., 1998 <i>Br J Pharmacol</i> , 125	
lisuride		
LY344864		
lysergol	Newman-Tancredi et al., 1999 <i>Naunyn Schmiedeberg's Arch Pharmacol</i> , 359	
2-Me-5-HT		
5-MeOT		
7-methoxy-1-naphthylpiperazine	Leysen et al., 1996 <i>Mol Pharmacol</i> , 50	
1-naphthylpiperazine		
naratriptan	Granas and Larhammar 1999 <i>Eur J Pharmacol</i> , 380	
8-OH-DPAT		
[³ H]-OH-DPAT		
olanzapine		
ORG-5222		
oxymetazoline		
pergolide		
rizatriptan		
roxindole		
Antagonists	References	
SB 224289	Roth et al., 2001 <i>Psychopharmacology (Berl)</i> , 157	
SB 272183		
SB 649915	Maroteaux et al., 1992 <i>Proc Natl Acad Sci U S A</i> , 89	
SB 714786	Granas and Larhammar 1999 <i>Eur J Pharmacol</i> , 380	
sertindole		
spiperone	Lesage et al., 1998 <i>Br J Pharmacol</i> , 123	
(+)-WAY 100135	Parker et al., 1996 <i>J Neurochem</i> , 67	
yohimbine		
yohimbine	Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124	
zotepine		

Fig. 16 (continued)

Serotonin 5-HT1B receptor	
Agonists	References
RU 24969	Shapiro et al., 2003 <i>Neuropsychopharmacology</i> , 28
SB 216641	
SL65.0155	
sumatriptan	Newman-Tancredi et al., 2000 <i>Mol Pharmacol</i> , 58
[³ H]sumatriptan	
terguride	Price et al., 1997 <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 356
TFMPP	Leysen et al., 1996 <i>Mol Pharmacol</i> , 50
tryptamine	
xanomeline	
ziprasidone	
zolmitriptan	

Fig. 16 (continued)

Serotonin 5-HT1D receptor			
Agonists	References	Antagonists	References
alniditan	Lesage et al., 1998 <i>Br J Pharmacol</i> , 123	m-CPP	Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303,
[³ H]alniditan		bufotenine	
aripiprazole		cyanopindolol	
BRL-15572	Shapiro et al., 2003 <i>Neuropsychopharmacology</i> y, 28	fluspirilene	Weinshank et al., 1992 <i>Proc. Natl. Acad. Sci. U.S.A.</i> , 89
bromocriptine		GR-127935	
cabergoline		haloperidol	
CGS-12066	Price, et al., 1997 <i>Naunyn Schmiedebergs Arch. Pharmacol.</i> , 356	ketanserin	Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124
clozapine		L-772,405	
CP-122288		metergoline	
5-CT	Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303,	methiothepin	Hamblin and Metcalf 1991 <i>Mol Pharmacol</i> , 40
dihydroergotamine		methysergide	
dimethyltryptamine		MPDT	
dipropyl-5-CT	Weinshank et al., 1992 <i>Proc. Natl. Acad. Sci. U.S.A.</i> , 89	ocaperidone	Leysen et al., 1996 <i>Mol Pharmacol</i> , 50,
donitriptan		9-OH-risperidone	
eletriptan	Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124	pipamperone	Millan et al., 2000 <i>J Pharmacol Exp Ther</i> , 293
[³ H]eletriptan		rauwolscine	
EMDT		risperidone	
F 11356	Domenech et al., 1997 <i>Naunyn Schmiedebergs Arch Pharmacol</i> , 356	ritanserlin	
GR 127935			
5-HT			

Fig. 16 (continued)

Serotonin 5-HT1D receptor		
Agonists	References	Antagonists
[³ H]5-HT lisuride	Shapiro et al., 2003 <i>Neuropsychopharmacology</i> , 28	S33084
LY344864		SB 224289
(+)-lysergic acid	Price, et al., 1997 <i>Naunyn-Schmiedeberg's Arch. Pharmacol.</i> , 356	SB 272183
lysergol		SB 277011-A
α -Me-5-HT	Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303,	SB 649915
2-Me-5-HT		SB 714786
5-MeOT	Weinshank et al., 1992 <i>Proc. Natl. Acad. Sci. U.S.A.</i> , 89	sertindole
7-methoxy-1-naphthylpiperazine		spiperone
1-naphthylpiperazine	Napier, et al., 1999 <i>Eur J Pharmacol</i> , 368,	(+)-WAY 100135
naratriptan		yohimbine
8-OH-DPAT	Glennon et al., 2000 <i>J Med Chem</i> , 43	
[³ H]8-OH-DPAT	Leysen et al., 1996 <i>Mol Pharmacol</i> , 50,	
olanzapine		
ORG-5222		
oxymetazoline		
pergolide		
quetiapine		
rizatriptan		
roxindole		

Fig. 16 (continued)

Serotonin 5-HT1D receptor	
Agonists	References
RU 24969	Price, et al., 1997 <i>Naunyn Schmiedeberg's Arch. Pharmacol.</i> , 356
SB 216641	Millan et al., 2002 <i>J Pharmacol Exp Ther</i> , 303,
SL65.0155	Weinshank et al., 1992 <i>Proc. Natl. Acad. Sci. U.S.A.</i> , 89
sumatriptan	Napier, et al., 1999 <i>Eur J Pharmacol</i> , 368
[³ H]sumatriptan	Phebus et al., 1997 <i>Life Sci</i> , 61
terguride	Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124
TFMPP	
tryptamine	
xanomeline	
ziprasidone	
zolmitriptan	

Fig. 16 (continued)

Serotonin 5-HT _{1E} receptor			
Agonists	references	Antagonists	References
m-CPP	Bai et al., 2004 <i>Eur J Pharmacol</i> , 484	fluspirilene	
BRL-15572		metergoline	
clozapine	Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124	methiothepin	Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124
5-CT		methylethylgonovine	
dihydroergotamine	Adham et al., 1993 <i>Proc Natl Acad Sci U S A</i> , 90,	methysergide	McAllister et al., 1992 <i>Proc Natl Acad Sci U S A</i> , 89,
DOI		1-naphthylpiperazine	
donitriptan	Parker et al., 1996 <i>J Neurochem</i> , 67	9-OH-risperidone	Adham et al., 1993 <i>Proc Natl Acad Sci</i> 90
eletriptan		rauwolscine	
EMDT	John et al., 1999 <i>J Pharmacol Exp Ther</i> , 290,	risperidone	Parker et al., 1996 <i>J Neurochem</i> , 67
ergonovine		sertindole	
ergotamine	Bai et al., 2004 <i>Eur J Pharmacol</i> , 484,	yohimbine	
5-fluorotryptamine		zotepine	
GR-127935	Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124		
5-HT			
[³ H]5-HT			
LY344864			
lysergol			

Fig. 16 (continued)

Serotonin 5-HT _{1E} receptor	
α -Me-5-HT	
2-Me-5-HT	
5-MeO-DMT	Baier et al., 2004 <i>Eur J Pharmacol</i> , 484
5-MeOT	
naratriptan	Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124
8-OH-DPAT	
olanzapine	Adham et al., 1993 <i>Proc Natl Acad Sci U S A</i> , 90,
ORG-5222	
quetiapine	Parker et al., 1996 <i>J Neurochem</i> , 67
rizatriptan	John et al., 1999 <i>J Pharmacol Exp Ther</i> , 290,
sumatriptan	
TFMPP	Bai et al., 2004 <i>Eur J Pharmacol</i> , 484,
tryptamine	Schotte et al., 1996 <i>Psychopharmacology (Berl)</i> , 124
xanomeline	
ziprasidone	
zolmitriptan	

Fig. 16 (continued)

Serotonin 5-HT1F receptor	
Agonists	References
2-Me-5-HT	Dhawan, et al., 1996 <i>Pharmacol. Rev.</i> , 48
5-MeO-DMT	
5-MeOT	Horan et al., 1993 <i>J Pharmacol Exp Ther</i> , 266
NAN 190	
naratriptan	Zajac et al., 1983 <i>Biochem. Biophys. Res. Commun.</i> , 111
8-OH-DPAT	
olanzapine	Wang et al., 1994 <i>FEBS Lett.</i> , 348,
quetiapine	
rizatriptan	Mollereau et al., 1994 <i>FEBS Lett</i> , 341,
sumatriptan	
sumatriptan	Hu et al., 1998 <i>J Neurosci</i> , 18,
TFMPP	
tryptamine	Pan et al., 2002 <i>J Neurophysiol</i> , 88
xanomeline	
zolmitriptan	

Fig. 16 (continued)

Serotonin 5-HT _{5A} receptor		
Agonists	References	Antagonists
5-CT	Matthes et al., 1993 <i>Mol. Pharmacol.</i> , 43	Rees et al., 1994 <i>FEBS Lett.</i> , 355,
[³ H]5-CT		
donitriptan	Grailhe et al., 2001 <i>Eur J Pharmacol.</i> , 418,	Matthes et al., 1993 <i>Mol. Pharmacol.</i> , 43
EMDT		Grailhe et al., 2001 <i>Eur J Pharmacol.</i> , 418
5-HT		
[¹²⁵ I]LSD	Erlender et al., 1993 <i>Proc Natl Acad Sci U S A</i> , 90,	Erlender et al., 1993 <i>Proc Natl Acad Sci U S A</i> , 90,
lysergic acid		
8-OH-DPAT	John et al., 1999 <i>J Pharmacol Exp Ther</i> , 290	Glennon et al., 2000 <i>J Med Chem</i> , 43
RU 24969		
sumatriptan	Rees et al., 1994 <i>FEBS Lett.</i> , 355,	Grailhe et al., 2001 <i>Eur J Pharmacol.</i> , 418
TFMPP		

Fig. 16 (continued)

METHODS FOR THE CLASSIFICATION AND DIAGNOSIS OF SCOLIOSIS THROUGH THE USE OF G1 PROTEIN RECEPTOR

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority, under 35 U.S.C. §119(e), of U.S. provisional application Ser. No. 61/104,442 filed on Oct. 10, 2008 and of U.S. provisional application Ser. No. 61/228,769, filed on Jul. 27, 2009, which are incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] N/A.

FIELD OF THE INVENTION

[0003] The present invention relates to the diagnosis of a predisposition to developing a scoliosis (e.g., adolescent idiopathic scoliosis (AIS)) and to screening assays for identifying compounds for treating scoliosis.

BACKGROUND OF THE INVENTION

[0004] Scoliosis is a medical condition in which a person's spine is curved from side to side, and may also be rotated. It is an abnormal lateral curvature of the spine. On an x-ray, the spine of an individual with a typical scoliosis may look more like an "S" or a "C" than a straight line.

