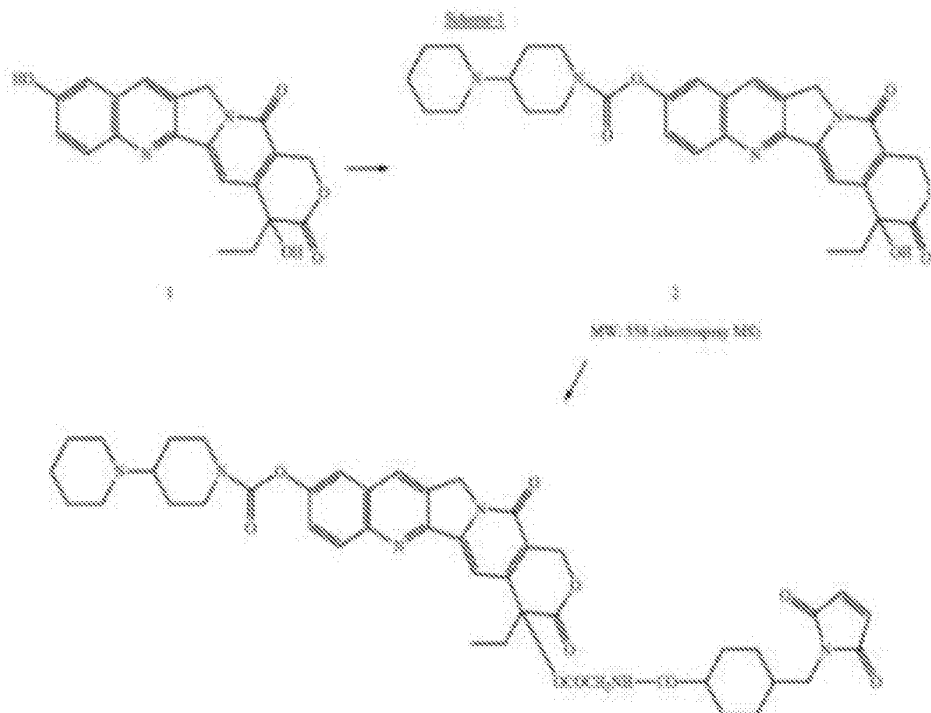

than a hydrazone; wherein said linker comprises an amino acid moiety and a thiol-reactive moiety and is linked to the chemotherapeutic moiety via the amino acid moiety and to the targeting moiety via the thiol-reactive moiety.

[0017] Another embodiment of the invention is a method of treating cancer, a malignancy, an autoimmune disease, an infection, or an infectious lesion with the immunoconjugates described herein"

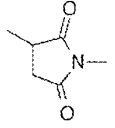
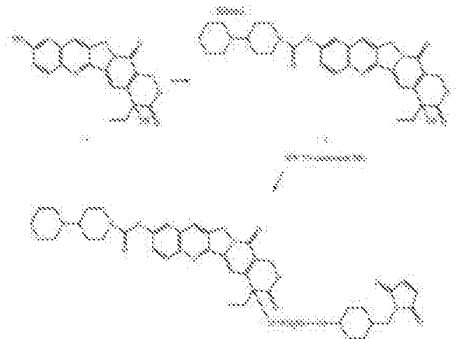
As shown, Govindan *et al.* discloses all the essential characteristics of the claimed antibody-drug conjugate. In addition, in paragraph [0052]:

"[0052] In a preferred embodiment of the present invention, antibodies, such as mAbs, are used that recognize or bind to markers or tumor-associated antigens that are expressed at high levels on target cells and that are expressed predominantly or only on diseased cells versus normal tissues, and antibodies that internalize rapidly. Antibodies useful within the scope of the present invention include mAbs with properties as described above (and show distinguishing properties of different levels of internalization into cells and microorganisms), and contemplate the use of, but are not limited to, in cancer, the following mAbs: LL1 (anti-CD74), LL2 (anti-CD22), RS7 (anti-epithelial glycoprotein-1(EGP-1)), PAM-4 and KC4 (both anti-MUC1), MN-14 (anti-carcinoembryonic antigen (CEA, also known as CD66e), Mu-9 (anti-colon-specific antigen-p), Immu 31 (an anti-alpha-fetoprotein), TAG-72 (e.g., CC49), Tn, J591 (anti-PSMA (prostate-specific membrane antigen)), G250 (an anti-carbonic anhydrase IX mAb) and L243 (anti-HLA-DR). Other useful antigens that may be targeted using these conjugates include HER-2/neu, BrE3, CD19, CD20 (e.g., C2B8, hA20, 1F5 Mabs) CD21, CD23, CD80, alpha-fetoprotein (AFP), VEGF, EGF receptor, P1GF, MUC1, MUC2, MUC3, MUC4, PSMA, gangliosides, HCG, EGP-2 (e.g., 17-1A), CD37, HLA-DR, CD30, Ia, A3, A33, Ep-CAM, KS-1, Le(y), 5100, PSA (prostate-specific antigen), tenascin, folate receptor, Thomas-Friedenreich antigens, tumor necrosis antigens, tumor angiogenesis antigens, Ga 733, IL-2, IL-6, T101, MAGE, antigen to which L243 binds, CD66 antigens, i.e. CD66a-d or a combination thereof" (emphasis added)

Similarly, the camptothecin derivatives with 4-(N-maleimidomethyl)-cyclohexane-1-carbonyl are disclosed in Govindan *et al.*



In addition, the linker would have been anticipated since the core succinimid had been divulged in *Govindan et al.*

Colombian application No. NC2016/0000187	Govindan et al.
<p>Claim 1 -(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-(NH-DX), -(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-(NH-DX), or -(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-NH-CH₂CH₂-O-CH₂CH₂-O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX), -(Succinimid-3-yl-N)- has a structure represented by the following formula: [Formula 2]</p> 	

In this regard, although the linker structure is not identical, there is no information disclosed in the description allowing to identify a surprising effect achieved by including the specific linker. Therefore, the technical effect is obvious in view of the teachings disclosed in the prior art.

In conclusion, for the opponent the claimed invention is obvious when considering the teachings disclosed in *Govindan et al.*

2. Technical Advice

After having reviewed the instant application and the opponent's arguments, we would like to draw your kind attention to the following facts:

Please note that according to our current practice, a defense brief may be filed for responding to the arguments of the opponents against the clarity, unity of invention and inventiveness of the present invention. In the defense brief, it could be included an amended claim set that allows to compare the characteristics of the invention with the state of the art.

Regarding the clarity, please note that set of claims has been amended based on the specific objections indicated by the Colombian examiner in the first formal office action and in order to comply the local requirements. In this way, we suggest maintaining the claims and waiting for the issuance of the substantive office action so that we could evaluate if the examiner indicated new objections and it is necessary to further amend the claims.

In connection to the unity of invention, in our opinion, an objection to the unity of the invention can only be established once the patentability assessment has been conducted. In this way, we do not consider valid the arguments indicated by the opponent regarding this requirement.

With respect to the arguments against the inventive step of the claimed invention in view of the teachings disclosed in *Govindan et al.*, if you consider pertinent, it could be filed arguments demonstrating that the technical characteristics disclosed in the set of claims would have not been obviously derived from the teachings disclosed in the cited document. Specifically, it would be helpful to expose technical data and arguments proving an advantage or unexpected technical effect achieved by the specific characteristics of the antibody-drug conjugate which could not be anticipated by the skilled person in the art. Please let us know if said data is available.

Please be advised that in case a response to the opposition is not filed, the application will be evaluated considering the arguments submitted by the opponent. After substantive examination, it will be issued an office action indicating the examiner's position and the arguments and documents which indeed affects the patentability of the invention, if any.

Please let us know how to proceed.

Deadline

Please be informed that the deadline for filing a response to the office action is **August 1, 2017**, which is extendable once, for 60 working days. Nonetheless, as we need a certain amount of time in order to prepare the response, we appreciate receiving your instructions by **July 25, 2017**. Otherwise, if we do not receive your instructions by the mentioned date, we will request the indicated extension of time. The costs involved on an extension of time are: official fee USD \$42, professional fees USD \$207 plus VAT (19%).

► **IMPORTANT NOTE:** Since failure to reply to an Office Action leads to the lapse of the application, in the absence of your instructions until **July 25, 2017**, we shall automatically file a time extension and will charge for this service.

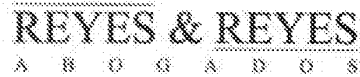
Divisional applications

Please be advised that according to our current practice, a divisional application can be filed anytime during prosecution of the parent application, before the issuance of a final decision. However, we clarify that a divisional application cannot contain subject matter which has been already evaluated by the Colombian examiner or overlaps subject matter of the parent application; otherwise, the division will be rejected by the Colombian PTO. In addition, as a recent adopted practice, when filing a divisional application, it is mandatory to limit the scope of the description (including the examples) to the specific subject matter claimed in the divisional claims.

If you have any comments or questions, please do not hesitate to contact us.

Very truly yours,

Paola Rúa
Patent Engineer
prua@clarkemodet.com.co



Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF: EXPEDIENTE NC2016/0000187

TÍTULO SOLICITADO: "CONJUGADO DE ANTICUERPO-FÁRMACO ANTI-RECEPTOR 2 DEL FACTOR DE CRECIMIENTO EPIDERMAL (HER2)"

PUBLICACIÓN: Gaceta 780 de 30 dic 2016 de 2015

PRIORIDAD: JA 2014-017777 31/01/2014
JA 2014-168944 22/08/2014
JA 2014-227886 10/11/2014

SOLICITANTE: DAIICHI SANKYO COMPANY,LIMITED

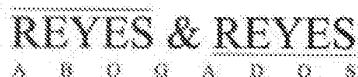
APODERADO: CAROLINA MERCEDES DAZA MONTALVO

TRÁMITE: PRESENTACION DE OPOSICIÓN

JUAN PABLO REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 92189 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **LABORATORIOS LEGRAND S.A.**, en ejercicio de lo dispuesto por el artículo 42 de la Decisión 486 de 2000, muy respetuosamente me permito formular la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente **NC2016/0000187**, por no cumplir con lo dispuesto en la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.



II. LEGÍTIMO INTERÉS

Asiste a **LABORATORIOS LEGRAND S.A.**, legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringiría injustificadamente el empleo de ciertas sustancias, derivados y procesos que a nuestro juicio carecen de nivel inventivo, en detrimento de sus legítimos intereses comerciales. De igual manera mis clientes están interesados, por su misma condición de industrias farmacéuticas, en evitar la concesión de privilegios de patente que incumplan los requisitos materiales de patentabilidad establecidos en las normas vigentes, y que supongan un riesgo para la libre competencia.

III. FUNDAMENTOS DE LA OPOSICIÓN

La solicitud colombiana en trámite **NC2016/0000187**, referida a un conjugado de anticuerpo-fármaco caracterizado porque un compuesto antitumoral está conjugado a un anticuerpo anti-HER2 en forma de una estructura fármaco-enlazador, no cumple los requisitos materiales para otorgársele el privilegio de patente de invención, tal como puede determinarse al valorar los siguientes argumentos:

Falta de claridad y concisión

La solicitud carece de concisión ya que las 16 reivindicaciones no definen específicamente las características técnicas esenciales del conjugado de anticuerpo-fármaco que busca protección, esto debido a que en las reivindicaciones principales se presentan diferentes enlazadores al anticuerpo seleccionables que a su vez comprenden una cantidad no determinada de compuestos.

Aunque las reivindicaciones incluyen las fórmulas y las secuencias del anticuerpo, éstas no definen todas y cada una de las posibles moléculas que buscan protección según las alternativas divulgadas, además, se encuentran definidas a partir de sustituciones y resultados opcionales lo que no aporta a la identificación de la molécula objeto de la solicitud en trámite. Por lo tanto, las modalidades pretendidas tampoco encuentran sustento por la descripción.

REYES & REYES

A B O G A D O S

Ahora bien, la reivindicación principal de la presente invención reclama como características técnicas esenciales tanto el compuesto de fórmula I, los enlazantes y anticuerpos (como Trastuzumab), idea que está alejada de la realidad pues como se verá más adelante, el estado de la técnica anticipaba este tipo de conjugados, así como los agentes antitumorales, los anticuerpos como trastuzumab, y la parte succinimid del agente enlazante.

La forma de redacción de la reivindicación principal no es clara ni concisa y tiende a confundir al lector, no es claro cual es la parte caracterizante de dicha invención, y por el contrario trata de reclamar derechos sobre materia que es ampliamente conocida como son los anticuerpos anti-HER2 (podría tratarse de trastuzumab) y/o derivados de campotecina.

Adicional a lo anterior, la solicitud en estudio tampoco cumple con el criterio de claridad por cuanto no se define la condición reductora en el método de las reivindicaciones 11 a 16:

11. Un método para preparar un conjugado de anticuerpo anti-HER2, caracterizado porque el anticuerpo anti-HER2 se trata en una condición reductora y después se hace reaccionar con un compuesto seleccionado del grupo de compuestos mostrado a continuación:

(maleimid-N-il)-CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂-O-CH₂CH₂-C(=O)-

GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX),

(maleimid-N-il)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-(NH-DX), or

(maleimid-N-il)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-(NH-DX),

en donde (maleimid-N-il)- es un grupo representado por la siguiente fórmula:

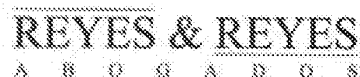
(...)

en donde el anticuerpo anti-HER2 comprende una cadena pesada una cadena ligera seleccionada del grupo:

una cadena pesada que consiste en una secuencia de aminoácidos que consiste en las posiciones 1 a 449 de SEQ ID NO: 1 y una cadena ligera que consiste en una secuencia de aminoácidos que consiste en las posiciones 1 a 214 de SEQ ID NO: 2 y

una cadena pesada que consiste en la secuencia de aminoácidos de SEQ ID NO: 1 y una cadena ligera que consiste en la secuencia de aminoácidos de SEQ ID NO: 2.

y en donde adicionalmente las secuencias pueden ser seleccionadas entre diferentes segmentos de la cadena de aminoácidos de las SEQ ID NO: 1 y 2.



Por lo tanto la materia objeto de las reivindicaciones excede lo desarrollado y corresponde a una extrapolación no razonable de las moléculas evaluadas.

De acuerdo a lo anterior, la solicitud falla en cumplir con lo dispuesto por el Artículo 30 de la D. 486 que precisa lo siguiente: - *Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción.*

Por otro lado, las reivindicaciones 11 – 16 que establecen el método de producción de un conjugado de anticuerpo anti-HER2, incluyen regiones funcionalmente diferentes que no se relacionan ni en su estructura ni en su función entre los conjugados de las reivindicaciones 1 – 11, por lo que la solicitud además incumple con el requisito de unidad de invención tal como lo determina el Art. 25 de la D. 486: -*La solicitud de patente sólo podrá comprender una invención o un grupo de invenciones relacionadas entre sí, de manera que conformen un único concepto inventivo.*

En este sentido, la solicitud no sólo comprende diferentes categorías desligadas entre sí de las características esenciales, sino que además incluye una cantidad no determinada de posibles invenciones al sumar a un método ya bastante amplio en el que se incluyen además moléculas comerciales, tales como el anticuerpo Trastuzumab, dentro de las secuencias reclamadas y que son objeto de evaluación en toda la memoria descriptiva de la solicitud.

Por otro lado, la solicitud en trámite pretende la protección de un medicamento de acuerdo a las siguientes características:

9. *Un fármaco antitumoral y/o fármaco anticancerígeno caracterizado porque contiene el conjugado de anticuerpo-fármaco de conformidad con la reivindicación 1.*

Sin embargo, se considera que tal descripción no es clara ni concisa y podría cobijar un sinnúmero de composiciones que comprenden conjugados con compuestos antitumorales. Así las cosas, la materia de las reivindicaciones y en general la solicitud en estudio no puede ser objeto de patente pues incumple las normas mínimas de presentación, como es la claridad, concisión, soporte y unidad de invención.

REYES & REYES

A B O G A D O S

La solicitud carece de nivel inventivo

Según la descripción, la solicitud trata de resolver el problema técnico objetivo de disminuir la toxicidad del tratamiento del cáncer con un antitumoral mediante un conjugado entre un compuesto antitumoral, un agente enlazante y anticuerpo anti-HER2 (podría ser trastuzumab):

En este contexto, los inventores crearon un enlazador con una estructura específica y exitosa en la obtención de un conjugado de anticuerpo-fármaco en el cual el anticuerpo anti-HER2 y exatecano son conjugados entre sí mediante el enlazador, y se confirma un excelente efecto antitumoral presentado por el conjugado para de este modo, completar la presente invención.

Materia que ya había sido revelada en el estado de la técnica, prueba de ello se encuentra en el siguiente documento publicado anteriormente a la fecha de presentación de la solicitud en estudio y que cito a continuación:

- Publicación Americana US 2016/0287722 A1 ***“Immunoconjugates with an Intracellularly-Cleavable Linkage”*** Serengulam V. Govindan, Summit, NJ [US] 2016-10-06, objeta el nivel inventivo de la solicitud en trámite ya que describe conjugados terapéuticos con mejorada capacidad de marcar varios tipos de células cancerígenas que contienen motivos diana y motivos terapéuticos:

SUMMARY OF THE INVENTION

[0010] In one embodiment, the invention relates to an immunoconjugate comprising:

[0011] (a) a targeting moiety;

[0012] (b) a chemotherapeutic moiety; and

[0013] (c) a linker binding to the targeting moiety via a thiol group, and to the chemotherapeutic moiety via an intracellularly-cleavable moiety other than a hydrazone. In another embodiment, the invention relates to an immunoconjugate comprising:

REYES & REYES

A B O G A D O S

[0014] (a) targeting moiety;
 [0015] (b) a chemotherapeutic moiety; and
 [0016] (c) a linker binding to the targeting moiety via a thiol group; and to the chemotherapeutic moiety via an intracellularly-cleavable moiety other than a hydrazone; wherein said linker comprises an amino acid moiety and a thiol-reactive moiety and is linked to the chemotherapeutic moiety via the amino acid moiety and to the targeting moiety via the thiol-reactive moiety.
 [0017] Another embodiment of the invention is a method of treating cancer, a malignancy, an autoimmune disease, an infection, or an infectious lesion with the immunocjugates described herein.