[0005] Spinal deformities and scoliosis in particular represent the most prevalent type of orthopedic deformities in children and adolescents, while idiopathic scoliosis (AIS) represents the most common form of scoliosis. The etiology of adolescent idiopathic scoliosis (AIS) is unclear. AIS affects mainly girls in number and severity but in spite of several studies suggesting a genetic predisposition, the form of inheritance remains uncertain (Axenovich T I et al., *Am J Med Genet* 1999, 86(4): 389-394; Wise C A et al., *Spine* 2000, 25(18): 2372-2380; Blank R D et al., *Lupus* 1999, 8(5): 356-360; Giampietro P F et al., *Am J Med Genet* 1999, 83(3):164-177). Several divergent perspectives have been postulated to better define this etiology (Machida M., *Spine* 1999, 24(24): 2576-2583; Roth J A et al., *J Biol Chem* 1999, 274(31): 22041-22047; Hyatt B A et al., *Nature* 1996, 384(6604): 62-65; von Gall C et al., *Eur J Neurosci* 2000, 12(3): 964-972). Genetics, growth hormone secretion, connective tissue structure, muscle structure, vestibular dysfunction, melatonin secretion, and platelet microstructure are major areas of focus. The current opinion is that there is a defect of central control or processing by the central nervous system (CNS) that affects a growing spine and that the spine's susceptibility to deformation varies from one individual to another.

[0006] There is unfortunately no method approved by the FDA yet to identify children or adolescents at risk of developing IS to predict which affected individuals require treatment to prevent or stop progression of the disease (Weinstein S L, Dolan L A, Cheng J C et al. Adolescent idiopathic scoliosis. *Lancet* 2008; 371:1527-37). Consequently, the application of current treatments, such as bracing or surgical correction, is delayed until the detection of a significant deformity or a demonstration of clear progression, resulting in a delayed and less-than-optimal treatment. (Society SR. Morbidity & Mortality Committee annual Report 1997.). Among patients with IS requiring treatment, 80 to 90% will

be treated by bracing and around 1% will need surgery to correct the deformity by spinal instrumentation and fusion of the thoracic and/or lumbar spine with the risk of having complications. (Weiss H R, Goodall D. Rate of complications in scoliosis surgery—a systematic review of the Pub Med literature. *Scoliosis*. 2008; 3:9). Today in the United States there are approximately one million children between ages 10 and 16 with some degree of IS. One out of every six children diagnosed with scoliosis will have a curve that progresses to a degree that requires active treatment. About 29,000 scoliosis surgeries are done every year in North America, resulting in significant psychological and physical morbidity. (Goldberg M S, Mayo N E, Poitras B et al. The Ste-Justine Adolescent Idiopathic Scoliosis Cohort Study. Part I: Description of the study. *Spine* 1994; 19:1551-61; Poitras B, Mayo N E, Goldberg M S et al. The Ste-Justine Adolescent Idiopathic Scoliosis Cohort Study. Part IV: Surgical correction and back pain. *Spine* 1994; 19:1582-8).

[0007] There is a need for methods for classifying subjects having diseases involving spinal deformities (e.g., scoliosis, such as AIS), for diagnosing a predisposition to scoliosis and for identifying compounds for preventing or treating these diseases.

[0008] The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0009] The present invention demonstrates for the first time that cells from scoliotic patients (idiopathic scoliosis patients) exhibit a broad impairment in G-protein coupled receptor (GPCR) signaling. This impairment was measured in several different GPCRs and in various cell types including bone-forming cells, muscle-forming cells as well as blood cells.

[0010] A variety of hormones, neurotransmitters and biologically active substances control, regulate, or adjust the functions of living bodies via specific receptors located in cell membranes. Many of these receptors mediate the transmission of intracellular signals by activating guanine nucleotide-binding proteins (G proteins) to which the receptor is coupled. Such receptors are generically referred to as G protein-coupled receptors ("GPCR"s). Binding of a specific signaling molecule to the GPCR can cause a conformational change in the receptor, resulting in a form that is able to bind and activate a G protein, thereby triggering a cascade of intracellular events that eventually leads to a biological response. Typically, GPCRs interact with G proteins to regulate the synthesis of intracellular second messengers such as cyclic AMP, inositol phosphates, diacylglycerol and calcium ions.

[0011] More specifically, in accordance with the present invention, there is provided a method (e.g., an in vitro method) for determining whether a test compound is useful for preventing or treating scoliosis (e.g., an idiopathic scoliosis (IS), such as Infantile Idiopathic Scoliosis, Juvenile Idiopathic Scoliosis, Adolescent Idiopathic Scoliosis (AIS) or adult scoliosis), said method comprising: (a) contacting a cell expressing a receptor (i.e. at least one receptor) coupled to an inhibitory guanine nucleotide-binding (G_i) protein with a ligand to said receptor in the presence or absence of said test compound; (b) determining the magnitude of the signal induced by said ligand in said cell; wherein a higher signal in the presence relative to the absence of said test compound is

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indicative that said test compound is useful for preventing or treating a scoliosis (e.g., an Idiopathic Scoliosis, such as AIS).

[0012] This method can be used for screening for compounds able to modulate G_i -protein-signaling impairment generally. It can however also be used to determine which compound is the most effective for modulating and in particular reducing or counteracting the G_i -protein-signaling impairment in cells from a specific group of patient or for a specific patient. Indeed, the most effective compound for these purposes may vary from one patient to the next. The method of screening of the present invention may therefore be used to identify which compound is the most effective in counteracting the G_i -protein-signaling impairment in a specific group of patients or in one patient in particular.

[0013] The present invention also provides a method (e.g., an in vitro method) for diagnosing a predisposition to developing a scoliosis (e.g., an Idiopathic Scoliosis such as Infantile Idiopathic Scoliosis, Juvenile Idiopathic Scoliosis or Adolescent Idiopathic Scoliosis (AIS)) in a subject comprising:

[0014] (a) contacting a cell expressing a receptor coupled to an inhibitory guanine nucleotide-binding (G_i) protein from said subject with a ligand to said receptor;

[0015] (b) determining the magnitude of the signal induced by said ligand in said cell;

[0016] (c) comparing said signal to a corresponding reference signal;

[0017] (d) determining said predisposition based on said comparison.

[0018] As used herein the terms “predisposition to developing a scoliosis” refer to a genetic or metabolic predisposition of a subject to develop a scoliosis (i.e. spinal deformity) and/or a more severe scoliosis at a future time.

[0019] In another aspect, the present invention provides a method of classifying a human subject having an idiopathic scoliosis, such as Infantile Idiopathic Scoliosis, Juvenile Idiopathic Scoliosis or Adolescent Idiopathic Scoliosis (AIS) comprising:

[0020] (a) contacting a cell expressing a receptor coupled to an inhibitory guanine nucleotide-binding (G_i) protein from said subject with a ligand to said receptor; and

[0021] (b) determining the magnitude of the signal induced by said ligand in said cell, whereby the results of the determining step enables the classification of the subject having idiopathic scoliosis (e.g., AIS) in one idiopathic scoliosis subgroup.

[0022] In an embodiment, the above-mentioned idiopathic scoliosis is AIS. In an embodiment, the AIS subject (i) is classified in subgroup 1 if the magnitude of the signal is about 34% or less relative to the magnitude of a corresponding signal measured in a cell from a control subject, (ii) is classified in subgroup 2 if the magnitude of the signal is between about 34% and about 57% relative to the magnitude of a corresponding signal measured in a cell from a control subject, and (iii) is classified in subgroup 3 if the magnitude of the signal is between about 57% to about 80% relative to the magnitude of a corresponding signal measured in a cell from a control subject.

[0023] As such, the method of the present invention may also be advantageously used to determine, for example, the type and/or severity of the disease or the nature of the defect underlying the disease (i.e., “classification” or “stratification”

of the subjects). This is particularly interesting if the most effective drug for treating or preventing idiopathic scoliosis (e.g., AIS) varies between subjects affected by idiopathic scoliosis of different types and/or severities. The method for classifying of the present invention may therefore permit a better selection of the drug to be used for a particular patient. This method is also useful to classify/stratify already diagnosed scoliotic patients (e.g., adult scoliosis).

[0024] In an embodiment, the above-mentioned subject is a likely candidate for developing a scoliosis, such as idiopathic scoliosis (e.g., Infantile Idiopathic Scoliosis, Juvenile Idiopathic Scoliosis or Adolescent Idiopathic Scoliosis (AIS)). As used herein the terms “likely candidate for developing scoliosis” include subjects (e.g., children) of which at least one parent has a scoliosis (e.g., adolescent idiopathic scoliosis). Among other factors, age (adolescence), gender and other family antecedent are factors that are known to contribute to the risk of developing a scoliosis and are used to a certain degree to assess the risk of developing a scoliosis. In certain subjects, scoliosis develops rapidly over a short period of time to the point of requiring a corrective surgery (often when the deformity reaches a Cobb’s angle $\geq 50^\circ$). Current courses of action available from the moment a scoliosis such as AIS is diagnosed (when scoliosis is apparent) include observation (when Cobb’s angle is around $10-25^\circ$), orthopaedic devices (when Cobb’s angle is around $25-30^\circ$), and surgery (over 45°). A more reliable determination of the risk of progression could enable to 1) select an appropriate diet to remove certain food products identified as contributors to scoliosis; 2) select the best therapeutic agent; and/or 3) select the least invasive available treatment such as postural exercises, orthopaedic device, or less invasive surgeries or surgeries without fusions (a surgery that does not fuse vertebra and preserves column mobility). The present invention encompasses selecting the most efficient and least invasive known preventive actions or treatments in view of the determined risk of developing scoliosis.

[0025] The methods of the invention may be performed using cell expressing one or more receptor(s) coupled to a G_i protein (also known as G_i alpha subunit). “Receptor” as used herein refers to wild-type receptors as well as to fragments and/or variants thereof that retains the activity (i.e. GPCR-mediated activity) of the wild-type receptors. GPCRs coupled to the G_i protein (hereinafter referred to as G_i PCRs) include, for example, CD47, serotonin receptors (5-HT), adenosine receptors, adrenergic receptors, cannabinoid receptors, histamine receptors, prostaglandin receptors and dopamine receptors. FIG. 15 presents a non exhaustive list of G_i PCRs suitable for use in the method of the present invention.

[0026] In an embodiment, the above-mentioned receptor coupled to a G_i protein is a serotonin receptor, an α -adrenergic receptor, an adenosine receptor, a cannabinoid receptor or any combination thereof. In a further embodiment, the above-mentioned receptor is 5-HT1A, α 2-AD, A3 or CB2. In another embodiment, the above-mentioned receptor is not a melatonin receptor (e.g., MT2).