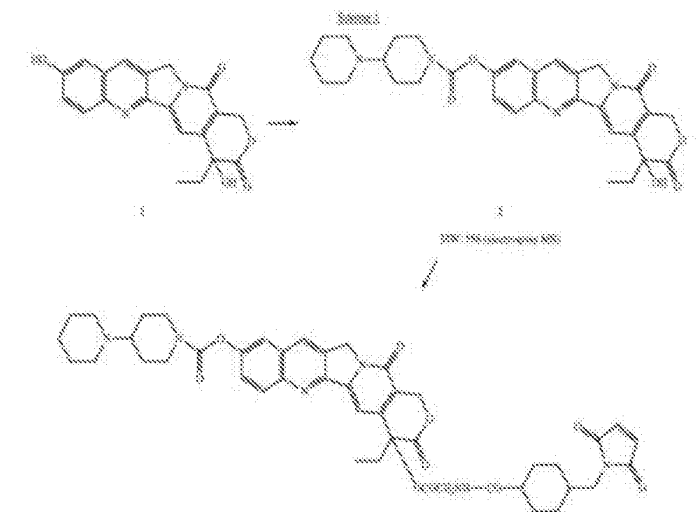
Como se puede ver en el sumario, *Govindan et al* revela las características esenciales del conjugado de anticuerpo reclamado, tales como el motivo diana, el motivo quimioterapéutico, el ligador o enlazante que comprende un grupo tiol, en el que se unen los anticuerpos anti-HER2 entre otros anticuerpos, tal como se puede observar en el siguiente fragmento:

[0052] In a preferred embodiment of the present invention, antibodies, such as mAbs, are used that recognize or bind to markers or tumor-associated antigens that are expressed at high levels on target cells and that are expressed predominantly or only on diseased cells versus normal tissues, and antibodies that internalize rapidly. Antibodies useful within the scope of the present invention include mAbs with properties as described above (and show distinguishing properties of different levels of internalization into cells and microorganisms), and contemplate the use of, but are not limited to, in cancer, the following mAbs: LL1 (anti-CD74), LL2 (anti-CD22), RS7 (anti-epithelial glycoprotein-1(EGP-1)), PAM-4 and KC4 (both anti-MUC1), MN-14 (anti-carcinoembryonic antigen (CEA, also known as CD66e), Mu-9 (anti-colon-specific antigen-p), Immu 31 (an anti-alpha-fetoprotein), TAG-72 (e.g., CC49), Tn, J591 (anti-PSMA (prostate-specific membrane antigen)), G250 (an anti-carbonic anhydrase IX mAb) and L243 (anti-HLA-DR). Other useful antigens that may be targeted using these conjugates include HER-2/neu, Brf3, CD19, CD20 (e.g., C2H8, hA20, 1F5 Mahs) CD21, CD23, CD80, alpha-feto-protein (AFP), VEGF, EGF receptor, P1GF, MUC1, MUC2, MUC3, MUC4, PSMA, gangliosides, HCG, EGP-2 (e.g., 17-1A), CD37, HLA-DR, CD30, Ia, A3, A33, Ep-CAM, KS-1, Le(y), 5100, PSA (prostate-specific antigen), tenascin, folate receptor, Thomas-Friedenreich antigens, tumor necrosis antigens, tumor angiogenesis antigens, Ga 733, IL-2, IL-6, T101, MAGE, antigen to which L243 binds, CD66 antigens, i.e. CD66a-d or a combination thereof. The CD66

REYES & REYES

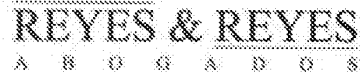
A B O G A D O S

De igual forma, los compuestos derivados de hidroxycamptotecina con 4-(N-maleimidometil) ciclohexano – 1 carbonil se divulgaban previamente en dicho documento:



Además, se desea resaltar que el agente enlazante se habría anticipado ya que el núcleo **succinimid** había sido revelado por Govindan:

Solicitud Colombiana NC2016/0000187	US 2016/0287722 A1
<p>Rv1:</p> <p>-(Succinimid-3-il-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-(NH-DX),</p> <p>-(Succinimid-3-il-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-(NH-DX), o</p> <p>-(Succinimid-3-il-N)-CH₂CH₂-C(=O)-NH-CH₂CH₂-O-CH₂CH₂-O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX),</p> <p>(Succinimid-3-il-N)- tiene estructura representada por la siguiente fórmula:</p> <p>{Fórmula 2}</p>	<p>→</p>



Ahora bien, aunque la estructura del enlazante no es idéntica, se observa que en la descripción no existe información suficiente que permita identificar un efecto sorprendente o novedoso obtenido por dicho enlazante, en comparación a otros enlazantes conocidos en el estado de la técnica, y se considera que el efecto logrado era obvio al emplear un anticuerpo como por ejemplo trastuzumab para dirigir el compuesto citotóxico hacia las células diana.

En conclusión, la reivindicación principal ya era conocida en el estado de la técnica si se tiene en cuenta:

- El compuesto antitumoral derivado de campotecinas así como su uso en conjugados de este tipo
- Anticuerpos anti-HER2 (podría tratarse de trastuzumab) así como su uso en conjugados
- La parte succinimid era ya usado por otros autores como Govindan

y dado que no existen datos que indiquen que el agente enlazador es el responsable de mejorar o aumentar el efecto biológico del tratamiento antitumoral no es posible considerar que la presente solicitud sea inventiva.

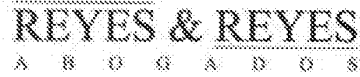
Rogamos se analicen los argumentos expuestos con el rigor y detenimiento habituales y que en consecuencia se decrete, además de la negación de la solicitud en comento, el archivo del expediente que la contiene

IV. FUNDAMENTOS DE DERECHO

Son fundamentos de derecho el Capítulo I, (Requisitos de Patentabilidad) y el artículo 42 (Oposiciones) de la Decisión 486 de 2000.

V. SOLICITUD DE PLAZO EXTRA PARA SUSTENTAR LA OPOSICIÓN

En desarrollo de lo dispuesto por el artículo 42, inciso 2° de la Decisión 486, solicito comedidamente a su Despacho el plazo adicional de sesenta días para sustentar la presente oposición. Para los anteriores efectos, me permito anexar la constancia de pago correspondiente.

**V. PRUEBAS**

Ruego que se tenga como tal, sin perjuicio de las que puedan presentarse al vencimiento de la prórroga solicitada, y de aquellas que el Despacho considere oficiosamente, el siguiente:

D1. US 2016/0287722 A1 “*Immunoconjugates with an Intracellularly-Cleavable Linkage*” Serengulam V. Govindan, Summit, NJ [US] 2016-10-06, portada, p 1–3, reivindicaciones

VI. ANEXOS

Para efectos del trámite correspondiente, me permito anexar al presente escrito los siguientes anexos:

1. Certificado de existencia y representación legal de **LABORATORIOS LEGRAND S.A.**.
2. Poder para actuar.
3. Constancia de pago de las tasas de tramitación de oposición.
4. Las mencionadas en el acápite de pruebas

VII. NOTIFICACIONES

El suscrito recibirá notificaciones en la secretaría de su Despacho o en mi oficina localizada en la Carrera 17 No. 88-23, Oficina 205, Fax (1) 621.25.42 de esta ciudad. Laboratorios Legrand S.A. las recibirá en la secretaría de su Despacho o en la Carrera 19-A No. 90-13, Oficina 901 de Bogotá D.C.

Señor Superintendente,

JUAN PABLO REYES VILLAMIZAR

C.C. 80.419.709 de Usaquén
T.P.A. 92.189 de C.S.J.



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/187,179	06/20/2016	Toshinori AGATSUMA	111119-0108	8341

22428	7590	10/21/2016
Foley & Lardner LLP 3000 K STREET N.W. SUITE 600 WASHINGTON, DC 20007-5109		

EXAMINER	
AKHOON, KAUSER M	

ART UNIT	PAPER NUMBER
1642	

NOTIFICATION DATE	DELIVERY MODE
10/21/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipdocketing@foley.com

Office Action Summary	Application No. 15/187,179	Applicant(s) AGATSUMA ET AL.	
	Examiner KAUSER AKHOON	Art Unit 1642	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on 06/20/2016.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☒ Claim(s) 1-8 is/are pending in the application.
5a) Of the above claim(s) ____ is/are withdrawn from consideration.

6) ☐ Claim(s) ____ is/are allowed.

7) ☒ Claim(s) 1-8 is/are rejected.

8) ☐ Claim(s) ____ is/are objected to.

9) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10) ☐ The specification is objected to by the Examiner.

11) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☐ All b) ☐ Some** c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. ____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date 06/27/2016.

3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____.

4) ☐ Other: ____.

Application/Control Number: 15/187,179

Page 2

Art Unit: 1642

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Claims 1-8 are currently pending.

Priority

Acknowledgment is made that this application is a 371 National Stage Entry of International Application PCT/JP14/006421, filed December 24, 2014, which claims priority to Japanese Patent Application No. 2013-267548, filed December 25, 2013.

Information Disclosure Statement

Applicant's Informational Disclosure Statement, filed on 06/27/2016 has been considered. The signed and initialed PTO 1449 has been mailed with this action.

Specification

The disclosure is objected to because of the following informalities: the Sequence Listing was submitted electronically as a text file, thus the specification must be amended to contain an incorporation-by-reference of the material in the ASCII text file in a separate paragraph identifying the name of the ASCII text file, the date of creation, and the size of the ASCII text file in bytes. See MPEP 502.05 L and Fed. Register (Oct. 27, 2009, 74(206): 55,208-55,209)..

Appropriate correction is required.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the

Application/Control Number: 15/187,179

Page 3

Art Unit: 1642

subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

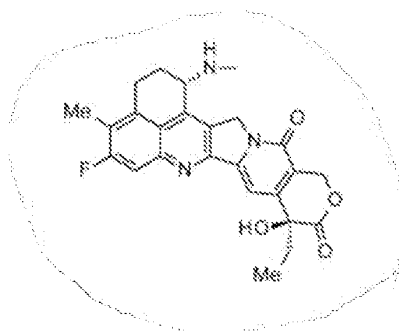
Claims 1-8 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Susaki et al. ("Susaki", US 7,041,818) in view of Govidan et al. ("Govidan", US2011/0293513, cited in IDS submitted 06/27/2016) and Flygare et al. ("Flygare", US2012/0121615).

Application/Control Number: 15/187,179

Page 4

Art Unit: 1642

Susaki teaches drug delivery systems (drug complexes) in which DX-8951 (exatecan,



Formula I ((1*S*,9*S*)-1-Amino-9-ethyl-5-fluoro-1,2,3,9,12,15-

hexahydro-9-hydroxy-4-methyl-10*H*,13*H*-benzo(de)pyrano(3',4':6,7)

indolizino(1,2-*b*)quinoline-10,13-dione)) is linked to a GGFG tetrapeptide. (Example 2, column

19, lines 50-60). Susaki teaches the drug bound by means of a spacer comprising GGFG

tetrapeptide (column 6, lines 1-15). Susaki teaches that the tetrapeptide GGFG as having a high

releasing rate (Table 1, column 10, line 8). Susaki teaches that the linker may be $-O-(CH_2)_n-$

$NH-$ where *n* is 1-16 (column 11, lines 48-52). Susaki teaches the use of the drug complex to

treat liver cancer (claim 18, column 5, lines 1-8). However, Susaki does not teach the antibody

drug conjugate with anti-TROP2 antibody and the linkers as instantly claimed.

Flygare teaches antibody drug conjugates using thiol reactive electrophilic functional

group, maleimidocaproyl attached to an amino acid spacer attached to a drug moiety

(maytansinoid) (page 16, paragraph [0144-0145] and Figure 1a).

Govidan teaches antibody drug conjugates having hRS7 which is an anti-TROP2

antibody with a drug (page 16, paragraph [0102] and Table 2, page 35).

Therefore, it would have been obvious to one having ordinary skill in the art at the time of the instant invention to have prepared the antibody drug conjugate using the Susaki exatecan linked to GGFG tetrapeptide compositions with maleimidocaproyl taught by Flygare with the

Application/Control Number: 15/187,179

Page 5

Art Unit: 1642

anti-TROP2 antibody of Govidan with a reasonable expectation of success. One having ordinary skill in the art would have been motivated to combine the teaching of Susaki, Flygare and Govidan because Susaki teaches that the GGFG tetrapeptide has a high releasing rate (Table 1, column 10, line 8) and Flygare teaches that maleimidocaproyl group is thio reactive group for conjugating antibodies to the drug.

Although Flygare, Govidan and Susaki lists all of the linkers required by the claim, all of the possibilities identified in Flygare, Govidan and Susaki are not found in any specific embodiment, one of ordinary skill in the art is able to "envisage" the claimed combination comprising the antibody drug conjugate from sufficiently limited or well delineated examples, (see MPEP 2131.02). Thereby meeting the limitations of claims 1 and 4-5.

Regarding claims 2 and 3, Govidan teaches that hRS7 antibody conjugates typically 5 to 7 drugs per antibodies (Table 2, page 35, paragraph [0195]).

Regarding claims 6 and 8, Susaki teaches the use of the drug complex to treat liver cancer (claim 18, column 5, lines 1-8).

Regarding claim 7, Susaki teaches pharmaceutical preparations (column 15, lines 25-36).

Thus, given the teachings of the prior art and level of a person of ordinary skill in the art at the time of Applicant's invention one would have had a reasonable expectation of success in practicing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

In the absence of any factual showing or unexpected results the presently claimed compositions are considered *prima facie* obvious over the prior art for the reasons stated above.

Application/Control Number: 15/187,179

Page 6

Art Unit: 1642

In light of the forgoing discussion, the subject matter defined by the claims would have been obvious within the meaning of 35 USC § 103(a).

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Application/Control Number: 15/187,179

Page 7

Art Unit: 1642

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Application No. 14/435,114

Claims 1-8, are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-48 of copending Application No. 14/435,114 in view of Govidan et al. ("Govidan", US2011/0293513).

Copending claims composition encompasses the same drug, overlapping linker system and antibodies, however do not teach anti-TROP2 antibody. Govidan teaches antibody drug conjugates having hRS7 which is an anti-TROP2 antibody with a drug (page16, paragraph [0102] and Table 2, page 35).

Therefore, it would have been obvious to have prepared the copending antibody drug conjugate using the anti-TROP2 antibody of Govidan with a reasonable expectation of success.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Application No. 14/436,458

Claims 1-8, are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-54 of copending Application No. 14/436,458 in view of Govidan et al. ("Govidan", US2011/0293513).

Application/Control Number: 15/187,179

Page 8

Art Unit: 1642

Copending claims composition encompasses the same drug, overlapping linker system and antibodies, however do not teach anti-TROP2 antibody. Govidan teaches antibody drug conjugates having hRS7 which is an anti-TROP2 antibody with a drug (page16, paragraph [0102] and Table 2, page 35).

Therefore, it would have been obvious to have prepared the copending antibody drug conjugate using the anti-TROP2 antibody of Govidan with a reasonable expectation of success.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Application No. 15/180,203

Claims 1-8 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-18 of copending Application No. 15/180,203 in view of Govidan et al. (“Govidan”, US2011/0293513).

Copending claims composition encompasses the same drug, overlapping linker system and antibodies, however do not teach anti-TROP2 antibody. Govidan teaches antibody drug conjugates having hRS7 which is an anti-TROP2 antibody with a drug (page16, paragraph [0102] and Table 2, page 35).

Therefore, it would have been obvious to have prepared the copending antibody drug conjugate using the anti-TROP2 antibody of Govidan with a reasonable expectation of success.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

No claims are allowed.

Application/Control Number: 15/187,179

Page 9

Art Unit: 1642

Any inquiry concerning this communication or earlier communications from the examiner should be directed to KAUSER M. AKHOON whose telephone number is (571) 270-1026. The examiner can normally be reached on M-F 8:30 AM-5:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Misook Yu can be reached on (571) 272-0839. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/K. A./

Examiner, Art Unit 1642

/PETER J REDDIG/

Primary Examiner, Art Unit 1642

Федеральная служба по интеллектуальной
собственности
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Форма N 10 ИЗ, ПМ, ПО-2015
100,181

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Москва
129090

Г&П 31-08-2017



001-0022797875

На № 2015113767 от 14.04.2015

Наш № 2015113767/10(021591)

При переписке просим ссылаться на номер заявки и
сообщить дату получения настоящей
корреспонденции от **29.08.2017**

ЗАПРОС

экспертизы по существу

(21) Заявка № 2015113767/10(021591)

(22) Дата подачи заявки 10.10.2013

(86) Заявка № РСТ/JP2013/006069 от 10.10.2013

(71) Заявитель(и) ДАЙИТИ САНКИО КОМПАНИ, ЛИМИТЕД, JP

(51) МПК

C07K16/28 (2006.01)



ВОПРОСЫ, ДОВОДЫ, ЗАМЕЧАНИЯ, ПРЕДЛОЖЕНИЯ

Отдел биохимии ознакомился с материалами заявки и сообщает следующее.

Заявитель просит выдать патент РФ с конвекционным приоритетом по заявке 2012-225887 от 11.10.2012, поданной в Патентное Ведомство Японии.

К рассмотрению представлены объекты:

1. Конъюгат антитело-лекарственное средство;
2. Лекарственное средство;
3. Противоопухолевое лекарственное средство и/или противораковое лекарственное средство;
4. Фармацевтическая композиция;
5. Способ лечения опухоли и/или рака;
6. Промежуточное соединение лекарственное средство-линкер;
7. Соединение;
8. Линкер.

Анализ формулы изобретения производится в соответствии с положениями Гражданского Кодекса РФ** (далее ГК) и Административного Регламента**** (Регламента), в части не противоречащей Кодексу.

Касательно п.1 формулы, экспертиза сообщает следующее:

Обращаем внимание на то, что:

- * запрашиваемые документы (заменяющие листы) необходимо представить в 2-х экземплярах;
- * информация, представленная в дополнительных материалах, может быть предоставлена для ознакомления третьим лицам (п. 27 Регламента**** п. 23 Регламента****);
- * сведения о состоянии делопроизводства по заявкам размещаются на сайте ФИПС по адресу «www.fips.ru» в разделе «Информационные ресурсы / Открытые реестры»;
- * информация об услугах, предоставляемых ФИПС на платной основе, размещена на сайте по адресу «www.fips.ru» в разделе «Услуги ФИПС»;
- * при изменении адреса для переписки необходимо сообщить об этом незамедлительно.

* Гражданский кодекс Российской Федерации в редакции 2008 года (применяется к заявкам, поданным до 01.10.2014).

** Гражданский кодекс Российской Федерации в редакции 2014 года (применяется к заявкам, поданным с 01.10.2014).

***Положение о патентных и иных пошлинах за совершение юридически значимых действий, связанных с патентом на изобретение, полезную модель, промышленный образец, с государственной регистрацией товарного знака и знака обслуживания, с государственной регистрацией и предоставлением исключительного права на наименование места происхождения товара, а также с государственной регистрацией отчуждения исключительного права на результат интеллектуальной деятельности или средство индивидуализации, залога исключительного права, предоставления права использования такого результата или такого средства по договору, перехода исключительного права на такой результат или такое средство без договора, утвержденное постановлением Правительства Российской Федерации от 10.12.2008 N 941 с изменениями.

**** Административный регламент исполнения Федеральной службой по интеллектуальной собственности, патентам и товарным знакам государственной функции по организации приема заявок на изобретение и их рассмотрения, экспертизы и выдачи в установленном порядке патентов Российской Федерации на изобретение утвержден приказом Министерства образования и науки Российской Федерации от 29 октября 2008 г. N 327, зарегистрированным Министерством юстиции Российской Федерации 20 февраля 2009 г. N 13413 (Бюллетень нормативных актов федеральных органов исполнительной власти, 2009, N 21).

***** Административный регламент исполнения Федеральной службой по интеллектуальной собственности, патентам и товарным знакам государственной функции по организации приема заявок на полезную модель и их рассмотрения, экспертизы и выдачи в установленном порядке патентов Российской Федерации на полезную модель утвержден приказом Министерства образования и науки Российской Федерации от 29 октября 2008 г. N 326, зарегистрированным Министерством юстиции Российской Федерации 24 декабря 2008 г. N 12977 (Бюллетень нормативных актов федеральных органов исполнительной власти, 2009, N 10).

- Пункт 1 формулы нарушает п. 10.8.3 Регламента, так как в п.1 формулы отсутствует назначение.
- Родовым понятием пункта 1 формулы является «конъюгат антитело - лекарственное средство», из чего следует, что в раскрытии пункта формулы должна быть приведена характеристика антитела. Из данной формулировки следует, что в качестве антитела может быть использовано любое антитело, что делает данный пункт формулы несоответствующим пп. 10.8(2), 10.8(4), 10.8.3 и 24.4(5) Регламента, так как материалами заявки не подтверждено использование любых антител. Таким образом, заявителю следует ограничить объем притязаний, охарактеризовав используемые антитела при помощи физико-химических или иных характеристик, как того требует п. 10.8.3 Регламента.
- Из формулировки родового понятия следует, что лекарственное средство может быть любым, не обязательно противоопухолевым, однако далее указывается противоопухолевое средство. По мнению экспертизы, более корректной формулировкой будет, например, «Конъюгат антитело – лекарственное средство, содержащее противоопухолевое соединение, где противоопухолевое соединение (далее по тексту)».

Таким образом, заявителю следует скорректировать данный пункт формулы в соответствии с вышеприведенными доводами.

Пункт 2 формулы нарушает требования единства терминологии (см. п. 10.11(4) Регламента), т.к в п.1 формулы заявителем используется «пептидный остаток», а в п.2 формулы «аминокислотный остаток». Заявителю следует скорректировать данный пункт формулы, приведя терминологию к единству.

Что касается пункта 13 формулы, касательно группы $-(NH-DX)$, имеющей структурную формулу, соответствующую противоопухолевому соединению, представленному в п.1 формулы, то, данную группу нельзя, в соответствии с материалами заявки, обобщать и включать в исходной понятие линкер. Кроме того, в материалах заявки, на стр.105 русский текст, описание, к данным соединениям, приведенным в п.13, применяется понятие «осколок лекарственное средство-фрагмент линкерной структуры». Заявителю следует скорректировать данный пункт формулы, либо убрав группу $-(NH-DX)$ из заявляемых соединений, либо скорректировать данный пункт формулы, внося в него признак «осколок лекарственное средство-фрагмент линкерной структуры» для соответствия единства терминологии (п. 10.11(4) Регламента). Аналогичные доводы касаются п.14 формулы.

Что касается независимого п.15 формулы, то анализ формулы показал, что изобретение по данному пункту формулы является частным случаем изобретения, представленного в п.1 формулы. Таким образом, заявителю целесообразно будет отказаться от рассмотрения данного пункта формулы за избеганием повторения одинаковых изобретений в формуле, либо скорректировать данный пункт формулы, указав отличия изобретения по п.15 от изобретения по п.1, учитывая так же вышеприведенные доводы, касающиеся пункта 1. Аналогичные доводы, касательно повторения изобретения, касаются пп. 16-21 формулы.

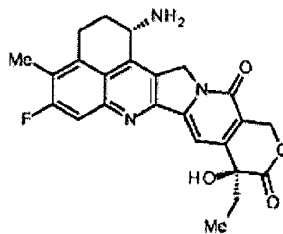
Что касается п.25 формулы, то признак «обладающее одним или более из свойств» является неясным, поскольку не ясно максимальное количество сочетаемых в антителе свойств. Заявителю следует скорректировать данный пункт формулы, в соответствии с пп. 10.8(2), 10.8(4) и 24.4(5) Регламента.

Пункт 26 формулы нарушает пп. 10.8(2) и 24.4(5) Регламента, т.к. не ясен объем заявляемого изобретения. Исходя из формулировки пункта следует, что в качестве опухолевых клеток могут выступать любые опухолевые клетки, однако материалами заявки не подтверждено использование любых клеток в качестве опухолевых. Заявителю следует скорректировать данный пункт формулы, ограничив объем притязаний относительно использования опухолевых клеток, не выходя за рамки исходных материалов.

В соответствии с п. 10.8.3 Регламента для изобретения по п.30 формулы необходимо приводить назначение. Заявителю следует скорректировать данный пункт формулы, приведя назначение лекарственного средства. Аналогичные доводы касаются п. 31 формулы.

В соответствии с пунктом 10.8.3 Регламента для изобретения по п.33 формулы необходимо приводить назначение и количественные характеристики входящих в состав ингредиентов. Заявителю следует скорректировать данный пункт формулы, приведя назначение фармацевтической композиции и количественное содержание входящих в состав ингредиентов.

Что касается пункта 35 формулы, то данный пункт формулы нарушает п.10.8.(4) Регламента, так как не ясно, признак «X представляет атом брома или атом йода», при условии дальнейшего раскрытия группы $-(NH-DX)$, представленной группой



в которой отсутствует атом брома или йода. Кроме того, в соответствии с п.10.8.3 Регламента для изобретения по п.35 формулы необходимо приводить назначение. Заявителю следует скорректировать данный пункт формулы, например, обозначив признак «X представляет атом брома или атом йода» иной буквой алфавита или обобщив данные атомы как «атом галогена», для избежания неясности.

В соответствии с п.10.8.3. Регламента для изобретения по п.46 необходимо приводить назначение. Аналогичные доводы касаются пп. 47-49 формулы.

Помимо вышесказанного, экспертиза сообщает, что анализ формулы изобретения показал, что она составлена с нарушением требований единства изобретения согласно Правилу 13.2 Инструкции РСТ по следующим обоснованиям.

Заявляемые изобретения не содержат ни одного общего особого технического признака.

Анализ формулы изобретения выявил несколько групп изобретений.

I группа изобретений включает в себя объекты по пп. **1, 15, 30, 31, 33, 34.**

II группа изобретений включает в себя объект по п. **35.**

III группа изобретений включает в себя объект по п. **46.**

IV группа изобретений включает в себя объект по п. **47.**

V группа изобретений включает в себя объект по п. **48.**

VI группа изобретений включает в себя объект по п. **49.**

В связи с вышеизложенным, экспертиза не может признать представленную формулу изобретения соответствующей единому изобретательскому замыслу, поскольку вышеуказанные группы изобретений не объединены особым единым техническим признаком, как того требует Правило 13.2 Инструкции РСТ.

Кроме того, формула изобретения составлена с нарушением требований единства изобретения согласно национальному законодательству. Согласно п.10.5 Регламента, единство изобретения признается соблюденным, если в формуле изобретения заявлена группа изобретений, одно из которых предназначено для получения, осуществления или использования другого, либо относится к объектам одного вида, одинакового назначения, обеспечивающим получение одного и того же технического результата (варианты)

Анализ формулы изобретения в соответствии с требованиями национального законодательства к единству изобретения позволил выявить аналогичные группы изобретений.

I группа изобретений включает в себя объекты по пп. **1, 15, 30, 31, 33, 34.**

II группа изобретений включает в себя объект по п. **35.**

III группа изобретений включает в себя объект по п. **46.**

IV группа изобретений включает в себя объект по п. 47.

V группа изобретений включает в себя объект по п. 48.

VI группа изобретений включает в себя объект по п. 49.

Согласно формуле изобретения, объекты, отнесенные в различные группы, не предназначены для получения, осуществления или использования друг друга, экспертиза не может признать их соответствующими требованию единства, как того требует п.1 ст.1375 Кодекса и п.10.5 Регламента.

Таким образом, экспертизой установлено нарушение требования единства изобретения, поэтому заявителю предлагается выбрать группу изобретений для дальнейшего рассмотрения в рамках настоящей заявки, либо уточнить объем притязаний, приведя формулу изобретения в соответствии с требованиями единства изобретения. На не вошедшие изобретения в данную для рассмотрения группу изобретений заявитель вправе подать выделенную заявку.

Таким образом, заявителю предлагается ознакомиться с доводами экспертизы и высказать свои соображения.

Если заявитель в **трёхмесячный срок со дня направления запроса** не представит запрашиваемые материалы или не подаст ходатайство о продлении этого срока, заявка будет признана отозванной. Срок представления запрошенных материалов может быть продлен, но не более чем на десять месяцев (общий срок продления не должен превышать десять месяцев) (п. 6 ст. 1386 Кодекса**)

Дополнительные материалы, поданные заявителем, не должны изменять заявку на изобретение по существу (п. 2 ст. 1378 Кодекса**).

Государственный
эксперт по
интеллектуальной
собственности II
категории отдела
биохимии ФИПС

Документ подписан электронной подписью
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Федеральная служба по интеллектуальной собственности
Федеральное государственное бюджетное учреждение
«Федеральный институт промышленной собственности»
(ФИПС)

ОТЧЕТ О ПОИСКЕ

1. ИДЕНТИФИКАЦИЯ ЗАЯВКИ		
Регистрационный номер	Дата подачи	
2015113767/10(021591)	10.10.2013	
PCT/JP2013/006069	10.10.2013	
Приоритет установлен по дате:		
☐ подачи заявки ☐ поступления дополнительных материалов от _____ к ранее поданной заявке № _____ ☐ приоритета по первоначальной заявке № _____ из которой данная заявка выделена ☐ подачи первоначальной заявки № _____ из которой данная заявка выделена ☐ подачи ранее поданной заявки № _____ ☒ подачи первой(ых) заявки(ок) в государстве-участнике Парижской конвенции (31) Номер первой(ых) заявки(ок) (32) Дата подачи первой(ых) заявки(ок) (33) Код страны		
1. 2012-225887	11.10.2012	JP
Название изобретения (полезной модели): ☒ - как заявлено; ☐ - уточненное (см. Примечания) КОНЪЮГАТ АНТИТЕЛО-ЛЕКАРСТВЕННОЕ СРЕДСТВО		
Заявитель: ДАЙИТИ САНКИО КОМПАНИ, ЛИМИТЕД, JP		
2. ЕДИНСТВО ИЗОБРЕТЕНИЯ		
☒ соблюдено ☐ не соблюдено. Пояснения: см. Примечания		
3. ФОРМУЛА ИЗОБРЕТЕНИЯ:		
☒ приняты во внимание все пункты		(см. Примечания)
☐ приняты во внимание следующие пункты:		
☐ принята во внимание измененная формула изобретения		(см. Примечания)
4. КЛАССИФИКАЦИЯ ОБЪЕКТА ИЗОБРЕТЕНИЯ (ПОЛЕЗНОЙ МОДЕЛИ) (Указываются индексы МПК и индикатор текущей версии)		
C07K 16/28 (2006.01)		
5. ОБЛАСТЬ ПОИСКА		
5.1 Проверенный минимум документации PCT (указывается индексами МПК)		
5.2 Другая проверенная документация в той мере, в какой она включена в поисковые подборки:		
5.3 Электронные базы данных, использованные при поиске (название базы, и если, возможно, поисковые термины): E-Library, Espacenet, PatSearch, PATENTSCOPE, RUPTO, NCBI, EMBL-EBI, Google, Google Scholar, PubMed, USPTO, ScienceDirect		
6. ДОКУМЕНТЫ, ОТНОСЯЩИЕСЯ К ПРЕДМЕТУ ПОИСКА		
Категория*	Наименование документа с указанием (где необходимо) частей, относящихся к предмету поиска	Относится к пункту формулы №
1	2	3
A	RU 2450008 C2 (ИНДЕНА С.П.А. (IT)) 10.05.2012	1-49
A	Charlotte F. McDonagh et al. "Engineered antibody-drug conjugates	1-49

(см. на обороте)

1	2	3
	with defined sites and stoichiometries of drug attachment", Protein Engineering, Design and Selection, V. 19 (7), P. 299-307, DOI 10.1093/protein/gzl013	
A	WO2006065533 A2 (SEATTLE GENETICS, INC.) 22.06.2006	1-49
A	US 20080050310 A1 (GENENTECH, INC) 28.02.2008	1-49
<p>*Особые категории ссылочных документов:</p> <p>«А» документ, определяющий общий уровень техники и не считающийся особо релевантным</p> <p>«Е» более ранний документ, но опубликованный на дату международной подачи или после нее</p> <p>«L» документ, подвергающий сомнению притязание(я) на приоритет, или который приводится с целью установления даты публикации другого ссылочного документа, а также в других целях (как указано)</p> <p>«О» документ, относящийся к устному раскрытию, использованию, экспонированию и т.д.</p> <p>«Р» документ, опубликованный до даты международной подачи, но после даты испрашиваемого приоритета</p> <p>«Т» более поздний документ, опубликованный после даты международной подачи или даты приоритета и не порочащий заявку, но приведенный для понимания принципа или теории, на которых основывается изобретение</p> <p>«Х» документ, имеющий наиболее близкое отношение к предмету поиска: заявленное изобретение не обладает новизной или изобретательским уровнем в сравнении с документом, взятым в отдельности</p> <p>«У» документ, имеющий наиболее близкое отношение к предмету поиска; заявленное изобретение не обладает изобретательским уровнем, когда документ взят в сочетании с одним или несколькими документами той же категории, такая комбинация документов очевидна для специалиста</p> <p>«&» документ, являющийся патентом-аналогом</p>		
7. ПРИМЕЧАНИЯ:		
8. УДОСТОВЕРЕНИЕ ОТЧЕТА		
Настоящий отчет состоит из 1 л.		К отчету приложены копии ссылок на л. в экз.
Дата действительного завершения поиска: 29.08.17		Должность и подпись уполномоченного лица:
<p>Поисковый орган: ФИПС Бережковская наб., 30, корп. 1, Москва, Г-59, ГСП-3, 125993 Телефон (8-499) 240- 60- 15. Факс (8-495) 531- 63- 18; e-mail: fips@rupto.ru</p>		ГЭ по ИС 2 кат. Кочнева И.К.

Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody–drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity

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Key words

Antibody–drug conjugate, bystander killing, HER2, T-DM, topoisomerase I inhibitor

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Antibody–drug conjugates deliver anticancer agents selectively and efficiently to tumor tissue and have significant antitumor efficacy with a wide therapeutic window. DS-8201a is a human epidermal growth factor receptor 2 (HER2)-targeting antibody–drug conjugate prepared using a novel linker-payload system with a potent topoisomerase I inhibitor, exatecan derivative (DX-8951 derivative, DXd). It was effective against trastuzumab emtansine (T-DM1)-insensitive patient-derived xenograft models with both high and low HER2 expression. In this study, the bystander killing effect of DS-8201a was evaluated and compared with that of T-DM1. We confirmed that the payload of DS-8201a, DXd (1), was highly membrane-permeable whereas that of T-DM1, Lys-SMCC-DM1, had a low level of permeability. Under a coculture condition of HER2-positive KPL-4 cells and negative MDA-MB-468 cells *in vitro*, DS-8201a killed both cells, whereas T-DM1 and an antibody–drug conjugate with a low permeable payload, anti-HER2-DXd (2), did not. *In vivo* evaluation was carried out using mice inoculated with a mixture of HER2-positive NCI-N87 cells and HER2-negative MDA-MB-468-Luc cells by using an *in vivo* imaging system. *In vivo*, DS-8201a reduced the luciferase signal of the mice, indicating suppression of the MDA-MB-468-Luc population; however, T-DM1 and anti-HER2-DXd (2) did not. Furthermore, it was confirmed that DS-8201a was not effective against MDA-MB-468-Luc tumors inoculated at the opposite side of the NCI-N87 tumor, suggesting that the bystander killing effect of DS-8201a is observed only in cells neighboring HER2-positive cells, indicating low concern in terms of systemic toxicity. These results indicated that DS-8201a has a potent bystander effect due to a highly membrane-permeable payload and is beneficial in treating tumors with HER2 heterogeneity that are unresponsive to T-DM1.

Antibody–drug conjugates (ADCs) represent a promising class of drugs with a wider therapeutic window than conventional chemotherapeutic agents, owing to efficient and specific drug delivery to antigen-expressing tumor cells. A first-generation ADC, gemtuzumab ozogamicin, an anti-CD33 antibody conjugated with a DNA alkylating agent calicheamicin, was approved by the FDA in 2000 for the treatment of CD33-positive relapsed AML.⁽¹⁾ However, 10 years after its approval, gemtuzumab ozogamicin was withdrawn from the market owing to a lack of clinical benefit in a post-marketing clinical trial.⁽²⁾ Two ADCs have been approved and are currently in the market; brentuximab vedotin, a CD30-targeting antibody in conjugation with a tubulin polymerization inhibitor monomethyl auristatin E, was approved in 2011 for the treatment of relapsed or refractory Hodgkin's lymphoma and systemic anaplastic large cell lymphoma.^(3–5) The introduction of trastuzumab emtansine (T-DM1), a human epidermal growth factor receptor 2 (HER2)-targeting ADC with a tubulin polymerization inhibitor DM1, was

approved in 2013 for HER2-positive breast cancer,^(6–8) proved that ADCs could target solid tumors in addition to hematological malignancies. More than 30 kinds of ADC programs are currently in clinical trials, and most of them are conjugated with the same type of payload, tubulin polymerization inhibitor.⁽⁹⁾ However, in the clinical trials of these ADCs, several dose-limiting toxicities were observed, and some were considered to be mediated by the free drugs in plasma.⁽¹⁰⁾ Currently, the development of a new linker-payload system focusing on different types of drugs has received attention⁽¹¹⁾ as improvements in terms of different antitumor spectrum, overcoming drug resistance to the conventional systems, and providing a greater safety profile would be much desired.