[0027] In an embodiment, the above-mentioned method is performed on more than one receptor coupled to a G_i protein. In another embodiment, the above-mentioned method is performed using more than one ligand specific for a receptor coupled to a G_i protein. In another specific embodiment, each ligand is specific to a different receptor coupled to a G_i protein (e.g., 2, 3, 4, 5 or 6 ligands). FIG. 16 presents a non exhaustive

list of GiPCRs ligands. In a specific embodiment, ligands for use in the present invention are not MT2 ligands.

[0028] Any sample (e.g., cells, tissues) in which one or more G_i -coupled receptors and/or G_i proteins may be expressed may be used in accordance with the methods of the present invention. The cells may naturally or recombinantly express one or more G_i -coupled receptor(s), and/or a G_i protein. For recombinant expression, a nucleic acid encoding a G_i PCR and/or a nucleic acid encoding a G_i protein is introduced into a suitable cell, and the cell is incubated under conditions that provide for expression of the protein(s). The cells used herein naturally express one or more receptors coupled to G_i proteins and were selected in part for their accessibility for collection from subjects. Hence, cells such as osteoblasts, osteoclasts, peripheral blood mononuclear cell (PBMC) (inherently including principally lymphocytes but also monocytes) and myoblasts are advantageously accessible and may conveniently be used in the methods of the present invention. Blood cells (e.g., PBMCs, platelets (thrombocytes), etc.) in particular are particularly accessible and provide for a more rapid testing. Any blood cell can be used for the methods of the present invention so long as it possesses at least one GPCR receptor coupled to a G_i protein. In an embodiment, the cells are obtained or derived from a subject having an idiopathic scoliosis (e.g., Infantile Idiopathic Scoliosis, Juvenile Idiopathic Scoliosis or Adolescent Idiopathic Scoliosis (AIS)). For the present invention, PBMCs do not need to be established in cultures since methods described herein can be performed in cell suspension with fresh or frozen PBMCs.

[0029] Any ligand of a given G_i -protein coupled receptor may be used in accordance with certain aspects of the present invention. Ligands (e.g., natural or synthetic) of GPCRs are well known in the art, and several of these ligands are commercially available (from Tocris Bioscience, for example). FIG. 15 presents a non exhaustive list of G_i PCR ligands suitable for use in the method of the present invention either alone or in combination with other ligands. In a specific embodiment, the above-mentioned ligand is a known agonist of the receptor. In an embodiment, the above-mentioned ligand is (a) 1-[3-(3,4-Methylenedioxyphenoxy)propyl]-4-phenyl-piperazine maleate (known as BP554 maleate) for the 5-HT1A receptor; (b) 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (known as UK14304) for the α_2 -AD receptor; (c) 1-Deoxy-1[6-[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-N-methyl- β -D-ribofuranuronamide (known as IB-MECA) for the A3 receptor; (d) N-Cyclohexyl-7-chloro-1[2-(4-morpholinyl)ethyl]quinolin-4(1H)-one-3-carboxamide (known as CB65) for the CB2 receptor.

[0030] It should be understood that a variety of different test compounds may be screened by the above methods. Test compounds encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 Daltons. Test compounds comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The test compounds often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Test compounds are also found among biomolecules including peptides (e.g., peptides targeting one or

more defective protein(s) involved in the transduction of the signal through receptors coupled to G_i proteins), nucleic acids (e.g., oligonucleotides such as antisense molecules targeting a defective gene involved in the transduction of the signal through receptors coupled to G_i proteins), antibodies, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Test compounds may be obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs.

[0031] In an embodiment, the reference signal is a signal obtained in a corresponding sample (e.g., a cell) obtained or derived from a control subject, such as a subject (e.g., age- and/or gender-matched) who has not developed a scoliosis (e.g., idiopathic scoliosis such as Infantile Idiopathic Scoliosis, Juvenile Idiopathic Scoliosis or Adolescent Idiopathic Scoliosis (AIS)), or who is not predisposed to developing a scoliosis. In that case, a lower signal in the cell from the subject relative to the corresponding reference signal is indicative that the subject has a predisposition to developing a scoliosis, whereas a higher or a substantially identical signal is indicative that the subject does not have a predisposition to developing a scoliosis.

[0032] In another embodiment, the above-mentioned reference signal is a signal obtained in a corresponding sample (e.g., a cell) obtained or derived from a subject (e.g., age- and/or gender-matched) who has developed a scoliosis (e.g., idiopathic scoliosis such as Infantile Idiopathic Scoliosis, Juvenile Idiopathic Scoliosis or Adolescent Idiopathic Scoliosis (AIS)), or who is known to be predisposed to developing a scoliosis. In that case, a lower or a substantially identical signal in the cell from the subject relative to the corresponding reference signal is indicative that the subject has a predisposition to developing a scoliosis, whereas a higher signal is indicative that the subject does not have a predisposition to developing a scoliosis.

[0033] In an embodiment, the corresponding sample is a cell of the same type (e.g., both the test sample and the reference sample(s) are lymphocytes) as that from the subject. In an embodiment, the assay will be assayed in plates (e.g., 96-wells, 384-wells, etc.) containing the test sample and a control sample. In another embodiment, the plates also contain reference samples from a healthy subject and from subjects of the groups 1, 2 and 3.

[0034] In an embodiment, a lower or higher signal refers to a difference of at least about 10%, in further embodiments at least about 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150% or 200% between the signal obtained with the test sample (sample obtained from the subject being tested) relative to the reference signal. In an embodiment, a substantially identical signal refers to a signal that differs by less than 10%,

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in further embodiments by less than 9%, 8%, 7%, 6% or 5%, as compared to the reference signal.

[0035] The changes in the magnitude of the signal induced by the ligand (e.g. an agonist) may be detected using any methods. Methods for measuring the magnitude or intensity of the signal (e.g., intracellular response) mediated through GiPCRs are well known in the art. The magnitude of the signal may be determined, for example, by measuring the level of a molecule, such as a second messenger (e.g., cAMP, Ca^{2+}) or a gene product (e.g., mRNA or protein) whose level is modulated following triggering of the receptor by a ligand. The magnitude of the signal may also be determined, for example, by measuring changes in protein-protein interactions (e.g., by fluorescence resonance energy transfer [FRET] or bioluminescence resonance energy transfer [BRET]) following triggering of the receptor by a ligand. Other methods to measure the magnitude or intensity of the signal mediated through GiPCRs include, for example, measurement of cAMP levels (Medhurst et al., 2003. In: J Neurochem., 84), measurement of thallium flux using GIRK-thallium Flux Assay (Niswender et al., 2008; In: Mol Pharmacol. 73(4)), Patch-clamp (Saugstad et al., 1996. In: J. Neurosci. 16), measurement of GTPyS binding using [^{35}S]GTPyS labeling assay (Riobo et al., 2006. In: Proc Natl Acad Sci USA, 103), and measurement of the changes in impedance (Peters et al., 2007. In: J Biomol. Screen. 12: 312-9). In an embodiment, the magnitude of the signal is determined using the changes in impedance that occurs in the cell following receptor triggering (e.g., cellular dielectric spectroscopy [CDS]). Such measurement may be made, for example, using the real-time cell electronic sensing (RT-CESTM) technology (ACEA Biosciences Inc., San Diego, Calif., USA) (Huang et al., Analyst, 2008, 133(5): 643-648; Solly et al., Assay Drug Dev. Technol, 2004, 2(4): 363-372) or using the CellKeyTM technology (MDS Sciex, Concord, Ontario, Canada) according to the method described below.

[0036] In embodiment, the methods are performed in a format suitable for high throughput assays, e.g., 96- or 384-well format, and suitable robots, (e.g., pipetting robots), and instrumentation may be used.

[0037] Also provided by the present invention are kits for practicing the above-mentioned methods. The kits may include, for example, one or more reagent(s) to determine a G_i protein-coupled receptor signal, as well as buffers, cells, control samples (e.g., sample from a healthy subject, reference samples of patients of the group 1, 2 and 3), containers, etc. for performing the subject assays. The various components of the kit may be present in separate containers or certain compatible components may be pre-combined into a single container, as desired.

[0038] In addition to above-mentioned components, the kits typically further include instructions for using the components of the kit to practice the methods. The instructions for practicing the subject methods are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging) etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote

source, e.g., via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

[0039] In another aspect, the present invention provides a method for preventing and/or treating a scoliosis (e.g., idiopathic scoliosis such as Infantile Idiopathic Scoliosis, Juvenile Idiopathic Scoliosis or Adolescent Idiopathic Scoliosis (AIS)) comprising administering an effective amount of a compound (and/or a composition comprising a compound) identified by the above-described screening method to a subject.

[0040] Compositions (e.g., pharmaceutical compositions) are prepared by formulating the compounds (e.g., peptides, small molecules) identified by the above-described screening method, with any suitable pharmaceutical carrier/excipient or combination of pharmaceutical carriers/excipients. Preparations may be made for administration by any route, such as intravenous, intramuscular, subcutaneous, oral, rectal, vaginal, transdermal, transmucosal, sublingual and the like.

[0041] In an embodiment, the above-mentioned subject is a mammal, in a further embodiment, a human.