DS-8201a is a novel HER2-targeting ADC prepared using our original linker-payload system, which is composed of a humanized anti-HER2 antibody and a novel topoisomerase I inhibitor, exatecan derivative (DX-8951 derivative, DXd), by enzymatically cleavable peptide-linker.⁽¹²⁾ It showed potent

antitumor activity that was attributable the combination of pharmacological effects of the antibody component and topoisomerase I inhibition. The stability in plasma was favorable and the safety profiles in rats and monkeys were well tolerated. Moreover, DS-8201a was effective against T-DM1-insensitive tumors owing to the different mechanism of action of the conjugated drug and higher drug-to-antibody ratio (DAR) than that of T-DM1. Based on these promising preclinical profiles, a phase I dose escalation study was initiated in August 2015 in Japan (trial registration ID: NCT02564900).

The ADCs in both clinical trials and market target hematological malignancies; solid tumors are difficult to target as the target antigen in tumor tissue is expressed heterogeneously.⁽⁹⁾ Recently, preclinical studies of some ADCs showed that “bystander killing” may be a solution for this issue, which could benefit not only antigen-expressing tumor cells but also adjacent antigen-negative cells through the transfer of released payload from the antigen-expressing cells to the neighboring antigen-negative cells.^(13–16) However, several reports showed that T-DM1 did not have a bystander killing effect *in vitro*.^(15,16) This could be another differentiation point between DS-8201a and T-DM1.

In this report, we investigated the bystander killing potential of DS-8201a and compared with that of T-DM1 *in vitro* and *in vivo* using a unique method that supports more accurate evaluation than the methods reported previously. Moreover, we assessed the effect of membrane permeability of payloads on bystander killing based on a hypothesis that the ability of cell penetration of released payload is correlated to the intensity of bystander killing. Notably, DS-8201a has high membrane-permeable drug payload and showed more potent bystander killing than T-DM1 and anti-HER2 ADC with low cell membrane-permeable payload of topoisomerase I inhibitor, suggesting its potential in targeting tumors with HER2 heterogeneity, such as gastric cancer.

Materials and Methods

Antibody–drug conjugates and chemotherapeutics. DS-8201a (anti-HER2-DXd (1)) and anti-HER2-DXd (2) were synthesized according to the conjugation procedure described in another report⁽¹⁷⁾ by using the anti-HER2 Ab produced with reference to the same amino acid sequence of trastuzumab and its DAR is approximately 7 to 8. The IgG-DXd (1) and IgG-DXd (2) were synthesized using a method similar to that for DS-8201a and anti-HER2-DXd (2) with purified human IgG (Bethyl Laboratories, Montgomery, TX, USA), resulting in comparable DAR. T-DM1 was purchased from Genentech Roche (San Francisco, CA, USA), and DXds (1) and (2) and Lys-SMCC-DM1 were synthesized in our laboratory.

Cell lines. The human gastric carcinoma cell line NCI-N87 and the human breast adenocarcinoma cell line MDA-MB-468 were purchased from ATCC (Manassas, VA, USA). The human breast cancer cell line KPL-4 was provided by Dr. Kurebayashi at the Kawasaki Medical University (Kurashiki, Japan). MDA-MB-468-Luc cells were established by transfecting them with the lentivirus vector (Valent BioSciences Corporation, Libertyville, IL, USA) carrying the luciferase gene. All cell lines were cultured with RPMI 1640 Medium (Thermo Fisher Scientific Inc. Waltham, MA, USA) supplemented with 10% heat-inactivated FBS at 37°C under 5% CO₂ atmosphere.

Distribution coefficient (log D, pH 7.4). Equal amounts of n-octanol and PBS (pH 7.4) were mixed, shaken, and left for more than 12 h. Each compound solution in DMSO (10 mM)

was added to the solution (final 100 μM containing DMSO 1%) and shaken vigorously for 30 min at room temperature. After centrifugation, the concentration of compound in each phase (upper, n-octanol phase; lower, aqueous phase) was measured by an LC-MS/MS system (API 4000; AB Sciex, Framingham, MA, USA). Log D was calculated using the following equation: Log D = log (peak area in n-octanol phase / peak area in aqueous phase).

Parallel artificial membrane permeability assay. Parallel artificial membrane permeability assay (PAMPA) was carried out using a Freedom EVO200 system (Tecan, Männedorf, Switzerland). The filter membrane of the acceptor plate (Stirwell PAMPA Sandwich; Pion, Billerica, MA, USA) was coated with GIT-0 lipid solution (Pion). Each compound solution in DMSO (10 mM) was added to Prisma HT buffer (Pion) to obtain 5-μM donor solutions (containing 0.05% DMSO, pH 5.0 and pH 7.4), and then placed on a donor plate. The acceptor plate was filled with an acceptor sink buffer (Pion). The donor plate was stacked onto the acceptor plate and incubated for 4 h at 25°C. After incubation, the concentrations of compounds in both plates were measured by an LC-MS/MS system (API 4000). The permeability coefficient (P_{eff}; 10^{−6} cm/s) was calculated using PAMPA Evolution DP software (Pion).

***In vitro* cell growth assay.** Cells were seeded in a 96-well plate at 1000 cells/well for KPL-4 and 2000 cells/well for MDA-MB-468. After overnight incubation, a serially diluted solution of each ADC was added. Cell viability was evaluated after 5 days using a CellTiter-Glo luminescent cell viability assay from Promega (Madison, WI, USA) according to the manufacturer's instructions. For coculture study, KPL-4 and MDA-MB-468 cells were seeded in a 6-well plate at 1 × 10⁵ cells and 3 × 10⁵ cells, respectively, in 2 mL/well culture medium. After overnight incubation, the supernatant was removed from the plate and each ADC diluent (10 nM) was added at 6 mL/well. Viable cells were detached from the plate after 5 days of culture, and the cell number in each well was determined using a cell counter. In order to determine the ratio of KPL-4 and MDA-MB-468 cells of the total viable cells, the cells were stained with anti-HER2/nuc FITC (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated on ice for 20 min. After washing, fluorescent signals of 2 × 10⁴ stained cells were measured using a flow cytometer. Based on the number and ratio of HER2-positive and HER2-negative cells in each treatment well, the number of KPL-4 or MDA-MB-468 cells was calculated.

***In vivo* xenograft studies.** All *in vivo* studies were carried out in accordance with the local guidelines of the Institutional Animal Care and Use Committee. Specific pathogen-free female C57BL/6-*Foxn1*^{nu/nu}/CrJ mice (BALB/c nude mice) aged 5 weeks were purchased from Charles River Laboratories Japan Inc. (Yokohama, Japan). All models were established by s.c. inoculation in the flanks of the mice. NCI-N87 and MDA-MB-468-Luc models were established by injecting 5 × 10⁶ and 1 × 10⁷ cells suspended in a Matrigel matrix (Corning Inc. Corning, NY, USA), respectively. After 6 days for NCI-N87, and 9 days for MDA-MB-468-Luc models, the tumor-bearing mice were randomized into treatment and control groups based on the tumor volume, and dosing initiated (day 0). Each ADC was given i.v. to the mice at a dose of 3 or 10 mg/kg, and a volume of 10 mL/kg. As a vehicle, PBS buffer (10 mM acetate buffer, 5% sorbitol, pH 5.5) was given at the same volume as the ADCs. The tumor volume was defined as 1/2 × length × width².

***In vivo* luciferase imaging.** Seven days after inoculating the mixture of 5 × 10⁶ NCI-N87 cells and 1 × 10⁷ MDA-MB-

468-Luc cells suspended in the Matrigel matrix into the right flank of the mice at a total volume of 100 μ L, the tumor-bearing mice were randomized into treatment and control groups based on the tumor volume, and dosing initiated (day 0). Each ADC or the vehicle was given i.v. to the mice. Luciferase activity of each mouse was measured using an *in vivo* imaging system (IVIS 200 Imaging System; PerkinElmer Waltham, MA, USA) twice a week in parallel with the measurement of tumor length 10 min after administering 150 mg/kg luciferin (Promega) i.v. The amount of luminescence was analyzed using analysis software (Living Image Software version 4.3.1; PerkinElmer) as average radiance (p/s/cm²/sr). For another study, MDA-MB-468-Luc cells at a density of 1.5×10^7 cells were inoculated into the left flank of the mice in addition to the inoculation of the mixture into the right flank. Seven days after inoculation, dosing and evaluation were undertaken in a similar manner as described above.

Immunohistochemistry. Fourteen days after drug treatment, tumors were collected and fixed in formalin. Immunohistochemistry for HER2 was carried out by using formalin-fixed, paraffin-embedded tumor samples and a HercepTest II kit (Dako A/S) by SRL Medisearch Inc. (Tokyo, Japan).

Statistical analysis. All statistical analyses carried out using SAS System Release 9.2 (SAS Institute, Cary, NC, USA). The IC₅₀ values were determined by sigmoid Emax model.

Results

Structure of anti-HER2 DXd and T-DM1 and cell membrane permeability of payloads. The structures of HER2-targeting ADCs

are shown in Figure 1. DS-8201a (anti-HER2-DXd (1)) and anti-HER2-DXd (2) are structurally composed of an anti-HER2 antibody, a tetrapeptide linker, and topoisomerase I inhibitors, DXds. The linker-payload was conjugated to the antibody through cysteine residues following the reduction of the disulfide bonds. DS-8201a and anti-HER2-DXd (2) were designed to be cleaved by lysosomal enzymes such as cathepsins B and L, which are highly expressed in tumor cells.^(18–20) DS-8201a has a self-immolative moiety between the tetrapeptide linker and payload part, which is rapidly hydrolyzed, resulting in the release of DXd (1) having a primary hydroxyl group, whereas anti-HER2-DXd (2) simply releases DXd (2) having an amino group. T-DM1 is an FDA-approved HER2-targeting ADC and is composed of trastuzumab, a non-cleavable linker, and a tubulin polymerization inhibitor, DM1. In tumor cells, T-DM1 is cleaved and releases Lys-SMCC-DM1, a linker-payload component that has the potential to inhibit tubulin polymerization.^(21–23) The cell membrane permeability of these released payloads was assessed based on Log D and Peff values. The Log D of DXd (1) was 2.3, which was higher than those of DXd (2) and Lys-SMCC-DM1 (both 0.2). In addition, the Peff of DXd (1) was the highest at both pH 5.0 and pH 7.4, and cytotoxicity of DXd (1) was the most potent owing to its high membrane permeability (Table 1). These results suggest that the membrane permeability of DXd (1) is higher than that of DXd (2) and Lys-SMCC-DM1; therefore, after DS-8201a is internalized into HER2-positive cells and releases DXd (1) into the cytoplasm, DXd (1) could penetrate to adjacent cells, hypothesizing a

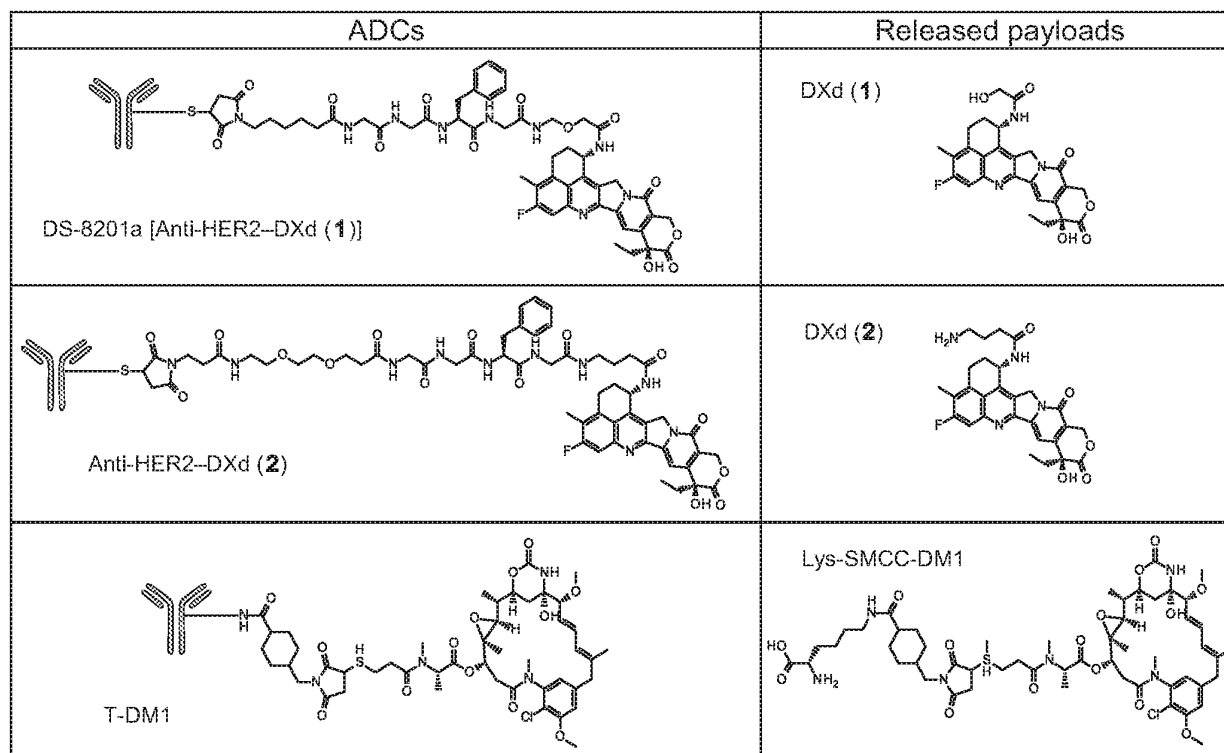


Fig. 1. Structures of antibody–drug conjugates (ADCs) and released payloads. DXd, DX-8951 derivative; HER2, human epidermal growth factor receptor 2; T-DM1, trastuzumab emtansine.

Table 1. Physicochemical parameters of antibody–drug conjugate payloads

		DXd (1)	DXd (2)	Lys-SMCC- DM1
Molecule weight		493.5	520.6	1103.7
Log D	pH 7.4	2.3	0.2	0.2
PAMPA, Peff	pH 5.0	13	<0.1	<0.1
	pH 7.4	12.2	<0.1	<0.1
Cytotoxicity, IC ₅₀ , nM	KPL-4	4.0	33.5	24.8
	MDA-MB-468	0.5	9.7	40.5

DXd, DX-8951 derivative; PAMPA, parallel artificial membrane permeability assay; Peff, permeability coefficient; T-DM1, trastuzumab emtansine.

bystander effect, whereas it would be difficult for DXd (2) and Lys-SMCC-DM1 to penetrate to adjacent cells.

In vitro cell growth inhibitory activity. The cell growth inhibitory activities of DS-8201a, anti-HER2-DXd (2), and T-DM1 were evaluated. As negative control, IgG-DXd (1) and IgG-DXd (2) were also evaluated. All HER2-targeting ADCs were effective against the HER2-positive KPL-4 cell line; however, control IgG ADCs were not (Fig. 2a). The IC₅₀ values of DS-8201a, anti-HER2-DXd (2), and T-DM1 were 109.7, 22.1, and 18.5 pM, respectively. None of the ADCs inhibited the growth of the HER2-negative MDA-MB-468 cell line (IC₅₀

values > 10 000 pM, Fig. 2a). As a reference, all released payloads showed growth inhibition in both KPL-4 and MDA-MB-468 cells (Table 1). Thus, HER2-specific cell growth inhibitory activity of all HER2-targeting ADCs was confirmed.

Bystander killing effect of DS-8201a in vitro. In order to confirm whether DS-8201a induced bystander killing, a coculture cell killing assay was carried out. The HER2-positive KPL-4 cells and the HER2-negative MDA-MB-468 cells were mixed and cultured at an appropriate ratio overnight. The cells were treated with ADC for 5 days, and then the number of living cells was determined. Concurrently, in order to determine the ratio of KPL-4 and MDA-MB-468 cells, HER2-expressing living cells were detected by flow cytometric analysis. As shown in Figure 2(b), the living cells clearly showed two peaks indicative of KPL4 and MDA-MB-468 cells. Based on the number of living cells and ratio of KPL-4 and MDA-MB-468 cells in each well, the number of KPL-4 or MDA-MB-468 cells was calculated. As a result, although anti-HER2-DXd (2) and T-DM1 killed only HER2-positive KPL-4 cells completely, DS-8201a killed both KPL-4 and MDA-MB-468 cells (Fig. 2c). Negative control ADCs did not induce cell killing compared to untreated wells. As DS-8201a has HER2-specific cytotoxicity, MDA-MB-468 cell killing could be caused by the released payload, DXd (1), suggesting the bystander killing effect of DS-8201a. However, anti-HER2-DXd (2) and T-DM1 did not show a bystander killing effect against HER2-negative cells, and it was supposed that

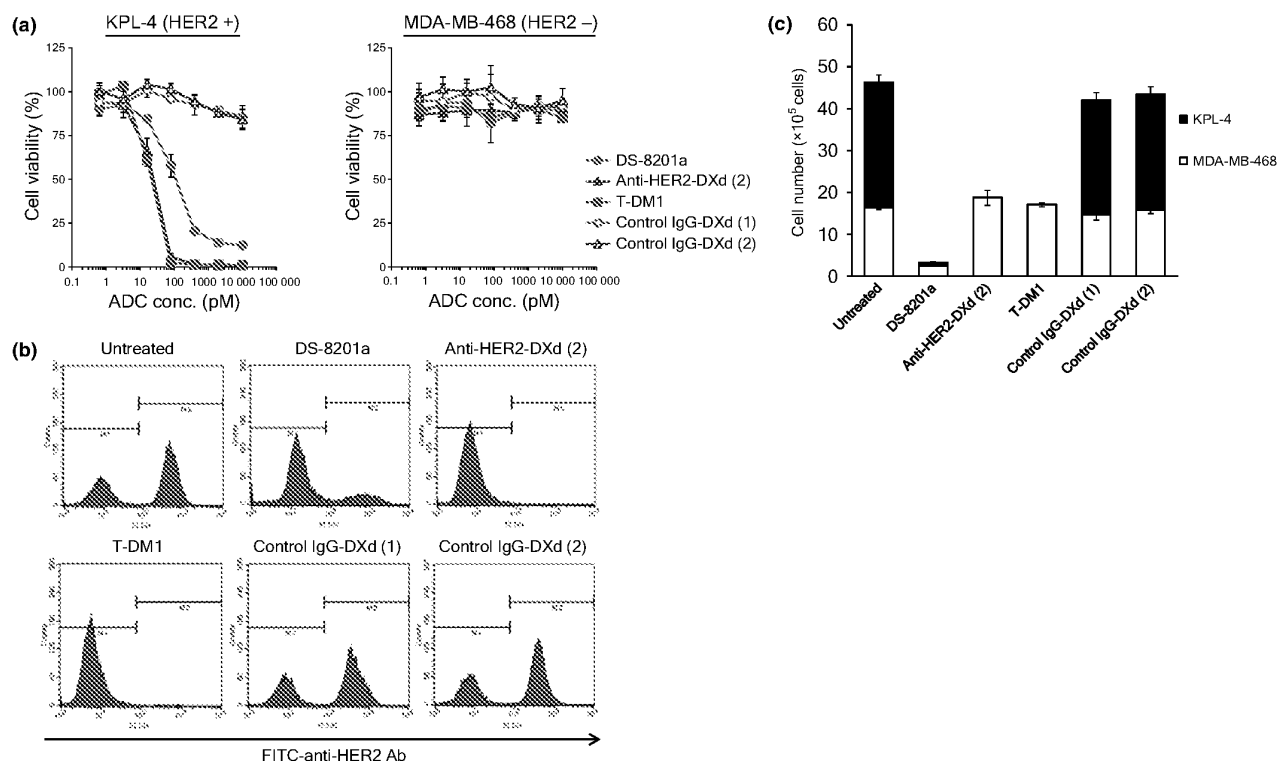
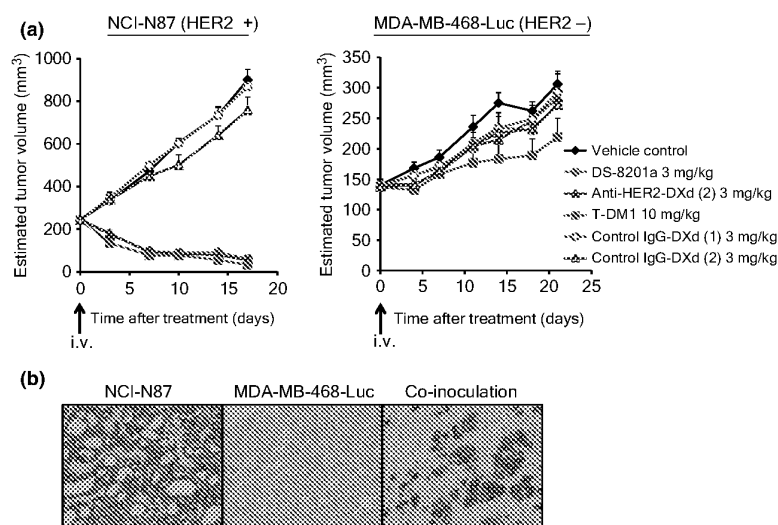


Fig. 2. Bystander killing effect of antibody–drug conjugates (ADCs) in coculture conditions *in vitro*. (a) Growth inhibitory activity of ADCs against KPL-4 and MDA-MB-468 cells. Tumor cells were treated with ADCs for 5 days and cell viability (%) was calculated. Each point represents the mean and SD ($n = 3$). KPL-4 and MDA-MB-468 cells were cocultured and treated with 10 nM ADCs for 5 days. After collecting adherent cells, cell number and ratio of human epidermal growth factor receptor 2 (HER2)-positive and HER2-negative cells were determined by a cell counter and a flow cytometer, respectively. (b) Data of flow cytometric analysis. (c) Numbers of KPL-4 and MDA-MB-468 viable cells. Each bar represents the mean and SD ($n = 3$). DXd, DX-8951 derivative; T-DM1, trastuzumab emtansine.

Fig. 3. Antitumor activity of antibody–drug conjugates (ADCs) and model establishment for bystander evaluation. (a) Antitumor activity of ADCs in NCI-N87 and MDA-MB-468-Luc xenograft models. The mean estimated tumor volume and SE ($n = 6$) are represented on the graph. (b) Human epidermal growth factor receptor 2 (HER2) expression in co-inoculated tumor. Eight days after s.c. inoculation, each tumor was collected and fixed with 10% neutral-buffered formalin. Immunohistochemistry of HER2 protein was carried out using the HercepTest II kit according to manufacturer's instructions. DXd, DX-8951 derivative; T-DM1, trastuzumab emtansine.



the membrane permeability of the payload is an important factor for bystander killing.

Bystander killing effect of DS-8201a *in vivo*. A novel *in vivo* evaluation system was established for *in vivo* assessment of the bystander killing observed in the *in vitro* study. MDA-MB-468-Luc cells were generated to detect the existence of HER2-

negative cells in a mixed tumor composed of HER2-positive cells and HER2-negative cells using an *in vivo* imaging system. First, the antitumor activity of each HER2-targeting ADC was evaluated against a HER2-positive model or a HER2-negative model. As the HER2-positive model, NCI-N87 cells were used. All HER2-targeting ADCs at single doses induced tumor

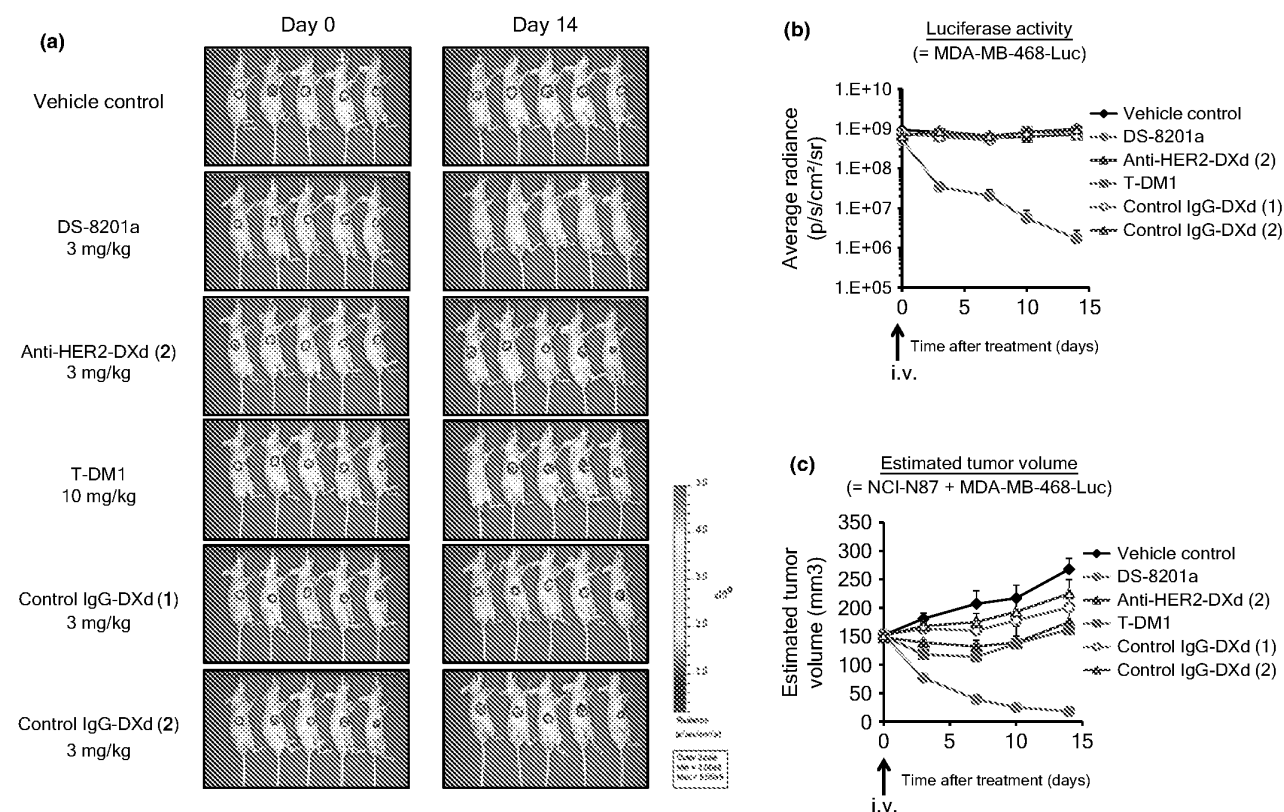


Fig. 4. Bystander killing in co-inoculated conditions *in vivo*. Seven days after inoculation of a mixture of both NCI-N87 and MDA-MB-468-Luc cells, mice were i.v. given antibody–drug conjugates (day 0). Luciferase activity was detected by *in vivo* imager after i.v. administration of substrate. (a) Imaging data of luciferase activity. (b) Luciferase activity. (c) Estimated tumor volume. SE ($n = 5$) represented on the graph. DXd, DX-8951 derivative; HER2, human epidermal growth factor receptor 2; T-DM1, trastuzumab emtansine.

regression in the NCI-N87 model, but not in the MDA-MB-468-Luc model, indicating HER2-dependent efficacy of the ADCs (Fig. 3a). In both models, control IgG-DXd were not effective. Second, we confirmed the heterogeneity of the mixed tumor generated by inoculating a mixture of NCI-N87 and MDA-MB-468-Luc cells through immunohistochemistry of HER2. As shown in Figure 3(b), both HER2-positive and HER2-negative cancer cells were observed in the mixed tumor; only HER2-positive cancer cells and host-derived stromal cells were observed in an NCI-N87 tumor. Then, each HER2-targeting ADC or control ADC was administered to the co-inoculated xenograft mice, and tumor volume and luciferase activity of each mouse were measured. In the DS-8201a group, an apparent reduction in luciferase signal was observed, indicating that MDA-MB-468-Luc cells were completely eradicated by DS-8201a (Fig. 4a,b). Similar results were not observed with other HER2-targeting ADCs or control ADCs. In terms of tumor volume changes, anti-HER2-DXd (2) and T-DM1 inhibited tumor growth but were less potent than DS-8201a (Fig. 4c), which could be attributed to the limited elimination of tumor, in particular only the HER2-positive population by anti-HER2-DXd (2) and T-DM1. In the anti-HER2-DXd (2)-treated and T-DM1-treated groups, the immunohistochemical analysis of treated tumors showed eradication of HER2-positive cells, and HER2-negative cancer cells occupied a large part of tumor tissue (Fig. S1). In the DS-8201a-treated group, almost all of the HER2-positive and HER-negative cells disappeared and there were little or no cancer cells remaining in the tumors. From these results, it was confirmed that DS-8201a showed antitumor activity against not only HER2-positive tumors but also HER2-negative tumors under the co-inoculated condition, whereas T-DM1 did not affect HER2-negative tumors.

Bystander effect on distant cells. It is still unclear whether the bystander killing of DS-8201a is observed only in HER2-negative cells adjacent to HER2-positive cells. Therefore, an effect on cells distant from HER2-positive cells was evaluated. A mixture of NCI-N87 and MDA-MB-468-Luc cells was inoculated into the right flank of mouse and only MDA-MB-468-Luc cells were inoculated into the left flank of the same mouse. Fourteen days after treatment with DS-8201a, luciferase signals in tumors of both sides were measured and

compared. The signal of co-inoculated tumor decreased, but that of mono-inoculated tumor did not change (Fig. 5a). On day 14, the signal of mono-inoculated tumors of DS-8201a treated mice was comparable to that of untreated mice (Fig. 5). These results indicated that the effect of DS-8201a on HER2-negative cells was observed only in the case of cells adjacent to HER2-positive cells, not distant cells.

Discussions

Generally, it is reported that ADCs including approved ones, T-DM1 and brentuximab vedotin, show antigen-dependent cytotoxicity *in vitro* and antitumor efficacy *in vivo*.^(24,25) Similarly, HER2-dependent efficacy of DS-8201a was confirmed in a previous report.⁽¹²⁾ In this study, we reported that DS-8201a kills both HER2-positive and HER2-negative cells under coculture conditions, an effect named bystander killing. In contrast, the low permeable payload-loaded HER2 ADC, anti-HER2-DXd (2), did not show any bystander killing effect in the same conditions even though the payload, DXd (2), has comparable topoisomerase I inhibitory activity (data not shown). Although the bystander effect of several ADCs has already been reported,^(13–16) this is the first study to investigate the relationship between bystander killing and membrane permeability of payload using two different kinds of topoisomerase I inhibitors. T-DM1 did not show evidence of bystander killing and it is supposed that the low permeability of Lys-SMCC-DM1, which is the active metabolite of T-DM1, is one of the reasons for the bystander killing effect, as shown in Table 1.

Recently, Roche announced that the phase II/III Gatsby trial, a clinical trial designed to support a new indication for T-DM1 in second-line treatment of HER2-positive advanced gastric cancer, did not meet its primary endpoint. The heterogeneity of HER2 expression in gastric cancer has been reported,⁽²⁶⁾ and it could be a potential reason for the failure. Bystander killing could address this limitation for this issue and DS-8201a can overcome the heterogeneity to show a significant clinical response in gastric cancer patients. Even in breast cancer, which is an approved indication of T-DM1, HER2 heterogeneity was reported⁽²⁷⁾ and a higher objective response rate of DS-8201a in

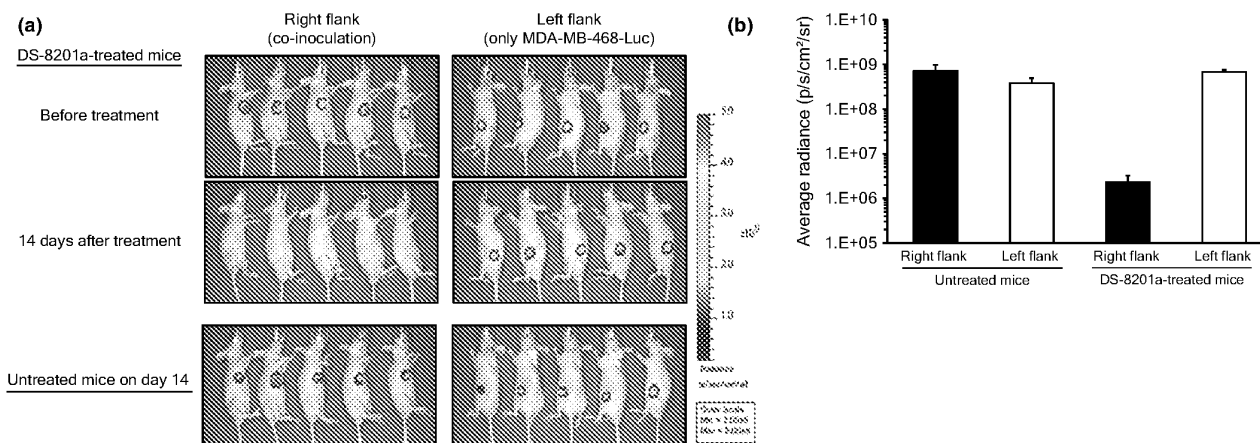


Fig. 5. Bystander killing effect on distant tumor. Nude mice were s.c. inoculated with a mixture of both NCI-N87 and MDA-MB-468-Luc cells into the right flank, and only MDA-MB-468-Luc cells into the left flank. Seven days after inoculation, mice were treated with i.v. DS-8201a at 3 mg/kg. Luciferase activity was detected by an *in vivo* imager after i.v. administration of substrate. (a) Imaging data of luciferase activity. (b) Mean luciferase activity and SE ($n = 5$) are represented on the graph.

a breast cancer patient group than T-DM1 is expected. Altogether, we conclude that the bystander killing potential of DS-8201a is a significant point of differentiation from T-DM1.

Most previous reports regarding the bystander effect of ADCs indicated only the results of *in vitro* experiments, which are closed systems that provide inadequate evaluation of cells adjacent to antigen-positive cells because the released payload could accumulate in the culture supernatant. Additionally, the reports of *in vivo* evaluation are limited owing to the difficulty of detecting antigen-negative cells. In this report, we undertook a novel *in vivo* imaging study and for the first time achieved a time-course observation of antigen-negative cells under co-inoculated conditions. The combination of *in vivo* imaging and the assessment of HER2 expression made it possible to detect both HER2-positive and HER-negative cells in one experiment. It helped us to understand that anti-HER2-DXd (2) and T-DM1 killed only HER2-positive cells *in vivo* (Fig. 4). However, it was difficult to control HER2 heterogeneity *in vitro* and *in vivo*; therefore we could use only KPL-4 cells for *in vitro* assays and NCI-N87 cells for *in vivo* assays. An improvement of experimental method is still required for assessment of bystander killing by various ADCs.

Moreover, the evaluation of the effect of released payload on distant cells, and not only neighboring cells, was also a breakthrough of this report. Safety concerns of the bystander effect have to be considered in addition to its advantages of efficacy; however, this was not evaluated in previous reports. Here, we clearly proved that the bystander killing of DS-8201a was observed only on cells neighboring HER2-positive cells, not distant cells, indicating that systemic toxicity or toxicity in distant normal tissues from tumor sites would

be low. However, effects on tumor-adjacent normal tissues are still unknown. There is one hypothesis that the safety concerns against these tissues, except for bone marrow cells and hematocytes, would be low because topoisomerase I inhibitors are effective against proliferative cells like cancer cells.

We showed that ADC conjugated with low-permeable payload did not provoke bystander killing. On the contrary, this type of ADC might be valuable for targeting hematological cancers because bystander killing could not be observed in circulating cancer cells and the released payloads with low permeability could accumulate in the cells, resulting in potent cytotoxicity. In fact, the cytotoxicity of anti-HER2-DXd (2) was more potent than that of DS-8201a (Fig. 2a). We may need to select an appropriate type of payload based on the target indication of ADC for potent antitumor efficacy.

The results of this study showed the potent bystander killing effect of DS-8201a owing to its high membrane-permeable payload and the potential of DS-8201a to target tumors with HER2 heterogeneity, such as gastric cancer, whereas T-DM1 does not show clinical benefit.

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Disclosure Statement

All the authors are employees of Daiichi Sankyo Co., Ltd.

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Original Article

Bystander killing effect of DS-8201a

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Human epidermal growth factor receptor 2 (HER2) expression status after antibody–drug conjugate (ADC) treatment.

Cancer Therapy: Preclinical

Clinical
Cancer
Research**DS-8201a, A Novel HER2-Targeting ADC with a Novel DNA Topoisomerase I Inhibitor, Demonstrates a Promising Antitumor Efficacy with Differentiation from T-DM1**

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Abstract

Purpose: An anti-HER2 antibody–drug conjugate with a novel topoisomerase I inhibitor, DS-8201a, was generated as a new antitumor drug candidate, and its preclinical pharmacologic profile was assessed.