[0042] Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] In the appended drawings: [renuméroté pages]

[0044] FIG. 1 presents the contribution of G_i proteins in melatonin signaling detected by CDS. Data in the graph were generated from maximum impedance responses and correspond to the mean $\pm$ SE of 3 independent experiments performed in triplicate;

[0045] FIG. 2 shows osteoblasts from control subjects (non-IS) and patients with IS were pre-treated with forskolin to induce adenylate cyclase activity and subsequent cAMP production. Cells were then challenged with increasing concentration of melatonin and the functional status of melatonin signaling was estimated by the ability of melatonin to reduce cAMP levels. Data were normalized with cAMP production induced by forskolin in the absence of melatonin;

[0046] FIG. 3 shows the response of different receptors coupled to G_i proteins expressed on osteoblasts obtained from AIS patients or control subjects following activation by a specific ligand, as measured by Cellular Dielectric Spectroscopy (CDS). Comparisons to control (*), Group 3 (ψ) or Group 2 (Ω) are indicated by: *, ψ , Ω , $p < 0.05$; **, $\psi\psi$, $\Omega\Omega$, $p < 0.01$; ***, $\psi\psi\psi$, $p < 0.001$;

[0047] FIG. 4 compares G_i coupled protein signaling dysfunction using different ligands presented as percentage of maximal response measured by Cellular Dielectric Spectroscopy (CDS). Increasing concentrations of melatonin, iodomelatonin, BP554, UK14304, IB-meca and CB65 were applied to osteoblasts from control subject (non-IS) and patients with IS (Groups 1, 2 and 3). The subsequent cellular response was measured by CDS in the CellKeyTM instrument. Curves were generated from maximum impedance magnitude. Data were normalized to maximal response in cells from control patients, and are expressed as mean $\pm$ SE of three independent experiments performed in duplicate;

[0048] FIG. 5 shows the response of different receptors coupled to G_i proteins expressed on myoblasts obtained from AIS patients or control subject following activation by a specific ligand, as measured by Cellular Dielectric Spectroscopy (CDS). Comparisons to control (*), Group 3 (ψ) or Group 2 (Ω) are indicated by: *, ψ , Ω , $p < 0.05$; **, $\psi\psi$, $p < 0.01$; ***, $\psi\psi\psi$, $p < 0.001$;

[0049] FIG. 6 compares G_i coupled protein signaling dysfunction using different ligands presented as percentage of maximal response measured by Cellular Dielectric Spectroscopy (CDS). Increasing concentrations of melatonin, iodomelatonin, BP554, UK14304, IB-meca and CB65 were applied to myoblasts from control subject (non-IS) and patients with IS (Groups 1, 2 and 3). The subsequent cellular response was measured by CDS in the CellKey™ instrument. Curves were generated from maximum impedance magnitude. Data were normalized to maximal response in cells from control patients, and are expressed as mean $\pm$ SE of three independent experiments performed in duplicate;

[0050] FIG. 7 shows the response of different receptors coupled to G_i proteins expressed on PBMCs obtained from AIS patients or control subjects following activation by a specific ligand, as measured by Cellular Dielectric Spectroscopy (CDS). Comparisons to control (*), Group 3 (ψ) or Group 2 (Ω) are indicated by: *, ψ , Ω , $p < 0.05$; **, $\psi\psi$, $\Omega\Omega$, $p < 0.01$; ***, $\psi\psi\psi$, $p < 0.001$;

[0051] FIG. 8 compares G_i coupled protein signaling dysfunction using different ligands presented as percentage of maximal response measured by Cellular Dielectric Spectroscopy (CDS). Increasing concentrations of melatonin, iodomelatonin, BP554, UK14304, IB-meca and CB65 were applied to PBMCs (containing principally lymphocytes) from control subjects (non-IS) and patients with IS (Groups 1, 2 and 3). The subsequent cellular response was measured by CDS in the CellKey™ instrument. Curves were generated from maximum impedance magnitude. Data were normalized to maximal response in cells from control patients, and are expressed as mean $\pm$ SE of three independent experiments performed in duplicate;

[0052] FIG. 9 shows that the altered response of G_i -coupled receptors to ligand stimulation is detected in PBMCs from family #24. The maximal impedance of all shown members of the family were measured by Cellular Dielectric Spectroscopy (CDS). *** means $p < 0.001$;

[0053] FIG. 10 shows that the altered response of G_i -coupled receptors to ligand stimulation is detected in PBMCs from four families: 23, 24, 29 and 39. The maximal impedance of all shown members of the families were measured by Cellular Dielectric Spectroscopy (CDS). *** means $p < 0.001$;

[0054] FIG. 11 shows ranges of values obtained in osteoblasts from control subjects and AIS patients belonging to functional groups 1, 2 and 3 using (A) 300 μ M melatonin, (B) 300 μ M iodomelatonin, (C) 300 μ M BP554, (D) 300 μ M UK14304, (E) 300 μ M IB-meca and (F) 300 μ M CB65, as measured by Cellular Dielectric Spectroscopy (CDS);

[0055] FIG. 12 shows ranges of values obtained in PBMCs from control subjects and A) AIS patients or B) asymptomatic at risk subjects patients, using 300 μ M of iodomelatonin as measured by Cellular Dielectric Spectroscopy (CDS). Each point represents the mean of three wells on individual subjects and the assay coefficient of variation found was typically less than 10%;

[0056] FIG. 13 A) shows the response of different receptors coupled to G_i proteins expressed on PBMCs obtained from asymptomatic at risk patients, AIS subjects and control subjects and following activation by a specific ligand, as measured by Cellular Dielectric Spectroscopy (CDS) at time zero (T_0) and eighteen months later (T_{18}). B) provides on a single graph the response to the melatonin receptor on PBMCs (containing principally lymphocytes) obtained from asymptomatic at risk patients;

[0057] FIG. 14 shows GPRC signaling in osteoblasts of a control subject and of AIS subjects (Groups 1, 2 and 3) using different ligands G_s , G_i and G_q to determine whether the signaling is specific to G_i -coupled receptor;

[0058] FIG. 15 shows a list of known G_i -protein coupled receptors; and

[0059] FIG. 16 shows a list of known ligands to G_i -protein coupled receptors.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0060] The present invention is illustrated in further details by the following non-limiting examples.

EXAMPLE 1

Clinical Characteristics of Subjects (Control, AIS and Asymptomatic)

[0061] The clinical characteristics of the examined healthy subjects (controls), AIS subject and asymptomatic subjects are presented in Tables I-III below, respectively.

TABLE I

Clinical data of healthy control subjects.		
Case Number	Gender	Age
5	Female	10.6
11	Male	11.3
20	Female	10.0
21	Female	10.4
23	Male	10.3
33	Male	10.1
43	Female	14.9
60	Female	14.4
61	Female	16.3
68	Female	12.9
69	Female	14.5
86	Female	13.7
4110	Female	17.3
4112	Male	13.6
4115	Female	14.7
4121	Male	13.8
4124	Female	12.8
4126	Female	14.6
4131	Male	15.5
4135	Male	15.8
4137	Female	16.8
4138	Female	13.1
4140	Male	14.9
4142	Female	15.3
4143	Female	13.1
4148	Male	14.8
4149	Female	15.4
4150	Female	15.2
4159	Male	14.0
4161	Male	13.3
4174	Female	12.9
4179	Female	16.6
4181	Male	15.9
4182	Female	15.7

TABLE I-continued

Clinical data of healthy control subjects.		
Case Number	Gender	Age
4190	Male	16.3
4191	Female	15.7
4193	Female	17.3
4195	Female	16.3
4200	Female	13.6
4205	Male	11.3
4206	Male	11.4
4209	Female	15.8

*All reported data is that from first collection date.

TABLE II

Clinical data of AIS patients				
Case Number Group	Gender	Age	Cobb Angle	Curve Pattern
113 3	Female	9.7	10	Right Thoracic
151 3	Female	13.1	31	Right Thoracic
155 2	Female	15.4	20	Left Thoracolumbar
159 3	Female	8.3	7	Left Lumbar
165 3	Female	10.8	26	Right Thoracic Left Lumbar
168 3	Female	14.2	16	Right Thoracic Left Thoracolumbar
208 3	Female	9.8	26	Left Thoracolumbar
254 3	Female	15.2	28	Left Thoracolumbar
271 3	Female	12.4	23	Right Thoracic
275 2	Female	11.0	27	Right Thoracic Left Lumbar
276 3	Female	13.1	19	Right Thoracic Left Lumbar
281 3	Female	12.6	7	Left Thoracolumbar
282 2	Female	9.7	20	Left Lumbar
302 3	Female	14.4	14	Right Thoracic Left Lumbar
306 2	Female	13.1	13	Right Thoracic Left Lumbar
327 1	Female	16.4	21	Right Thoracic
336 3	Female	14.0	6	Right Thoracic Left Lumbar
404 3	Female	14.6	25	Right Thoracic
425 3	Female	24.6	52	Left Thoracolumbar
475	Female	13.2	19	Left Thoracolumbar
541 2	Male	15.3	13	Right Thoracic
542 2	Female	15.6	21	Right Thoracic Left Thoracolumbar
543 2	Female	10.9	10	Right Thoracic Left Thoracolumbar
544 2	Female	15.1	8	Left Thoracic
548 3	Female	15.6	16	Right Thoracolumbar Right Lumbar
1020 3	Female	19.9	59	Right Thoracic Left Lumbar

TABLE II-continued

Clinical data of AIS patients				
Case Number Group	Gender	Age	Cobb Angle	Curve Pattern
1060 3	F	12.0	48	Right thoracic Left lumbar
1198 3	Female	16.3	20	Right Thoracolumbar
1311 2	F	15.52	42	Right thoracic
1332 3	F	13.11	35	Right thoracic
1385 2	Female	16.1	27	Left Thoracic Right Thoracic
1468 3	Male	15.5	69	Left Lumbar Right Thoracolumbar
1474 1	Male	17.9	54	Right Thoracic Left Lumbar
1488 1	Male	15.4	87	Right Thoracic
1513 3	Female	15.6	11	Right Thoracic Left Lumbar
1520 3	Female	14.6	54	Right Thoracic Left Thoracolumbar
1545 3	Female	13.5	57	Right Thoracic
1549 1	Female	14.8	24	Right Thoracic Left Thoracolumbar
1550 2	Female	12.3	60	Right Thoracic Left Lumbar
1552 3	Female	18.5	30	Left Thoracic Right Thoracic
1554 3	Female	14.7	49	Left Lumbar Right Thoracolumbar
1557 2	Female	12.1	83	Right Thoracic Left Lumbar
1562 3	Female	19.1	51	Right Thoracic Left Lumbar
1566 3	Female	12.2	53	Left Thoracic Right Thoracic

*All reported data is that from first collection date.

TABLE III

Clinical data of asymptomatic at-risk subjects studied.		
Case Number Group Number	Gender	Age
3004 2	Female	8.6
3006 3	Male	5.8
3009 2	Female	3.0
3018 3	Female	11.3
3023 3	Female	9.5
3027 2	Female	14.1
3030 3	Male	10.7
3040 3	Male	9.7
3043 3	Female	4.7
3054 1	Female	8.2

TABLE III-continued

Clinical data of asymptomatic at-risk subjects studied.		
Case Number Group Number	Gender	Age
3057 3	Female	15.3
3058 3	Female	8.0
3062 3	Male	14.5
3065 3	Female	12.2
3067 3	Female	12.0
3068 3	Male	12.0
3070 2	Female	8.5
3071 3	Male	5.5
3080 3	Female	10.0
3081 3	Male	16.2
3082 3	Female	7.6
3095 3	Male	10.7
3096 3	Female	8.5
3098 3	Male	12.5
3099 3	Female	10.4
3115 3	Male	10.6
3116 3	Female	9.5
3123 3	Female	10.8
3124 3	Male	15.0

TABLE III-continued

Clinical data of asymptomatic at-risk subjects studied.		
Case Number Group Number	Gender	Age
3159 3	Female	5.1

*All reported data is that from first collection date.

Clinical Characteristics of Subjects From Which Cells Were Isolated for Examples 3-4 and 8-10.