Experimental Design: *In vitro* and *in vivo* pharmacologic activities of DS-8201a were evaluated and compared with T-DM1 in several HER2-positive cell lines and patient-derived xenograft (PDX) models. The mechanism of action for the efficacy was also evaluated. Pharmacokinetics in cynomolgus monkeys and the safety profiles in rats and cynomolgus monkeys were assessed.

Results: DS-8201a exhibited a HER2 expression-dependent cell growth-inhibitory activity and induced tumor regression with a single dosing at more than 1 mg/kg in a HER2-positive gastric cancer NCI-N87 model. Binding activity to HER2 and ADCC activity of DS-8201a were comparable with unconjugated anti-

HER2 antibody. DS-8201a also showed an inhibitory activity to Akt phosphorylation. DS-8201a induced phosphorylation of Chk1 and Histone H2A X, the markers of DNA damage. Pharmacokinetics and safety profiles of DS-8201a were favorable and the highest non-severely toxic dose was 30 mg/kg in cynomolgus monkeys, supporting DS-8201a as being well tolerated in humans. DS-8201a was effective in a T-DM1-insensitive PDX model with high HER2 expression. DS-8201a, but not T-DM1, demonstrated antitumor efficacy against several breast cancer PDX models with low HER2 expression.

Conclusions: DS-8201a exhibited a potent antitumor activity in a broad selection of HER2-positive models and favorable pharmacokinetics and safety profiles. The results demonstrate that DS-8201a will be a valuable therapy with a great potential to respond to T-DM1-insensitive HER2-positive cancers and low HER2-expressing cancers. *Clin Cancer Res*, 22(20): 5097–108. ©2016 AACR.

Introduction

HER2 is a member of the EGFR family of transmembrane receptors (1) and overexpresses in a broad number of cancer types, such as bladder, breast, cervical, cholangio, colorectal, endometrial, esophageal, gastric, head and neck, liver, lung, ovarian, and salivary gland cancers (2, 3). Especially, amplification and overexpression of HER2 occurs in 25% to 30% of the instances of human breast cancer and are associated with a poor prognosis (4, 5). Several HER2-targeting therapies such as trastuzumab (6, 7), lapatinib (8), pertuzumab (9), and T-DM1 (10) have been approved worldwide for patients with HER2-positive

tumors, which are defined as either IHC 3+ or IHC 2+/FISH-positive. However, there is no HER2-targeting therapy targeting HER2-weak-positive tumors such as IHC 2+/FISH-negative and IHC 1+.

Among the HER2-targeting drugs, T-DM1 is the only antibody–drug conjugate (ADC) in the market which is composed of trastuzumab and a tubulin polymerization inhibitor, DM1 (11). In its phase III clinical trial, EMILIA study, T-DM1 was shown to prolong survival and improve quality of life in a well-balanced manner with higher levels of efficacy and safety in HER2-positive breast cancer patients (10).

Other than T-DM1, SGN-35 (Brentuximab vedotin) has been approved (12) and more than 30 kinds of ADC programs are currently in clinical trials (13). However, most of them are applied to linker-payload systems similar to T-DM1 or SGN-35 (Brentuximab vedotin), which carry tubulin polymerization inhibitors (DM1 and MMAE, respectively). In the clinical trials of these tubulin inhibitor–conjugated ADCs, several dose-limiting toxicities such as thrombocytopenia, neutropenia, and neuropathy were observed, and some were considered to be mediated by the free drugs in plasma (14). Considering these situations, an improvement of linker-payload systems in terms of different antitumor spectrum, overcoming drug resistance to the

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Ogitani et al.

Translational Relevance

Most of the antibody–drug conjugates (ADC) in the market and in clinical trials are conjugated with the same type of drug, tubulin polymerization inhibitor. Currently, the development of a new drug linker system focusing on different types of drugs has been progressing. DS-8201a is a HER2-targeting ADC structurally composed of a humanized anti-HER2 antibody, enzymatically cleavable peptide linker, and a novel topoisomerase I inhibitor. DS-8201a showed potent antitumor activity based on the combination of pharmacologic effects of the antibody component with topoisomerase I inhibition. The stability in plasma was favorable and the safety profiles in rats and cynomolgus monkeys were acceptable into clinical trials. Moreover, DS-8201a was effective against T-DM1–insensitive tumors and HER2 low-expressing tumors. These data suggest that DS-8201a has the potential to respond to not only T-DM1–refractory breast cancer and HER2-positive patients, but IHC 1+ and 2+/FISH–negative patients for whom current HER2-targeting therapies are ineffective.

conventional systems, and providing a greater safety profile would be much desired.

Using our original linker-payload technology, we have designed DS-8201a, a new HER2-targeting ADC composed of a humanized anti-HER2 antibody, enzymatically cleavable peptide-linker, and a novel topoisomerase I inhibitor. Topoisomerase I inhibitors such as irinotecan (CPT-11) bind to topoisomerase I–DNA cleavable complexes and stabilize them, resulting in the induction of double-strand DNA breaks and apoptosis (15). In a clinical setting, these are widely used for colorectal (16), gastric (17), and other cancers (18–21). We previously developed exatecan mesylate (DX-8951f) as a novel topoisomerase I inhibitor which had more potent efficacy than CPT-11 against various tumor xenograft models, including CPT-11–resistant tumors *in vivo* (22), and a phase III trial in pancreatic cancer was conducted (23). A DX-8951 derivative (DXd) was used as the conjugated drug for DS-8201a, aiming to provide an additional and promising pharmacologic option for current HER2-targeting therapy.

In addition, our linker-payload system enabled conjugation with 8 molecules of DXd per antibody. The drug-to-antibody ratio (DAR) of current ADCs ranges from 2 to 4 as a higher DAR could have some problems, including fast clearance of the ADC *in vivo*, and a higher exposure to the released drug in plasma associated with off-target toxicities (24). In the case of T-DM1, systemic clearance of T-DM1 was more rapid than clearance of total trastuzumab in both cynomolgus monkeys and humans, and released DM1 was detectable (25, 26). DS-8201a is a stable and homogeneous DAR 8 ADC without the drawbacks above and is expected to steadily deliver much of the potent topoisomerase I inhibitor to the tumors.

Herein, we describe the preclinical profile of DS-8201a, including efficacy, pharmacokinetics, and safety. DS-8201a showed excellent antitumor activity against a T-DM1–insensitive model and HER2-low expression models, and a favorable pharmacokinetics and safety profile, suggesting its potential to address unmet medical needs in HER2-expressing patients.

Materials and Methods**Antibodies and ADCs**

The anti-HER2 Ab was a human monoclonal IgG1 produced with reference to the same amino acid sequence as trastuzumab, and DS-8201a was synthesized according to the conjugation procedure described in another report (27) using the anti-HER2 Ab; its DAR was 7.7, as determined by reverse-phase chromatography (RPC). The drug distribution was analyzed by hydrophobic interaction chromatography (HIC). T-DM1 was purchased from Genentech or synthesized according to a previous report (28). Human IgG1 was purchased from Eureka Therapeutics Inc. Control IgG-ADC was synthesized using the same method as DS-8201a, resulting in a comparable DAR. Anti-HER2 ADC with DAR 3.4 was synthesized with a similar method to that of DS-8201a.

Cell lines

The human gastric carcinoma cell line NCI-N87, the human breast adenocarcinoma cell lines SK-BR-3 and MDA-MB-468, and the human pancreatic cancer cell lines Capan-1 and CFPAC-1 were purchased from ATCC. The human breast cancer cell line JIMT-1 was purchased from DSMZ. The human breast cancer cell line KPL-4 was provided from Dr. Kurebayashi at the Kawasaki Medical University (Okayama, Japan). The human gastric cancer cell line GCIY was provided by the Institute of Physical and Chemical Research (Japan). All cell lines except for MDA-MB-468 were cultured with appropriate mediums (RPMI1640 medium for KPL-4, NCI-N87, and GCIY, McCoy 5A Medium for SK-BR-3, DMEM for JIMT-1, and IMDM for Capan-1 and CFPAC-1) supplemented with 10% heat-inactivated FBS at 37°C and 5% CO₂ atmosphere. MDA-MB-468 was cultured with Leibovitz's L-15 medium supplemented with 10% heat-inactivated FBS at 37°C in a free gas exchange with atmospheric air.

Topoisomerase I inhibitory assay

SN-38 was purchased from Tokyo Chemical Industry Co., Ltd. and DX-8951f and DX-8951 derivative (DXd) was synthesized in-house. The inhibitory activities of SN-38, DX-8951f, and DXd against human topoisomerase I were evaluated by a topoisomerase I–mediated DNA relaxation assay according to a previous report (29). Briefly, recombinant human topoisomerase I was incubated with each drug for 5 minutes. Then, supercoiled DNA pBR322 was added and incubated at 25°C for 60 minutes. After the electrophoresis of the mixture on an agarose gel, the amount of the supercoiled DNA was measured with a CCD imager.

Cytotoxic assay

Cells were seeded to a 96-well plate at 1,000 cells per well. After overnight incubation, each diluted substance was added. Cell viability was evaluated after 6 days using a CellTiter-Glo Luminescent Cell Viability Assay from Promega Corp. according to the manufacturer's instructions. For the detection of HER2 expression in each cell line, cells were incubated on ice for 30 minutes with FITC Mouse IgG1, κ Isotype Control, or anti-HER2/neu FITC from Becton, Dickinson and Company. After washing, the labeled cells were analyzed by FACSCalibur (Becton, Dickinson and Company). Relative mean fluorescence intensity (rMFI) was calculated by the following equation:

$$\frac{\text{MFI of anti-HER2 Ab-FITC}}{\text{MFI of isotype control-FITC}}$$

Cell line and patient-derived xenograft studies

All *in vivo* studies were performed in accordance with the local guidelines of the Institutional Animal Care and Use Committee. Detailed study procedures are written in the supplement. Briefly, each cell suspension or tumor fragment was inoculated subcutaneously into specific pathogen-free female nude mice. When the tumor had grown to an appropriate volume, the tumor-bearing mice were randomized into treatment and control groups based on the tumor volumes, and dosing was initiated on day 0. Each substance was administered intravenously to the mice. Tumor growth inhibition (TGI, %) was calculated according to the following equation:

$$100 \times \frac{\text{Average tumor volume of the treated group}}{\text{Average tumor volume of the control group}}$$

Pharmacokinetics of DS-8201a in cynomolgus monkeys

Concentrations of DS-8201a and the total antibody in plasma were determined with a validated ligand-binding assay; the lower limit of quantitation was 0.100 µg/mL. Concentrations of DXd in plasma were determined with a validated liquid chromatography-tandem mass spectrometry (LC/MS-MS) method; the lower limit of quantitation was 0.100 ng/mL. DS-8201a was intravenously administered at 3.0 mg/kg to male cynomolgus monkeys. Plasma concentrations of DS-8201a, total antibody, and DXd were measured up to 672 hours postdose.

***In vitro* stability of DS-8201a in plasma**

The release rate of DXd from DS-8201a at the concentration of 10 µg/mL at 37°C up to 21 days was evaluated in mouse, rat, monkey, and human plasma.

ELISA

For a binding assay, immunoplates were coated with 2.5 µg/mL His-tagged HER2-ECD protein (Sino Biological Inc.) in coating buffer and kept overnight at 4°C. After washing, the plates were blocked and each serially diluted substance was added to the wells. After incubation for 1.5 hours at 37°C, the plates were washed and incubated with HRP-conjugated anti-human IgG secondary antibody for 1 hour at 37°C. After washing, TMB solution was added and A₄₅₀ in each well was measured with a microplate reader. For the detection of phosphorylated Akt (pAkt), SK-BR-3 cells were preincubated in a 96-well plate for 4 days and then incubated with each substance for 24 hours. After incubation, the cells were lysed and intercellular pAkt and total Akt were detected by using a PathScan Phospho-Akt1 (Ser473) Sandwich ELISA Kit and PathScan Total-Akt1 Sandwich ELISA Kit (Cell Signaling Technology, Inc.) according to the manufacturer's instructions. Relative pAkt of each sample well was calculated by dividing treated normalized pAkt values by untreated normalized pAkt values.

ADCC evaluation

Antibody-dependent cell-mediated cytotoxicity (ADCC) activities were evaluated using human peripheral blood mononuclear cells (PBMC) derived from a donor as effector cells and the SK-BR-3 cells as target cells. The effector cells (2×10^5 cells) and the ⁵¹Cr-labeled target cells (1×10^4 cells) were incubated with each substance, and the indicating effector:target (E:T) ratio was

20:1. After 4 hours of incubation, ADCC activity was measured by radioactivity in the culture supernatant.

Immunoblotting

KPL-4 cells were treated with each substance. After 24, 48, or 72 hours, the cells were harvested and lysed with M-PER lysis buffer containing Halt Protease & Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific Inc.).

The samples were loaded and separated by SDS-PAGE and blotted onto polyvinylidene difluoride membranes. The membranes were blocked, and probed overnight with anti-phospho-Chk1 (Ser345; 133D3) rabbit mAb, anti-Chk1 (2G1D5) mouse mAb, anti-cleaved PARP (Asp214) antibody, anti-β-actin (8H10D10) Mouse mAb (Cell Signaling Technology, Inc.), anti-phospho-Histone H2A.X (Ser139) antibody (Millipore), and anti-Histone H2A.X antibody (Abcam Plc.) at 4°C. Then, the membranes were washed and incubated with fluorescence-labeled secondary antibodies for 10 minutes using SNAP intra-dermally (Millipore). The fluorescence signal was detected using an Odyssey imaging system (LI-COR, Inc.).

Toxicity studies in rats and monkeys

DS-8201a was intravenously administered intermittently at 3-week intervals over a 6-week period to Crl:CD(SD) rats or cynomolgus monkeys (Table 1). Clinical signs, body weight, food consumption, and clinical pathology were monitored throughout the study. A necropsy was conducted on the day after the last administration. The reversibility of the toxic changes was assessed in a subsequent 9-week recovery period in rats and in a subsequent 6-week recovery period in cynomolgus monkeys.

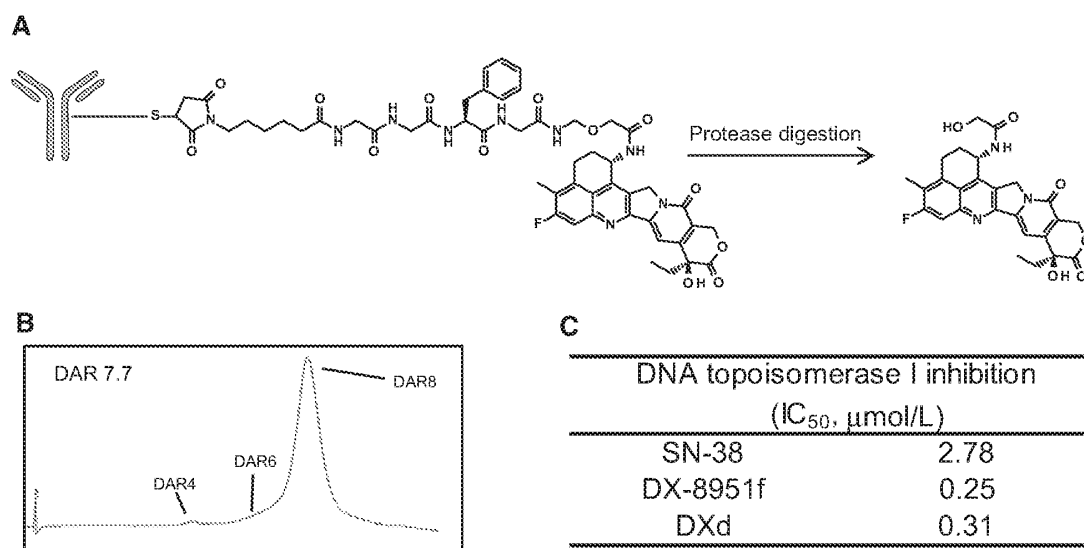
Statistical analysis

All statistical analysis except for toxicity studies was performed using SAS System Release 9.1.3 and 9.2 (SAS Institute Inc.). Statistical analysis in toxicity studies was performed using MUIS-COT (Yukms Co., Ltd.) All IC₅₀ and ED₅₀ values were determined by a Sigmoid Emax model, and dose dependency was evaluated by a Spearman rank correlation coefficient hypothesis test.

Results**Structure of DS-8201a**

The structure of DS-8201a is shown in Fig. 1A. DS-8201a is a HER2-targeting ADC and is composed of an anti-HER2 antibody and a derivative of DX-8951 (DXd), a topoisomerase I inhibitor, which are bound together by a maleimide glycyl-glycyl-phenylalanine-glycyl (GGFG) peptide linker. The linker-payload is conjugated with the antibody via the cysteine residues after the interchain disulfide bonds are reduced with a reducing agent, tris (2-carboxyethyl) phosphine hydrochloride (TCEP HCl). As the tetrapeptide is decomposed by lysosomal enzymes such as cathepsins B and L which are highly expressed in tumor cells (30–34), it is supposed that DS-8201a is cleaved by lysosomal enzymes and releases DXd, which attacks target molecules specifically in tumor cells after it binds to HER2 receptors and is internalized in tumor cells. By using RPC, the DAR of DS-8201a was determined as approximately 8, which is the theoretical maximum drug loading number for conventional interchain cysteine conjugation. Therefore, homogeneous drug distribution was observed in the HIC chart

Ogitani et al.

**Figure 1.**

Structure and characterization of DS-8201a. **A**, the chemical structure of the linkage of DS-8201a. **B**, the conjugated drug distribution by the reverse-phase chromatographic analysis. **C**, comparison of cell-free DNA topoisomerase I inhibitory activity among SN-38, DX-8951f and DXd. Each value represents the mean ($n = 2$ for SN-38 and DXd, $n = 8$ for DX-8951f).

(Fig. 1B). We confirmed that DXd is more potent in inhibitory activity in topoisomerase I than SN-38 as well as DX-8951f, as measured by a topoisomerase I-mediated DNA relaxation assay (Fig. 1C).