[0062] The institutional review boards of The Sainte-Justine Hospital, The Montreal Children's Hospital, The Shriners Hospital for Children in Montreal and McGill University approved this study. Parents or legal guardians of all participants gave written informed consent, and minors gave their assent. All patients with IS were examined by one of the six orthopedic surgeons participating in this study. A person was deemed to be affected if history and physical examination were consistent with the diagnosis of IS and a minimum of a ten degree curvature in the coronal plane with vertebral rotation was found by radiograph. A summary of demographics and clinical characteristics of these subjects are shown in Table IV below. These subjects are also included in Table II above.

[0063] A number of young asymptomatic children born from one parent affected by IS were also enrolled in the study to evaluate the performance of the test in the asymptomatic population. Each subject was examined by the same orthopedic surgeon in a clinic for early detection of IS at Sainte-Justine University Hospital. These subjects are also included in Table III above.

[0064] Healthy children were recruited as controls in Montreal's elementary schools after an informed written consent was obtained from their parents or legal guardians; minors also gave their assent. This recruitment was approved by The Montreal English School Board, The Affluent School Board and all institutional review boards mentioned above. Each normal subject was also examined by the same orthopedic surgeon using Adam's forward bending-test with a scoliometer (Table IV) to rule out any hidden scoliosis before entering the study. These subjects are also included in Table I above.

TABLE IV

Summary of clinical characteristics of the AIS subjects presented in Examples 3-4 and 8-10.						
Subjects	Mean Age (Years)	Mean Single Scoliosis Cobb Angle (°)			Mean Double Scoliosis Cobb Angle (°) Thoracic +	Heredity
		Thoracic	Lumbar	Thoracolumbar	Lumbar	
IS severely affected (N = 19)	14.8 ± 2.3	72 ± 21	—	59 ± 14	48.6 ± 18.9 (thoracic angle) 48.9 ± 14.8 (lumbar angle)	4/19
IS moderately affected (N = 25)	13.0 ± 2.2	N = 11 18.8 ± 6.7	N = 0 18.0 ± 2.8	N = 2 15.7 ± 8.9	N = 2 18.8 ± 9.4 (thoracic angle) 17.6 ± 11.3 (lumbar angle)	8/25
Healthy control subjects (N = 42)	14.1 ± 2.1	N = 7 —	N = 2 —	N = 7 —	N = 8 —	0
At-risk control subjects (N = 30)	10.2 ± 3.2	—	—	—	—	30/30

EXAMPLE 2

Material and Methods

[0065] The response of G_i protein-coupled receptors was investigated in various cell types (osteoblasts, myoblasts and PBMCs (including principally lymphocytes)) obtained from patients with clinically well-defined AIS and compared with an age- and gender-matched control subjects (not affected by a scoliosis).

[0066] Three populations were investigated: patients with IS, healthy controls without any family history of scoliosis, and asymptomatic offspring, born from at least one scoliotic parent, who are considered at-risk of developing scoliosis. A group of 44 patients with IS (19 AIS patients with curvatures greater than 45° and 25 AIS patients with curvatures between 10° and 29°) (Table II above), as well as 42 healthy control subjects (Table I above) and 30 asymptomatic children at-risk of developing scoliosis (Table III above) were recruited and examined every 6 months. All subjects were Caucasians.

Osteoblasts and Myoblasts

[0067] Bone specimens were obtained intraoperatively from vertebrae (varying from T3 to L4 according to the surgical procedure performed), while with trauma cases used as non-scoliotic controls bone specimens were obtained from other anatomical sites (tibia or femur, and in one case from an iliac crest biopsy). Osteoblasts and myoblasts isolated as previously described (Moreau A, Wang D S, Forget S et al. Melatonin Signaling Dysfunction in Adolescent Idiopathic Scoliosis. *Spine* 2004; 29:1772-81; Azeddine et al., 2007 *Molecular determinants of melatonin signaling dysfunction in adolescent idiopathic scoliosis*. Clin Orthop Relat Res. 2007; 462:45-52), were cultured at $37^\circ\text{C}/5\%\text{CO}_2$ in MEM supplemented with 10% fetal bovine serum (FBS) and 100 $\mu\text{g/mL}$ streptomycin. Prior to the experiment, cells were seeded into the CellKey™ standard 96-well plates at a density of 5×10^4 /well and grown for 24 h and incubated in standard conditions ($37^\circ\text{C}/5\%\text{CO}_2$). In some plates, Pertussis toxin (100 ng/mL, Sigma, Oakville, ON, Canada) treatment was performed 16 h before the CDS assay. Following overnight incubation, plates were placed onto the CellKey™ system and growth medium was exchanged to assay buffer (Hanks, Balanced Salt Solution containing 20 mM HEPES and 0.1% BSA) before starting the experiment. Cells were then allowed to equilibrate at room temperature for 30 min. Pre-addition measurements were made for 5 min to obtain a baseline reading. Then, ligands (melatonin, 2-Iodomelatonin, BP554 maleate, UK14304, IB-MECA and/or CB65) were added simultaneously to all 96 wells using an integrated fluidics system. Activation of endogenous receptors resulted in a change in impedance that started occurring immediately after fluid addition and mixing. The quick update rate (2 s.) of the CellKey™ system allows detection of these immediate changes in impedance. Impedance measurements were collected for 10 min to 15 min at 28°C . after ligand addition to monitor cellular responses to ligand interaction and to generate CellKey™ response profiles that are characteristic of the signaling mechanisms used.

PBMCs

[0068] Blood samples were obtained from patients and control groups and collected in blood collection tubes containing EDTA and then centrifuged on a Ficoll-Plaque (GE

Healthcare, Mississauga, ON, Canada) solution to obtain PBMCs (containing principally lymphocytes). PBMC fractions were preserved frozen in FBS with 10% DMSO and kept in liquid nitrogen until thawed and assayed. PBMCs were harvested from stock tissue culture flask and washed three times with assay buffer. Cells were isolated from the heparinized peripheral by ficoll-Hypaque density gradient centrifugation, and cultured in RPMI 1640 medium supplemented with 10% FBS, 100 $\mu\text{g/mL}$ streptomycin and 1% of phytohemagglutinin. Following 48 h of incubation at $37^\circ\text{C}/\text{CO}_2$ cells were washed three times with assay buffer and seeded into the CellKey™ small 96-well plates. Typically, an average of 1×10^5 cells to 1.5×10^5 were added to each well and allowed to settle for 30 min at room temperature. This resulted in a single layer of cells on top of the electrodes. After the cells settled, the cell plate was placed on the instrument and experiments were initiated in the manner described below after the acquisition of 5 min of baseline measurements. All experiments were conducted at 28°C .

Cellular Dielectric Spectroscopy (CDS)

[0069] The response induced by the various ligands to their receptors was measured by cellular dielectric spectroscopy (CDS) using the CellKey™ technology. (MDS Sciex, San Francisco, Calif., USA). The CellKey™ system integrates a proprietary impedance measurement system, custom 96-well microliter plate, onboard 96-well fluidics, and environment control and custom acquisition and analysis software in a full solution package (MDS Sciex) that is compatible with commercially available robotic platforms. Small voltage at 24 frequencies, from 1 KHz to 10 MHz, is applied to a monolayer of cells settled in a 96-well microplate containing electrodes at the bottom, and the resultant current is measured at an update rate of 2 s. The system is thermally regulated and experiments can be conducted between 28°C and 3°C ., and for examples presented herein was typically 28°C . Onboard fluid additions and exchanges are handled by 96-well head fluid delivery device with a range of 5-500 μL . In addition to the cell plate location, the CellKey™ system includes two additional stations for 96-or 384-well compound plates. All aspects of assay set up, execution, and data acquisition and analysis are controlled by CellKey™ software.

[0070] Agonist and antagonist activity of natural or synthetic ligands may be accurately quantified under conditions of low receptor expression (when using primary and first passage cells derived from patients, for example) using the CellKey™ technology.

[0071] cAMP Assay in Osteoblasts

[0072] Osteoblasts from patients with IS and control subjects were seeded in quadruplicate on 24-well plates (1×10^5 cells per well) and tested as previously described (Moreau A, Wang D S, Forget S et al. Melatonin Signaling Dysfunction in Adolescent Idiopathic Scoliosis. *Spine* 2004; 29:1772-81). All assays were performed in duplicate.

[0073] Statistical Analysis

[0074] Three experiments in duplicate were carried out for each subject, and results are mean $\pm$ SE of these measurements. The assay coefficient of variation was found typically less than 10%. All concentration response curves were analyzed by non-linear regression using GraphPad™ (San Diego, Calif.). For statistical analysis, multiple comparisons were performed with One-way analysis of variance (ANOVA) followed by a post-hoc test of Newman-Keuls. Probability values less than 0.05 were taken as significant.

Ex. 2034

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[0075] Data are presented as mean±SE. Multiple comparisons of means were performed with one-way analysis of variance (ANOVA) followed by a post-hoc test of Dunnett, using GraphPad™ Prism 4.0 software. Only P values<0.05 were considered significant.

EXAMPLE 3

CDS Detecting Relative Part of Gi Protein-Mediated Pathway in Melatonin Signaling

[0076] The ability of CDS to detect the relative part of the Gi protein-mediated pathway in melatonin signaling was determined by blocking this pathway with pertussis toxin (PTX). This toxin ADP-ribosylates the α_i subunit of the heterotrimeric Gi protein and thereby prevents its activity. The MG63 osteoblast cell line was serum-starved for 16 h in the absence or presence of 100 ng/mL of PTX to inhibit the α_i subunit of Gi protein. Cells were then stimulated with 10 uM of melatonin or iodomelatonin at 37° C. for 5 min. Cellular responses were measured with CellKey™ as described in the Material and Methods section.

[0077] Results presented in FIG. 1 show that the PTX treatment dramatically inhibits the cellular response measured by CDS in MG63 osteoblast cell line following stimulation with either melatonin or iodomelatonin. The inhibition extent was about 75% for melatonin and 90% for iodomelatonin, indicating the major effects Gi protein have on melatonin signaling measured by CDS.

EXAMPLE 4

Comparison of Melatonin Impairment Measured by cAMP and CDS

[0078] First osteoblasts derived from healthy controls and three severely affected IS patients (surgical cases exhibiting a Cobb's angle>45°) were pre-treated with forskolin to induce adenylate cyclase activity and subsequent cAMP production. Cells were then challenged with increasing concentration of melatonin and the functional status of melatonin signaling was estimated by the ability of melatonin to reduce cAMP levels. Data were normalized with cAMP production induced by forskolin in the absence of melatonin. As expected, osteoblasts from IS patients exhibited high cAMP production in response to melatonin subsequently to forskolin stimulation when compared to those from healthy control subjects (FIG. 2). Furthermore, the extent of this production was different between the three IS patients. These data are consistent with previous findings, (Goldberg M S, Mayo N E, Poitras B et al. The Ste-Justine Adolescent Idiopathic Scoliosis Cohort Study. Part I: Description of the study. *Spine* 1994; 19:1551-61.) confirming thus the occurrence of a melatonin signaling dysfunction of different degrees in the osteoblasts of these patients.

[0079] The ability of a CDS assay to detect this defect was then assessed by studying the effect of various concentrations of melatonin and iodomelatonin on CDS response of these cells. Increasing concentrations of melatonin or iodomelatonin were applied to osteoblasts and PBMCs (including principally lymphocytes) from healthy control subjects (Control) and patients with IS (Groups 1, 2 and 3). The subsequent cellular response was measured by CDS in the CellKey™ instrument. Curves were generated from maximum impedance magnitude. Data were normalized to maximal response in cells from control patients, and were expressed as

mean±SE of three independent experiments performed in duplicate. Results obtained showed that melatonin and iodomelatonin evoked similar responses in a concentration-dependent manner in osteoblasts from both control and IS patients. However, the magnitude of response was significantly lower in IS osteoblasts (FIG. 4). On the other hand, a significant difference in the extent of CDS response was also observed between the three IS patients. These results indicate that the dysfunction of melatonin signaling in IS and the disparity of this defect among patients can be detected by CDS in IS patients.

[0080] Interestingly, the screening of PBMCs in response to melatonin or iodomelatonin, using CDS, revealed similar features than those obtained with osteoblasts (FIGS. 4 and 8), demonstrating that melatonin signaling dysfunction also occurs in PBMCs.

EXAMPLE 5

Comparison of the Response of Different Gi-Coupled Receptors in Various Cell Types Isolated from IS vs. Control Subjects

[0081] As shown in FIGS. 3 to 8, osteoblasts, myoblasts and PBMCs isolated from AIS patients and stimulated with ligands specific for different Gi-protein coupled receptors (MT2, 5-HT1A, α_2 -AD, A3 or CB2) have an altered response (i.e. change in impedance following stimulation) as compared to that of cells from a normal, non-scoliotic subject, thus demonstrating that IS patients have a generalized impairment in Gi-protein-mediated signaling. As shown in Table V below, an impaired Gi-protein coupled receptor response was measured in cells from all IS subjects (phase I and phase II) tested. Also, cells from 12/31 asymptomatic at risk subjects (subjects having an IS parent) showed a defect in Gi-protein coupled receptor response. Interestingly, 4 out of the 12 subjects in which impaired Gi-protein coupled receptor response was detected later developed symptoms of scoliosis, as determined by X-rays analysis, demonstrating that the impaired/defective Gi-protein coupled receptor response may be used for the prognosis of IS (or of a predisposition to developing IS).

TABLE V

Summary of data obtained with control, AIS and asymptomatic at risk subjects				
Patients	Healthy Control	Asymptomatic at risk	AIS Phase II (10•-44•)	AIS Phase I (≥45•)
Total number	42	31	25	14
Unaffected	100%	62% (19/31)	0	0
Affected	0%	38% (12/31)	100%	100%
Group 1	0%	3% (1/31)	4% (1/25)	21% (3/14)
Group 2	0%	6% (2/31)	32% (8/25)	21% (3/14)
Group 3	0%	29% (9/31)	64% (16/25)	58% (8/14)

EXAMPLE 6

Measurement of Gi-Coupled Receptor Responses in Cells Obtained From Members of an AIS Family

[0082] FIG. 9-10 shows that an altered response of different Gi-coupled receptors is detected in PBMCs (including principally lymphocytes) isolated from both the IS affected mother and daughter, but not in cells from the unaffected

father and son. Interestingly, the Gi-coupled receptor responses measured in cells obtained from the mother and daughter are very similar (Group 2, as defined below), suggesting that the defect has been genetically transmitted by the mother to the daughter. Also, the defect is maintained in the mother although she underwent surgery to reduce spine curvature. The clinical data for the daughter are as follows: Age 13.5, curve pattern: right thoracic, Cobb angle: 6.

EXAMPLE 7

Stratification/Classification of AIS Subjects Based on Gi-Coupled Receptor Responses Measured in Osteoblasts

[0083] FIG. 11 shows the range of mean values obtained for control (n=3) and IS subjects (n=9) using the different Gi-coupled receptor ligands as measured in osteoblasts. The minimal (min) and maximal (max) values represent the mean

of three independent measurements performed in duplicate (six values in total) for a given patient/control subject. All AIS subject tested showed a lower G_i -coupled receptor response as compared to control subjects, but it was observed that AIS subjects typically show three types of response (as represented by the three different curves for AIS subjects in FIGS. 3 to 8), and thus may be classified into three groups. AIS subjects having the lowest values (low G_i -coupled receptor response) were classified as subgroup 1, AIS subject having the highest values ("high" G_i -coupled receptor response, but always lower than control subjects) were classified as subgroup 3, and AIS subjects having values between those of Groups 1 and 3 ("intermediate" G_i -coupled receptor response) were classified as subgroup 2. The data (dZiec values and % of control) obtained using various ligands are summarized in Table VI below. Duplicates (n1 and n2) of three independent experiments are presented.

TABLE VI

Stratification/classification of AIS subjects based on Gi-coupled receptor responses						
dZiec values/% of control						
Subject	Exper. 1		Exper. 2		Exper. 3	
	n1	n2	n1	n2	n1	n2
Melatonin						
CTR#60	55.81/—	53.31/—	63.72/—	67.72/—	59.25/—	61.03/—
AIS#1474	16.53/29.6%	13.03/24.4%	15.18/23.8%	15.18/22.4%	17.34/29.3%	16.12/26.4%
AIS#1550	28.28/50.7%	30.78/57.7%	29.43/46.2%	32.43/47.9%	29.34/49.5%	31.12/51%
AIS#1020	29.38/52.6%	31.88/59.8%	40.33/63.3%	39.33/58.1%	34.34/58%	36.12/59.2%
CTR#61	68.79/—	66.29/—	80.84/—	84.84/—	74.30/—	76.08/—
AIS#1549	17.30/25.1%	16.80/25.3%	18.41/22.8%	20.41/24.1%	17.34/23.3%	19.12/25.1%
AIS#1468	44.92/65.3%	46.42/70%	54.79/67.8%	54.79/64.6%	49.34/66.4%	51.12/67.2%
AIS#1513	38.90/56.5%	39.40/59.4%	50.81/62.9%	51.81/61.1%	44.34/59.7%	46.12/60.6%
CTR#69	76.12/—	73.62/—	89.58/—	93.58/—	82.34/—	84.12/—
AIS#1488	9.79/12.9%	11.29/15.3%	10.91/12.2%	10.91/11.7%	12.34/15%	12.12/14.4%
AIS#1198	23.94/39.3%	31.44/42.7%	31.91/35.6%	25.91/27.7%	27.41/33.3%	29.19/34.7%
AIS#1385	—	—	—	—	—	—
Iodomelatonin						
CTR#60	142.55/—	139.55/—	149.20/—	161.20/—	152.18/—	142.58/—
AIS#1474	33.02/23.2%	32.02/23.0%	38.33/25.7%	32.33/20.1%	39.97/26.3%	36.37/25.5%
AIS#1550	55.99/39.3%	48.99/35.1%	60.90/40.8%	62.90/39.0%	62.74/41.2%	59.14/41.5%
AIS#1020	69.57/48.8%	66.57/47.7%	85.25/57.1%	85.32/60.3%	83.71/55.0%	74.11/52.0%
CTR#61	130.88/—	127.88/—	138.31/—	150.31/—	140.90/—	131.30/—
AIS#1549	9.21/7.0%	6.21/4.9%	18.70/13.5%	20.70/13.8%	20.25/14.4%	20.65/15.7%
AIS#1468	91.38/69.8%	88.38/69.1%	101.69/73.5%	113.69/75.6%	102.83/73.0%	93.23/71.0%
AIS#1513	83.92/64.1%	80.92/63.3%	94.08/68.0%	106.08/70.6%	95.30/67.6%	85.70/65.3%
CTR#69	150.99/—	147.99/—	161.10/—	173.10/—	162.34/—	152.74/—
AIS#1488	25.43/16.8%	27.43/18.5%	30.31/18.8%	27.31/15.8%	19.17/11.8%	19.57/12.8%
AIS#1198	61.05/40.4%	61.05/41.3%	65.29/40.5%	70.29/40.6%	60.97/37.6%	57.37/37.6%
AIS#1385	66.51/44.1%	67.51/45.6%	79.93/49.6%	81.93/47.3%	79.02/48.7%	69.42/45.4%
IB-meca						
CTR#60	148.22/—	160.22/—	172.73/—	180.73/—	161.48/—	169.48/—
AIS#1474	31.25/21.1%	30.25/18.9%	36.61/21.2%	37.61/20.8%	29.93/18.5%	37.93/22.4%
AIS#1550	65.47/44.2%	67.47/42.1%	81.23/47.0%	79.23/43.8%	69.35/42.9%	77.35/45.6%
AIS#1020	74.19/50.1%	76.19/47.6%	87.32/50.6%	85.32/47.2%	76.76/47.5%	84.76/50.0%
CTR#61	127.27/—	139.27/—	155.44/—	163.44/—	142.36/—	150.36/—
AIS#1549	5.11/4.0%	17.11/12.3%	26.78/17.2%	34.78/21.3%	16.94/11.9%	24.94/16.6%
AIS#1468	98.77/77.6%	110.77/79.5%	121.90/78.4%	129.90/79.5%	111.34/78.2%	119.34/79.4%
AIS#1513	78.36/61.6%	90.36/64.9%	104.12/67.0%	112.12/68.6%	92.24/64.8%	100.24/66.7%
CTR#69	154.52/—	166.52/—	179.10/—	187.10/—	167.81/—	175.81/—
AIS#1488	39.34/25.5%	41.34/24.8%	47.91/26.8%	45.91/24.5%	39.63/23.6%	47.63/27.1%
AIS#1198	63.58/41.1%	75.58/45.4%	85.63/47.8%	93.63/50.0%	75.61/45.1%	83.61/47.6%
AIS#1385	73.49/47.6%	75.49/45.3%	85.15/47.5%	83.15/44.4%	75.32/44.9%	83.32/47.4%