Inhibition of cancer cell growth by DS-8201a

The inhibitory activity of DS-8201a against cancer cell growth was compared with an anti-HER2 Ab and control IgG-ADC-conjugated with DXd against various human cancer cell lines *in vitro*. HER2 expression on the cell surface of the cell lines KPL-4, NCI-N87, SK-BR-3, and MDA-MB-468 was firstly evaluated by flow cytometric analysis (Fig. 2A). The relative MFIs of KPL-4, NCI-N87, and SK-BR-3 were 95.7, 101.6, and 56.2, respectively, suggesting that HER2 is clearly expressed on the cell surfaces, whereas the relative MFI was 1.0 for MDA-MB-468, indicating no expression in the MDA-MB-468. Remarkable inhibitory activity to the cell growth was observed for DS-8201a against HER2-positive KPL-4, NCI-N87, and SK-BR-3, with the IC₅₀ values of 26.8, 25.4, and 6.7 ng/mL, respectively, whereas no such inhibition was seen against MDA-MB-468 with the IC₅₀ value of >10,000 ng/mL (Fig. 2B). Although the anti-HER2 Ab showed cell growth-inhibitory activity against NCI-N87 and SK-BR-3, these activities were rather weaker than that of the DS-8201a; the IC₅₀ values of NCI-N87 and SK-BR-3 were 204.2 and 65.9 ng/mL, respectively (Fig. 2B). Also, control IgG-ADC did not show cell growth-inhibitory activities in any of the four cell lines (Fig. 2B), although all four cell lines were sensitive to the payload, DXd (IC₅₀: 1.43 nmol/L–4.07 nmol/L). These results indicate that the cell growth-inhibitory activity of DS-8201a was remarkably enhanced by drug conjugation to the anti-HER2 Ab, and also that DS-8201a shows target-specific growth inhibition against HER2-positive cell lines.

Antitumor activity *in vivo*

The *in vivo* antitumor activity of DS-8201a was evaluated in a HER2-positive NCI-N87 xenograft model. DS-8201a induced tumor growth inhibition in a dose-dependent manner and tumor regression with a single dosing at more than 1 mg/kg without inducing any abnormalities in the general condition or body weight changes of the mice (Fig. 2C). In the same model, 4 mg/kg administration of anti-HER2 Ab partially inhibited the tumor growth, indicating 31% of tumor growth inhibition (TGI) compared with the control group on day 21 (Fig. 2D). On the other hand, DS-8201a clearly showed more potent antitumor efficacy, indicating 99% TGI at the same dose of 4 mg/kg, so that the enhancement of efficacy by drug conjugation was observed *in vivo* as well as in the *in vitro* models (Fig. 2D). Moreover, it is suggested that the *in vivo* efficacy of DS-8201a depends on its HER2 binding, as no inhibition of tumor growth was seen for the control-IgG ADC (Fig. 2D).

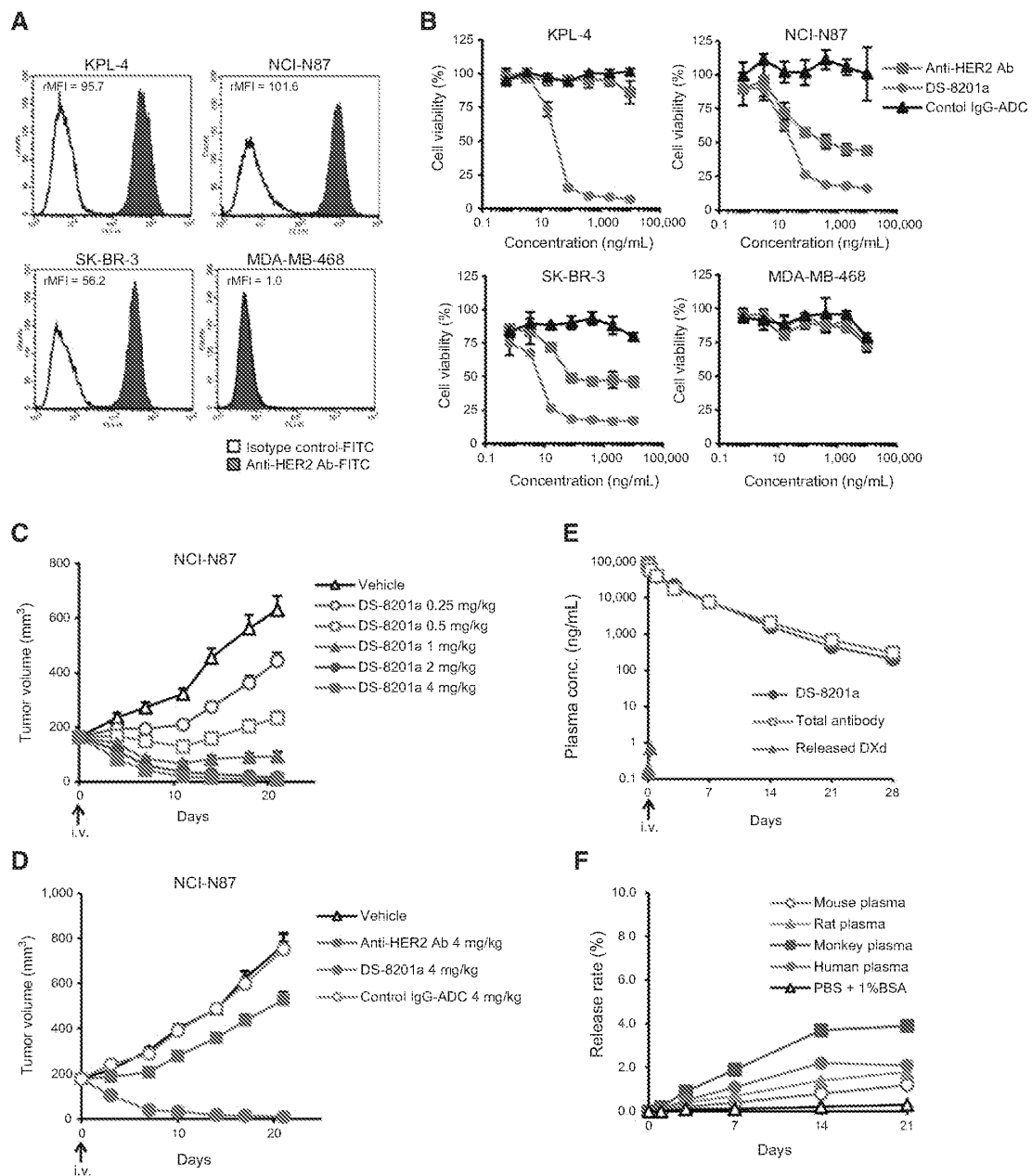
Pharmacokinetics in cynomolgus monkeys

The plasma DS-8201a concentrations decreased exponentially after a single intravenous administration of DS-8201a. The volume of distribution at steady state (V_{ss}) of DS-8201a and total antibody was close to the plasma volume (data not shown). No clear difference was observed in the pharmacokinetic profile between DS-8201a and the total antibody, indicating that the peptide-linker of DS-8201a is stable in plasma even at DAR 8 (Fig. 2E). A low level of DXd was detected only at the limited time points (Fig. 2E).

In vitro stability in plasma

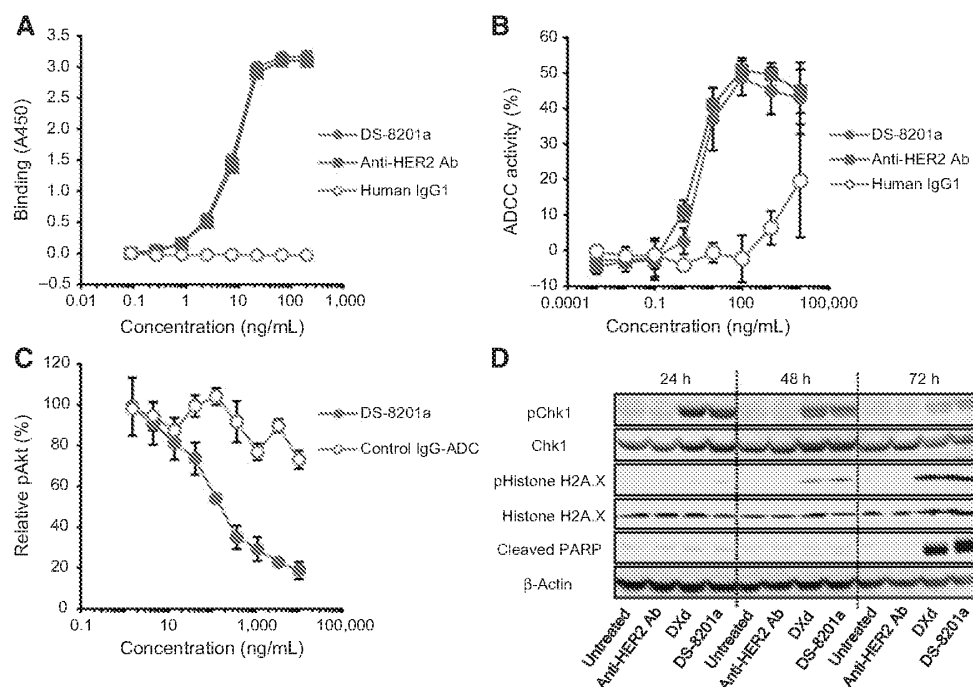
The release rates of DXd from DS-8201a ranged from 1.2% to 3.9% on day 21 in mouse, rat, monkey, and human plasma (Fig.

Preclinical Efficacy of DS-8201a, a Novel HER2-Targeting ADC

**Figure 2.**

In vitro and *in vivo* efficacy and stability in plasma of DS-8201a. **A**, HER2 expression in several cancer cell lines. **B**, *in vitro* cell growth inhibitory activity in the cell lines. The cells were treated with DS-8201a, anti-HER2 Ab, and control IgG-ADC for 6 days. Each point represents the mean and SD ($n = 3$). **C** and **D**, antitumor efficacy of DS-8201a in NCI-N87 xenograft model. The tumor-bearing mice were intravenously administered with DS-8201a, control IgG-ADC, and anti-HER2 Ab on day 0. Each point represents the mean tumor volume and SE ($n = 10$). **E**, pharmacokinetics of DS-8201a in cynomolgus monkeys. DS-8201a was intravenously administered to cynomolgus monkeys at the dose of 3 mg/kg ($n = 3$). **F**, *in vitro* stability of DS-8201a in plasma.

Ogitani et al.

**Figure 3.**

Mechanisms of action of DS-8201a. **A**, binding to HER2 determined by ELISA. **B**, ADCC activity. ^{51}Cr -labeled SK-BR-3 cells and PBMCs were mixed and incubated with each substance for 4 hours. Result of one of three donors is represented. **C**, inhibitory activity of pAkt phosphorylation. Intracellular pAkt levels in SK-BR-3 cells treated with DS-8201a and control IgG-ADC were measured by ELISA. **D**, induction of DNA damage and apoptosis. KPL-4 cells were treated with DS-8201a (10 $\mu\text{g/mL}$), anti-HER2 Ab (10 $\mu\text{g/mL}$), and DXd (10 nmol/L) for 72 hours. Several proteins were detected by Western blotting. Each point represents the mean and SD ($n = 3$).

2F), and these were comparable or rather lower than those of other ADCs, such as T-DM1, SGN-35 (Brentuximab vedotin), and inotuzumab ozogamicin (35–37). These results indicate that DS-8201a is stable in plasma.

Mechanism of action of DS-8201a

To confirm the effect of the drug conjugation with the anti-HER2 Ab on binding activity to HER2, K_d values of DS-8201a and the anti-HER2 Ab were determined by ELISA using HER2 ECD protein (Fig. 3A). K_d values were 7.3 ng/mL for DS-8201a and 7.8 ng/mL for the anti-HER2 Ab, indicating that drug conjugation did not affect HER2 binding. The major mechanisms of action of trastuzumab are thought to involve an ADCC activity by binding to FcγRIII on immune effector cells (38, 39), and a downregulation of phosphorylated Akt (pAkt), which upregulates p27 expression and inhibits cell proliferation (40). We investigated whether DS-8201a retains these same mechanisms of action as trastuzumab. Next, ADCC activity was measured by the detection of SK-BR-3 cell lysis mediated by human PBMCs. DS-8201a showed ADCC activity, resulting in 48.6% of maximum cytotoxicity with an EC_{50} of 3.8 ng/mL (Fig. 3B), and this effect was similar to the anti-HER2 Ab. Regarding the inhibition of phosphorylation of Akt, DS-8201a induced the downregulation of the intracellular pAkt (Ser473) in the SK-BR-3 cells in a dose-dependent manner after treatment for 24 hours

(Fig. 3C). In the same condition, the control IgG ADC did not affect the pAkt status. In SK-BR-3 cells, the treatment of trastuzumab decreased about 70% of the Akt phosphorylation (41), suggesting that DXd conjugation may enhance the effect of unconjugated anti-HER2 antibody on pAkt. These evaluations of ADCC and pAkt indicate that DS-8201a retains the functions of trastuzumab after DXd conjugation.

On the other hand, DNA damage and apoptosis induced by topoisomerase I inhibition were evaluated by detecting phosphorylations of Chk1 and Histone H2A.X (the markers of DNA damage; refs. 42, 43), and cleaved PARP (the marker of apoptosis; ref. 44) in the KPL-4 cells in the presence of DS-8201a, the anti-HER2 Ab, or DXd using the Western blotting method (Fig. 3D). An amount of 10 $\mu\text{g/mL}$ of DS-8201a induced the phosphorylations of Chk1 and Histone H2A.X, and PARP cleavage. DXd induced the same changes as DS-8201a. Conversely, 10 $\mu\text{g/mL}$ of anti-HER2 Ab did not induce a change in any of the objective proteins at any time point, including the untreated group. These results indicate that DS-8201a induced DNA damage and apoptosis in the same manner as DXd, and suggests that these changes were caused by the topoisomerase I inhibition activity of the released DXd from DS-8201a.

Therefore, DS-8201a is considered to exhibit HER2-specific cell growth inhibition and antitumor activity via a novel mechanism of action that combined the pharmacologic

Table 1. Summary of repeated dose toxicity studies in rats and monkeys

Species	Crl:CD(SD) rats	Cynomolgus monkeys
Doses	0, 20, 60 and 197 mg/kg	0, 10, 30 and 78.8 mg/kg
Regimens	Intravenous, every 3 weeks	Intravenous, every 3 weeks
	Days 1, 22, 43 (3 times in total)	Days 1, 22, 43 (3 times in total)
No. of animals	10/sex/group (Main): all dose groups	3/sex/group (Main): all dose groups
	5/sex/group (Recovery): 60 and 197 mg/kg groups	2/sex/group (Recovery): 30 and 78.8 mg/kg groups
Lethal dose	>197 mg/kg	78.8 mg/kg (1 female died)
Body weight	≤60 mg/kg: normal	≤30 mg/kg: normal
	197 mg/kg: low body weight gain	78.8 mg/kg: decreased in 1 male and 1 female
Hematology	20 mg/kg: normal	≤30 mg/kg: normal
	≥60 mg/kg: decreased RBC and WBC parameters	78.8 mg/kg: decreased RBC parameters
Target organs and tissues	≥20 mg/kg: intestines, testis	≥10 mg/kg: intestines
	≥60 mg/kg: bone marrow, thymus, lymph node, skin, kidney, incisor	≥30 mg/kg: lung, skin, testis
		78.8 mg/kg: bone marrow, kidney
STD ₁₀ /HNSTD	STD ₁₀ : >197 mg/kg	HNSTD: 30 mg/kg

Abbreviations: RBC, red blood cell; WBC, white blood cell.

activities of an anti-HER2 antibody with those of the topoisomerase I inhibitor, DXd.

Safety profile of DS-8201a

A repeated intravenous dosing (every 3 weeks for 3 doses) study was conducted in cynomolgus monkeys, the cross-reactive species for DS-8201a, and in rats (antigen–non-binding species; Table 1). In the rat study, no deaths or life-threatening toxicities were found at dose levels up to 197 mg/kg, the maximum dose. Therefore, the severely toxic dose of 10% in animals (STD₁₀) was considered to be >197 mg/kg. In the monkey study, one female at the highest dose of 78.8 mg/kg was euthanized due to moribundity on day 26. The cause of the moribundity appeared to be the deteriorated condition of the animal, which included decreased body weight and food consumption, as well as bone marrow toxicity and intestinal toxicity. Microscopic findings in the intestines, bone marrow and lungs in the surviving monkeys are shown in Supplementary Table S1. Gastrointestinal toxicity and bone marrow toxicity are typical dose-limiting factors in the clinical use of topoisomerase I inhibitors. The effects of DS-8201a on the intestines were very slight, and severe changes were not pronounced in any animal at up to 78.8 mg/kg. The bone marrow toxicity was produced only at 78.8 mg/kg, and was accompanied by decreases in reticulocyte ratios. No abnormalities in leukocyte and erythrocyte counts were observed in monkeys at 10 and 30 mg/kg. The repeated dose of DS-8201a caused moderate pulmonary toxicity in monkeys at 78.8 mg/kg, and findings graded as slight or very slight after the 6-week recovery period at ≥30 mg/kg. On the basis of the mortality and severity of the findings above, the highest non-severely toxic dose (HNSTD) for monkeys was considered to be 30 mg/kg. DS-8201a was well tolerated at the doses up to 197 mg/kg in rats and 30 mg/kg in monkeys following the repeated administration corresponding to the clinical regimen, and the nonclinical safety profile was acceptable for entry into human trials.