TABLE VI-continued

Stratification/classification of AIS subjects based on Gi-coupled receptor responses						
dZiec values/% of control						
Subject	Exper. 1		Exper. 2		Exper. 3	
	n1	n2	n1	n2	n1	n2
CB65						
CTR#60	143.28/—	155.28/—	173.77/—	181.77/—	159.53/—	167.53/—
AIS#1474	36.30/25.3%	38.30/24.7%	46.79/26.9%	44.79/24.6%	37.55/23.5%	45.55/27.2%
AIS#1550	55.13/38.5%	57.13/36.8%	66.49/38.3%	64.49/35.5%	56.81/35.6%	64.81/38.7%
AIS#1020	61.81/43.1%	73.81/47.5%	74.95/43.1%	82.95/45.6%	69.38/43.5%	77.38/46.2%
CTR#61	144.08/—	156.08/—	168.59/—	176.59/—	157.34/—	165.34/—
AIS#1549	36.48/25.3%	28.48/18.2%	32.23/19.1%	40.23/22.8%	30.36/19.3%	38.36/23.2%
AIS#1468	82.16/57.0%	94.16/60.3%	107.91/64.0%	115.91/65.6%	96.04/61.0%	104.04/62.9%
AIS#1513	107.73/74.8%	119.73/76.7%	129.10/76.6%	137.10/77.6%	119.41/75.9%	127.41/77.1%
CTR#69	141.68/—	153.68/—	163.73/—	171.73/—	153.70/—	161.70/—
AIS#1488	41.30/29.2%	43.30/28.2%	54.43/33.2%	52.43/30.5%	43.87/28.5%	51.87/32.1%
AIS#1198	72.90/51.5%	84.90/55.2%	94.57/57.8%	102.57/59.7%	84.73/55.1%	92.73/57.3%
AIS#1385	66.15/46.7%	78.15/50.9%	84.86/51.8%	92.86/54.1%	76.50/49.8%	84.50/52.3%
BP554						
CTR#60	140.49/—	152.49/—	162/—	170/—	152.25/—	160.25/—
AIS#1474	39.94/28.4%	41.94/27.5%	51.45/31.8%	50.45/29.7%	43.7/28.7%	46.7/29.1%
AIS#1550	74.64/53.1%	86.64/56.8%	91.46/56.5%	99.46/58.5%	84.05/55.2%	92.05/57.4%
AIS#1020	62.57/44.5%	64.57/42.3%	78.63/48.5%	76.63/45.1%	66.6/43.7%	74.6/46.6%
CTR#61	132.45/—	144.45/—	154.6/—	162.6/—	144.53/—	152.53/—
AIS#1549	16.43/12.4%	28.43/19.7%	38.57/24.9%	46.57/28.6%	28.5/19.7%	36.5/23.9%
AIS#1468	84.51/63.8%	96.51/66.8%	104.4/67.5%	112.4/69.1%	95.45/66.0%	103.45/67.8%
AIS#1513	91.15/68.8%	103.15/71.4%	113.38/73.3%	121.38/74.6%	103.27/71.5%	111.27/72.9%
CTR#69	145.69/—	157.69/—	165.87/—	173.87/—	156.78/—	164.78/—
AIS#1488	44.06/30.2%	46.06/29.2%	54.24/32.7%	52.24/30.0%	45.15/28.8%	53.15/32.3%
AIS#1198	59.83/41.1%	64.83/41.1%	74.63/45.0%	70.63/40.6%	63.73/40.6%	71.73/43.5%
AIS#1385	73.41/50.4%	68.41/43.4%	86.15/51.9%	85.15/49.0%	72.78/46.4%	73.78/44.8%
UK14304						
CTR#60	100.78/—	106.78/—	125.27/—	127.27/—	111.02/—	119.02/—
AIS#1474	20.6/20.4%	20.6/19.3%	23.35/18.6%	24.35/19.1%	19.48/17.5%	19.48/16.4%
AIS#1550	53.62/53.2%	45.62/42.7%	56.75/45.3%	44.75/35.2%	46.19/41.6%	54.19/45.5%
AIS#1020	42.74/42.4%	39.74/37.2%	43.5/34.7%	51.5/40.5%	41.62/37.5%	49.62/41.7%
CTR#61	90.39/—	94.39/—	102.1/—	110.1/—	92.75/—	101.75/—
AIS#1549	27.08/30.0%	29.08/30.8%	34.45/33.7%	36.45/33.1%	32.76/35.3%	36.76/36.1%
AIS#1468	65.72/72.7%	67.72/71.7%	77.77/76.2%	75.77/68.8%	67.75/68.8%	75.75/73.0%
AIS#1513	58.45/64.7%	60.45/64.0%	71.58/70.1%	69.58/63.2%	61.02/65.8%	69.02/67.8%
CTR#69	103.67/—	105.67/—	98.38/—	100.38/—	94.02/—	92.02/—
AIS#1488	26.24/25.3%	25.55/24.2%	25.92/26.3%	18.92/18.8%	26.55/28.2%	26.24/28.5%
AIS#1198	44.16/42.6%	46.16/43.7%	56.22/57.1%	54.22/54.0%	46.19/49.1%	54.19/58.9%
AIS#1385	—	—	—	—	—	—

TABLE VII

Differences observed between minimal and maximal values for each group and minimal and maximal values from control subjects for various ligands in osteoblasts. Values were calculated from the data shown in FIGS. 11A to F.			
		Absolute dZiec	% of control signal (min/max)
Melatonin			
Group 1	min diff.	18/60	30%
	max diff.	11/83	13%
Group 2	min diff.	37/60	62%
	max diff.	28/83	34%
Group 3	min diff.	50/60	83%
	max diff.	45/83	54%

TABLE VII-continued

Differences observed between minimal and maximal values for each group and minimal and maximal values from control subjects for various ligands in osteoblasts. Values were calculated from the data shown in FIGS. 11A to F.			
		Absolute dZiec	% of control signal (min/max)
Iodomelatonin			
Group 1	min diff.	35/120	29%
	max diff.	15/159	9%
Group 2	min diff.	84/120	70%
	max diff.	57/159	36%
Group 3	min diff.	101/120	84%
	max diff.	90/159	57%

TABLE VII-continued			
Differences observed between minimal and maximal values for each group and minimal and maximal values from control subjects for various ligands in osteoblasts. Values were calculated from the data shown in FIGS. 11A to F.			
		Absolute dZiec	% of control signal (min/max)
PB554			
Group 1	min diff.	49/128	38%
	max diff.	32/166	19%
Group 2	min diff.	88/128	69%
	max diff.	67/166	40%
Group 3	min diff.	107/128	84%
	max diff.	99/166	60%
UK14304			
Group 1	min diff.	32/98	33%
	max diff.	21/115	18%
Group 2	min diff.	50/98	51%
	max diff.	45/115	39%
Group 3	min diff.	71/98	72%
	max diff.	60/115	52%
IBmeca			
Group 1	min diff.	43/132	33%
	max diff.	20/172	12%
Group 2	min diff.	80/132	61%
	max diff.	73/172	42%
Group 3	min diff.	115/132	87%
	max diff.	98/172	55%
CB65			
Group 1	min diff.	47/138	34%
	max diff.	34/169	20%
Group 2	min diff.	88/138	64%
	max diff.	60/169	36%
Group 3	min diff.	123/138	89%
	max diff.	100/169	55%

EXAMPLE 8

Stratification/Classification of IS Subjects Based on GI-Coupled Receptor Responses Measured in PBMCs

[0084] A cohort of individuals with (n=45) (see Table II) or without AIS (n=42) (See Table I) was tested to determine the sensitivity and specificity of the cell-based assay with CDS by investigating the functional status of melatonin signaling in PBMCs (including principally lymphocytes) from each of these individuals.

[0085] PBMCs were isolated from control subjects, IS subjects as described in Example 2 above. The signaling effect of 300 μM of iodomelatonin as measured by Cellular Dielectric Spectroscopy (CDS) was determined.

[0086] FIG. 12A shows that all patients with IS were less responsive to iodomelatonin when compared to healthy volunteers tested (control). Similar results were obtained with melatonin (data not shown), thereby indicating a high selectivity for this assay. The minimal magnitude measured in PBMCs from healthy volunteers in response to iodomelatonin was 124 ohms under experimental conditions. None of the AIS patients tested exhibited a CDS response reaching such magnitude. The maximal magnitude measured in IS patients was 110 ohms, while the minimum was 16 ohms. Melatonin signaling responses to iodomelatonin in normal/healthy subjects was thus greater than 120 ohms. A CDS response range

between 10 and 40 ohms for IS patients was classified in group 1, 40 and 80 ohms in group 2, and finally between 80 and 120 ohms for IS patients in group 3. Of all AIS patients tested, 11% (5/44), 30% (13/44) and 59% (26/44) were diagnosed as belonging to the functional groups 1, 2 and 3, respectively. According to their CDS response, there was no ambiguity in classifying these patients. The mean of CDS response for group 1 was 27.86±4.18 and 65.71±3.42 for group 2, while it was 97.94±1.36 for group 3 and 155.17±3.91 for the control group. These differences were highly significant (P<0.001). These results demonstrate that the functional test based on the evaluation of melatonin signaling in PBMCs with the CDS assay effectively distinguishes patients with IS not only from healthy individuals but also from different functional groups. These results demonstrate the sensitivity, specificity and dynamic range of this functional test as a diagnostic tool. See also Table VII showing Differences observed between minimal and maximal values for each group and minimal and maximal values for control subjects for iodomelatonin in PBMCs.

TABLE VIII			
Differences observed between minimal and maximal values for each group and minimal and maximal values for control subjects for iodomelatonin in PBMCs. Values were calculated from the data shown in FIG. 12A.			
		Absolute dZiec	% of control signal (min/max)
Iodomelatonin			
Group 1	min diff.	36/124	29%
	max diff.	16/225	7%
Group 2	min diff.	75/124	60%
	max diff.	49/225	22%
Group 3	min diff.	110/124	89%
	max diff.	86/1125	38%

EXAMPLE 9

Predicting the Risk of Developing IS in PBMCs

[0087] Healthy controls (n=42) (Table I) or subjects considered at risk of developing this disease (n=30) (Table III) were tested to determine the ability of this assay to predict the risk of developing IS.

[0088] PBMCs were isolated asymptomatic at-risk subjects as described in Example 2 above. The signaling effect of 300 μM of iodomelatonin as measured by Cellular Dielectric Spectroscopy (CDS) was determined in these cells and compared with that measured in the healthy controls described in Example 6 above.

[0089] The functional screening of PBMCs from asymptomatic children born from at least one scoliotic parent revealed that the melatonin signaling defect can be detected in this cohort (FIG. 12B). Of all asymptomatic children tested, 60% (19/31) exhibited a CDS response in the range of normal magnitudes (>120 ohms), whereas the CDS response was reduced in 40% (12/31). The latter group were considered more susceptible to developing scoliosis. Among these 12 children at high risk, 4 (33%) have developed a spinal deformity 24 months later, exhibiting a mean Cobb angle of 11.7° as confirmed by radiography (data not shown). None of the 19 in the normal range have developed a scoliosis.

[0090] It is known that the prevalence of IS is increased in asymptomatic children born from at least one affected parent. Results presented above corroborate this fact and confirm that

this category of children is more susceptible of developing IS. The risk of developing scoliosis was predicted in 40% of asymptomatic children tested (i.e. 12/31), and 33% of them developed a spinal deformity several months later. For instance the then asymptomatic patient #3116 in Table III (who was then 9 years old), at age 11 presented a curve pattern right thoracic and left lumbar with a Cobb angle of 9-14°.

[0091] The lack of spinal deformity manifestation in the remaining 67% of the suspected children is likely related to their age. Indeed, the deformity of the spine is recognized to occur in children between 10 and 16 years of age. However, the mean age of asymptomatic children enrolled in the present study was 10.2±3.2 years, and they have been followed up for 24 months. Therefore, not all tested children would have reached the appropriate age for developing the spinal deformity during this period.

[0092] Among all tested children (including those of age 10 or older (scoliosis appears around in the age 10)), neither asymptomatic children exhibiting normal melatonin signaling nor control children without familial history of scoliosis have developed any spinal deformity during the study period.

EXAMPLE 10

Measurement of Gi-Coupled Receptor Responses in Asymptomatic at Risk Subjects

[0093] FIG. 13A and B shows that the defect in G_i -coupled receptors response may be detected over a period of 18 months in PBMCs (including principally lymphocytes) of asymptomatic at risk subjects (n=31) (subjects having a parent affected by AIS). Also, the amplitude of the defect is maintained over this period (i.e. subjects showed a very similar response curve at the first time point (t_0) and 18 months later (t_{18})), suggesting that the defect does not evolve over time.

EXAMPLE 11

Measurement of Gi-Coupled Receptor Responses in Osteoblasts

[0094] Osteoblasts were seeded into the CellKey™ standard 96-well microplate at a density of 5×10^4 /150 μ L per well and incubated in standard conditions (37° C./ 5% CO₂). Following overnight incubation, the plate was placed onto the CellKey™ system and growth medium was replaced by assay buffer (Hanks, Balanced Salt Solution containing 20 mM HEPES and 0.1% BSA) before starting the experiment. Cells were then allowed to equilibrate at room temperature for 30 min. At the end of this period, the plate was placed onto the system and pre-addition measurements were made for 5 min to obtain a baseline reading. Then, forskolin, isoproterenol, melatonin or bradykinin were added simultaneously to all 96 wells with an integrated fluidics system. The compound addition resulted in change in impedance that started occurring immediately after fluid addition and mixing. Impedance measurements were collected for 15 min at 28° C. FIG. 14 shows that GPCR signaling in AIS is specific to G_i coupled receptor. The difference in response observed for G_s -coupled GPCR in the presence of isoproterenol as well in the production of cAMP in the presence of forskolin between each groups is explained by the absence of functional G_i . There is approximately 10 times more G_i than G_s in the cells. Therefore, if some G_i is non-functional, it cannot inhibit the production of

cAMP in the presence of forskolin and thus, the G_s -coupled response also appears affected.

[0095] Although the present invention has been described hereinabove by way of specific embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

1. A method for determining whether a test compound is useful for treating a scoliosis, said method comprising:

- (a) contacting a cell expressing a receptor coupled to an inhibitory guanine nucleotide-binding (G_i) protein with a ligand to said receptor in the presence or absence of said test compound;
- (b) determining the magnitude of the signal induced by said ligand in said cell;

wherein a higher signal in the presence relative to the absence of said test compound is indicative that said test compound is useful for treating a scoliosis.

2. The method of claim 1, wherein said scoliosis is an idiopathic scoliosis.

3. the method of claim 1, wherein said idiopathic scoliosis is adolescent idiopathic scoliosis (AIS).

4. The method of claim 1, wherein said cell is obtained from a subject suffering from AIS.

5. The method of claim 1, wherein said signal is the change in impedance of said cell.

6. The method of claim 5, wherein said change in impedance is measured by cellular dielectric spectroscopy (CDS).

7. The method of claim 1, wherein said cell is an osteoblast, a myoblast or a peripheral blood mononuclear cell (PBMC).

8. The method of claim 1, wherein said cell is a PBMC.

9. The method of claim 8, wherein said cell is a lymphocyte.

10. The method of claim 1, wherein said receptor is a serotonin receptor, an α -adrenergic receptor, an adenosine receptor or a cannabinoid receptor.

11. The method of claim 10, wherein (i) said serotonin receptor is 5-HT1A; (ii) said α -adrenergic receptor is α 2-AD; (iii) said adenosine receptor is A₃; or (iv) said cannabinoid receptor is CB2.

12-14. (canceled)

15. The method of claim 1, wherein said ligand is an agonist.

16. The method of claim 11, wherein (i) said serotonin receptor is 5-HT 1A and said ligand is 1-[3-(3,4-Methylenedioxypheoxy)propyl]-4-phenyl-piperazine maleate (BP554 maleate); (ii) said α -adrenergic receptor is α 2-AD and said ligand is 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinolalinamine (UK14304); (iii) said adenosine receptor is A₃ and said ligand is 1-Deoxy-1-[6-[[3-(iodophenyl)methyl]amino]-9H-purin-9-yl]-N-methyl- β -D-ribofuranuronamide (IB-MECA); or (iv) said cannabinoid receptor is CB2 and said ligand is N-Cyclohexyl-7-chloro-1-[2-(4-morpholinyl)ethyl]quinolin-4(1H)-one-3-carboxamide (CB65).

17-19. (canceled)

20. The method of claim 1, comprising determining the magnitude of the signal induced by at least two different ligands.

21. The method of claim 1, comprising determining the magnitude of the signal for at least two different receptors coupled to a G_i protein.

22. A method for diagnosing a predisposition to developing a scoliosis in a subject comprising:

- (a) contacting a cell expressing a receptor coupled to an inhibitory guanine nucleotide-binding (G_i) protein from said subject with a ligand to said receptor;
- (b) determining the magnitude of the signal induced by said ligand in said cell;
- (c) comparing said signal to a corresponding reference signal; and
- (d) determining said predisposition based on said comparison.

23. The method of claim **22**, wherein said scoliosis is an idiopathic scoliosis.

24. The method of claim **23**, wherein said idiopathic scoliosis is adolescent idiopathic scoliosis (AIS).

25. The method of claim **22**, wherein said signal is the change in impedance of said cell.

26. The method of claim **25**, wherein said change in impedance is measured by cellular dielectric spectroscopy (CDS).

27. The method of claim **22**, wherein said cell is an osteoblast, a myoblast or a peripheral blood mononuclear Cell (PBMC).

28. The method of claim **22**, wherein said cell is a PBMC.

29. The method of claim **28**, wherein said cell is a lymphocyte.

30. The method of claim **22**, wherein said receptor is a serotonin receptor, an α -adrenergic receptor, an adenosine receptor or a cannabinoid receptor.

31. The method of claim **30**, wherein (i) said serotonin receptor is 5-HT1A; (ii) said α -adrenergic receptor is α 2-AD; (iii) said adenosine receptor is A_3 ; or (iv) said cannabinoid receptor is CB2.

32-34. (canceled)

35. The method of claim **22**, wherein said ligand is an agonist.

36. The method of claim **31**, wherein (i) said serotonin receptor is 5-HT1A and said ligand is 1-[3-(3,4-Methylenedioxyphenoxy)propyl]-4-phenyl-piperazine maleate (BP554 maleate); (ii) said α -adrenergic receptor is α 2-AD and said ligand is 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (UK14304); (iii) said adenosine receptor is A_3 and said ligand is 1-Deoxy-1-[6-[[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-N-methyl- β -D-ribofuranuronamide (IB-MECA); or (iv) said cannabinoid receptor is CB2 and said ligand is N-Cyclohexyl-7-chloro-1-[2-(4-morpholinyl)ethyl]quinolin-4(1H)-one-3-carboxamide (CB65).

37-39. (canceled)

40. The method of claim **22**, comprising determining the magnitude of the signal induced by at least two different ligands.

41. The method of claim **22**, comprising determining the magnitude of the signal for at least two different receptors coupled to a G_i protein.

42. The method of claim **24**, wherein the subject is a likely candidate for developing adolescent idiopathic scoliosis.

43. A method of classifying a human subject having idiopathic scoliosis comprising:

- (a) contacting a cell expressing a receptor coupled to an inhibitory guanine nucleotide-binding (G_i) protein from said subject with a ligand to said receptor; and
- (b) determining the magnitude of the signal induced by said ligand in said cell, whereby the results of the determining step enables the classification of the subject having idiopathic scoliosis in one subgroup.

44. The method of claim **43**, wherein said idiopathic scoliosis is adolescent idiopathic scoliosis (AIS).

45. The method of claim **43**, further comprising selecting a treatment for the subject based on the results of the determining step.

46. The method of claim **43**, wherein said signal is the change in impedance of said cell.

47. The method of claim **46**, wherein said change in impedance is measured by cellular dielectric spectroscopy (CDS).

48. The method of claim **43**, wherein said cell is an osteoblast, a myoblast or a peripheral blood mononuclear Cell (PBMC).

49. The method of claim **43**, wherein said receptor is a serotonin receptor, an α -adrenergic receptor, an adenosine receptor or a cannabinoid receptor.

50. The method of claim **49**, wherein (i) said serotonin receptor is 5-HT1A; (ii) said α -adrenergic receptor is α 2-AD; (iii) said adenosine receptor is A_3 ; or (iv) said cannabinoid receptor is CB2.

51-53. (canceled)

54. The method of claim **43**, wherein said ligand is an agonist.

55. The method of claim **50**, wherein (i) said serotonin receptor is 5-HT1A and said ligand is 1-[3-(3,4-Methylenedioxyphenoxy)propyl]-4-phenyl-piperazine maleate (BP554 maleate); (ii) said α -adrenergic receptor is α 2-AD and said ligand is 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (UK14304); (iii) said adenosine receptor is A_3 and said ligand is 1-Deoxy-1-[6-[[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-N-methyl- β -D-ribofuranuronamide (IB-MECA); or (iv) said cannabinoid receptor is CB2 and said ligand is N-Cyclohexyl-7-chloro-1-[2-(4-morpholinyl)ethyl]quinolin-4(1H)-one-3-carboxamide (CB65).

56-58. (canceled)

59. The method of claim **43**, comprising determining the magnitude of the signal induced by at least two different ligands.

60. The method of claim **43**, comprising determining the magnitude of the signal for at least two different receptors coupled to a G_i protein.

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