Antitumor activity of DS-8201a in low HER2-expressing tumors

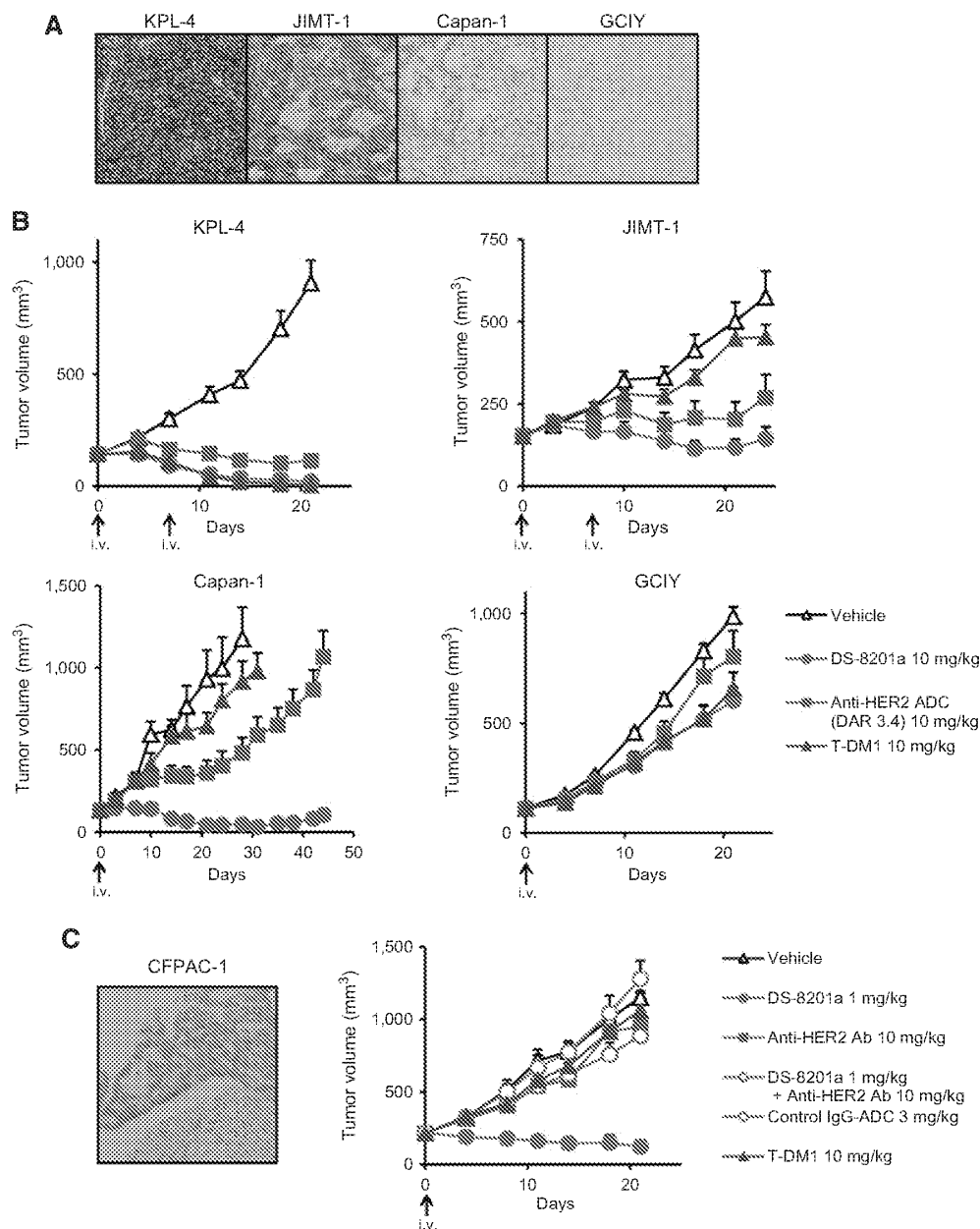
T-DM1 has been approved for HER2-positive metastatic breast cancer patients, defined as being HER2 IHC 3+ or IHC 2+/FISH-positive according to the current guidelines (45), and there are still clinical unmet needs in FISH-negative, HER2 1+ and 2+ populations for HER2-targeting therapies. Therefore, the antitumor activity of DS-8201a was evaluated in various

mice xenograft models with different HER2 expression levels; KPL-4 (strong positive), JIMT-1 (moderate positive), Capan-1 (weak positive), and GCIY (negative) (Fig. 4A and B). Anti-HER2 ADC with the same drug-linker as DS-8201a and about half the DAR (DAR 3.4) was also evaluated to investigate the effect of DAR on antitumor activity. While T-DM1 was effective against only the KPL4 model, DS-8201a was effective against all HER2-positive models with KPL4, JIMT-1, and Capan-1. Both ADCs were not effective in the GCIY model. Anti-HER2 ADC (DAR 3.4) inhibited tumor growth against all HER2-positive models, and the efficacy was HER2 expression-dependent. A stronger efficacy was apparently observed for DS-8201a than anti-HER2 ADC (DAR 3.4) in the HER2 weak-positive Capan-1 model. These results suggest that the high DAR ADC, DS-8201a, enables the delivery of sufficient payload amounts into cancer cells, indicating cytotoxicity even with low HER2 levels. In case of HER2 strong-positive models, even a low DAR ADC is able to deliver a sufficient amount of payload for cell death. DS-8201a was effective in tumors with broader HER2 levels due to its high DAR, approximately 8. To confirm HER2-specificity of DS-8201a in a HER2 low-expressing model, a competitive inhibition study was performed in a HER2 low CFPAC-1 model (Fig. 4C). The efficacy of DS-8201a was cancelled by the prior treatment of the anti-HER2 Ab, and the control IgG-ADC did not inhibit tumor growth at a 3-fold higher dose than DS-8201a. From these results, the HER2 specificity of DS-8201a in a HER2 low-expressing model was confirmed.

Comparison with T-DM1 in PDX models

In addition to the cell line-based xenograft models, several PDX evaluations were performed to assess clinical benefits more precisely. In a gastric cancer PDX model, NIBIO G016, DS-8201a demonstrated potent antitumor activity with tumor regression, but T-DM1 did not (Fig. 5A). As the HER2 status in this model was IHC 3+/FISH+, it is supposed that this difference in antitumor efficacy between DS-8201a and T-DM1 is based on the different sensitivity of payload due to dissimilar mechanism of action of each payload. In breast cancer PDX models, although both DS-8201a and T-DM1 were effective in the HER2 IHC 2+/FISH-positive ST225 model, complete tumor regression was observed on day 21 in 3 of 5 mice treated with DS-8201a, not T-DM1 (Fig. 5B). Furthermore, DS-8201a showed an antitumor activity in HER2 low-expressing ST565 and ST313 models with HER2 IHC 1+/FISH-negative

Ogitani et al.

**Figure 4.**

Antitumor activity of DS-8201a against tumors with low HER2 level. **A**, HER2 IHC in several xenografted tumors. **B**, antitumor activity of DS-8201a compared with T-DM1 and anti-HER2 ADC (DAR 3.4). **C**, competitive study by anti-HER2 Ab against the efficacy of DS-8201a. Each point represents the mean and SE ($n = 5-6$).

expression (Fig. 5C and D), but T-DM1 did not. This result indicated a similar tendency to the cell line-based xenograft models such as Capan-1 and CFPAC-1 (Fig. 4B and C). Consequently, DS-8201a showed more potent antitumor activity

than T-DM1 in all 4 of these models with several HER2 expression levels. These results suggest that DS-8201a has a differentiable potential from T-DM1, which shows effectiveness in T-DM1-insensitive and HER2 low-expressing tumors,

Preclinical Efficacy of DS-8201a, a Novel HER2-Targeting ADC

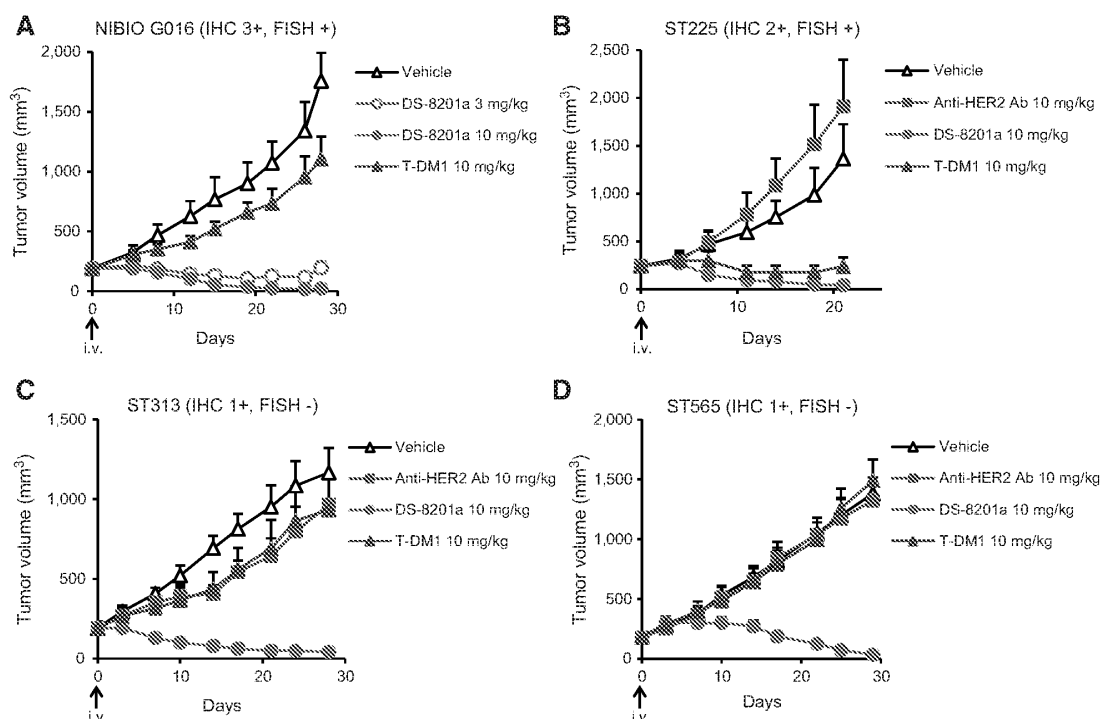


Figure 5. The effect of DS-8201a compared with T-DM1 in several PDX models. **A**, gastric cancer PDX model with HER2 IHC 3+/FISH-positive. **B**, breast cancer PDX models with HER2 IHC 2+/FISH-positive. **C** and **D**, breast cancer PDX models with HER2 IHC 1+/FISH-negative. Each point represents the mean and SE ($n = 5-6$).

resulting from the different mechanisms of action of the conjugated drug and the high DAR of DS-8201a.

Discussion

Most of the ADCs currently in the market and in clinical development carry tubulin polymerization inhibitors such as T-DM1 and SGN-35 (Brentuximab vedotin; ref. 13). We synthesized a novel ADC with a topoisomerase I inhibitor, which has a different mechanism of action from tubulin polymerization inhibitors, and a novel self-immolative linker system using an aminomethylene (AM) moiety. Although other cleavable linker systems applied to SGN-35 (Brentuximab vedotin) and several ADCs release amino group-containing payloads, this AM self-immolative linker system is able to release DXd containing the hydroxyl group from DS-8201a. Moreover, this novel linker-payload system enables a reduction in the hydrophobicity of the ADC and helps increase its DAR. In the case of T-DM1, lysine conjugation and noncleavable systems are used, and it is quite a different system from DS-8201a. DS-8201a showed potent HER2-specific efficacy both *in vitro* and *in vivo*, and by drug conjugation maintained the functional effects of trastuzumab equal to those of T-DM1 (46). Furthermore, the safety profiles of DS-8201a in rats and cynomolgus monkeys showed DS-8201a as being well tolerated.

These favorable profiles are thought to be attributed to the high stability of DS-8201a in plasma, even as an ADC with DAR 8. ADCs with greater DAR (DAR 6 and 8) are generally unstable and show higher clearances, which can result in decreasing efficacy and increasing toxicity (24). In fact, in a mouse study, an anti-CD30 ADC conjugated with monomethyl auristatin E (MMAE) with DAR 8 did not improve the *in vivo* efficacy compared with the ADC with DAR 4 due to the accelerated plasma elimination of the ADC, and the therapeutic index was decreased 2-fold by the lowering of the MTD (24). Our newly developed drug linker system enabled the anti-HER2 ADC to be stable in plasma and have preferable pharmacokinetics regardless of its high DAR, exhibiting a potent *in vivo* antitumor activity dependent upon the increased DAR. In the pharmacokinetic/pharmacodynamic analysis of DS-8201a, the human pharmacokinetic profiles following repeated administration of DS-8201a every 3 weeks for 3 cycles were simulated using the monkey pharmacokinetic data. On the basis of the simulated C_{trough} , 0.8 mg/kg every 3 weeks of DS-8201a is expected to indicate some efficacy in the clinical setting (data not shown). The repeated dose monkey study with a every 3 weeks regimen revealed that DS-8201a caused no severe toxicities at up to 30 mg/kg (HNSTD), indicating a wide therapeutic window.

The achievement of the DAR 8 ADC synthesis contributed to the antitumor effect on tumors with a low HER2 level. DS-

Ogitani et al.

8201a with DAR 8 showed potent antitumor activity in HER2 low-expressing models, though the anti-HER2 ADC with DAR 3.4 did not. As the efficacy in HER2 low-expressing models was observed in a HER2-dependent manner, it was suggested that DS-8201a enables the delivery of more DXd to HER2 low-expressing tumor cells than a lower DAR ADC. On the other hand, T-DM1 was not effective in these models, and the lower DAR of T-DM1 (DAR 3.5) than DS-8201a was considered to be one of the reasons. These results indicate the expansion of the patient population for DS-8201a treatment. Although HER2-positive patients who are defined as either IHC3+ or IHC2+/FISH-positive are treated with current HER2-targeting therapies, breast cancer patients who are defined as IHC1+ and IHC2+/FISH-negative are over 50% of all the breast cancer patients (47) and there is no HER2-targeting therapy indicating any discernible benefit for them. DS-8201a has the potential to show a clinical response toward the IHC1+ and IHC2+/FISH-negative populations as well.

In the PDX model, DS-8201a showed potent antitumor activity against T-DM1 primary insensitive cancer. MDR1 (p-glycoprotein) mediated efflux is known as one of the mechanisms having less sensitivity to tubulin polymerization and depolymerization inhibitors in the market, such as vincristine and paclitaxel, respectively (48, 49). Similarly, it was reported that DM1 is a substrate for MDR1 (37) and an anti-EpCAM-SMCC-DM1, which utilizes the same linker-payload as T-DM1, is also ineffective against MDR1-expressing tumors (50). On the other hand, DX-8951f exhibits potent antitumor activity in several xenograft models regardless of MDR1-expression, including a vincristine-resistant cell (22) and we have preliminary data that DXd showed cytotoxicity against MDR1-expressing cells. From these facts, DS-8201a is supposed to be an MDR1 poor substrate and has the possibility to overcome the primary resistance to T-DM1.

According to a phase II trial of T-DM1 (TDM4558g), however, the primary resistance to T-DM1 may be relatively infrequent, indicating around 20% (51), and the major population of T-DM1 prior treated patients is considered to have an acquired resistance to T-DM1 (52). As one of the mechanisms of acquired resistance to T-DM1, a loss of sensitivity to DM1, and a downregulation of HER2 protein were reported on the basis of preclinical assessment (53, 54). In the assessment, the sensitivity to CTP-11 in trastuzumab-maytansinoid conjugate (similar to T-DM1)-resistant MDA-MB-361 cells did not change, whereas about 3- to 4-fold loss of sensitivity to DM1-SMe was observed (53). Therefore, we greatly expect that DS-8201a is effective against tumors with acquired resistance to T-DM1, unless HER2 completely disappears from the cell membrane. It is because DS-8201a could be effective against HER2-low tumors.

The mechanism of action of the payload is also known to affect the safety profile of ADCs. In a clinical setting, the major adverse events of T-DM1 are thrombocytopenia and peripheral neuropathy as typical toxicities of tubulin inhibitor-conjugated ADCs (10, 26, 51). A platelet decrease was observed in a monkey study with T-DM1 (25), which is associated with

thrombocytopenia limiting the MTD of T-DM1 to 3.6 mg/kg every 3 weeks in the clinic (26). Irreversible axonal degeneration by T-DM1 was noted in monkeys as well (25), which was considered to be correlated with peripheral neuropathy in humans (26). A repeated dose of DS-8201a to monkeys induced no toxicities suggestive of the thrombocytopenia or peripheral neuropathy which were observed in the monkey studies with T-DM1. DS-8201a caused pulmonary toxicity in monkeys at ≥ 30 mg/kg (every 3 weeks for 3 doses), while it was not observed in rats (antigen-nonbinding species). Similarly, T-DM1 caused infiltration of mononuclear cells into the interstitium of the lung in cynomolgus monkeys at ≥ 10 mg/kg (every 3 weeks for 4 doses), which was not seen in rats (25). The evidence of HER2 protein expression on cell membranes of epithelial cells in the lung in humans (55), and the absence of the pulmonary findings in rats seems to suggest that the pulmonary effects of T-DM1 and DS-8201a may be mediated by HER2 expression in the lung.

The preclinical safety profile warranted clinical investigation, and the phase I dose escalation was initiated in August 2015 in Japan (Trial registration ID: NCT02564900). In the phase I dose expansion, the tolerability and efficacy against T-DM1 prior treated breast cancer patients, trastuzumab prior treated gastric cancer patients, and HER2 low-positive breast cancer patients will be assessed. The results stated in the current article support the view that DS-8201a can become an attractive and promising HER2-targeting ADC with the novel topoisomerase I inhibitor, which provides an additional treatment option to current HER2-targeting therapies; it is effective not only against breast cancer, but also many types of HER2-expressing cancers, including T-DM1-refractory and low HER2-expressing cancers, and exhibits a great efficacy in patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Writing, review, and/or revision of the manuscript: Y. Ogitani, T. Aida, K. Hagihara, J. Yamaguchi, M. Oitate, S. Arakawa, R. Atsumi, T. Nakada

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Clinical Cancer Research

DS-8201a, A Novel HER2-Targeting ADC with a Novel DNA Topoisomerase I Inhibitor, Demonstrates a Promising Antitumor Efficacy with Differentiation from T-DM1

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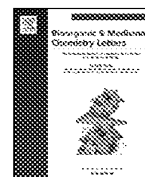
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Wide application of a novel topoisomerase I inhibitor-based drug conjugation technology



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ABSTRACT

To establish a novel and widely applicable payload-linker technology for antibody–drug conjugates (ADCs), we have focused our research on applying exatecan mesylate (DX-8951f), a potent topoisomerase I inhibitor, which exhibits extensive antitumor activity as well as significant myelotoxicity, as the payload part. Through this study, we discovered a promising exatecan derivative (DX-8951 derivative, DXd), that has the characteristics of low membrane permeability and shows considerably less myelotoxicity than that shown by exatecan mesylate in an in vitro human colony forming unit–granulocyte macrophage assay. DXd was further used for drug conjugation by using commercially or clinically useful monoclonal antibodies to evaluate the potency of the ADC. The result revealed that the DXd-ADCs targeting CD30, CD33, and CD70 were effective against each of their respective target-expressing tumor cell lines. Moreover, a novel DXd-ADC targeting B7-H3, which is a new target for ADCs, also showed potent antitumor efficacy both in vitro and in vivo. In conclusion, this study showed that this novel topoisomerase I inhibitor-based ADC technology is widely applicable to a diverse number of antibodies and is expected to mitigate myelotoxicity, thereby possibly resulting in better safety profiles than that of existing ADC technologies.

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Antibody–drug conjugates (ADCs) represent a promising class of drugs with a wider therapeutic index (TI) than conventional chemotherapeutic agents owing to their efficient and specific drug delivery to antigen-expressing tumor cells. Two ADCs have been approved and are currently in the market. One of these is brentuximab vedotin (ADCETRIS), a CD30-targeting antibody conjugated with a tubulin polymerization inhibitor monomethyl auristatin E (MMAE), which was approved in 2011 for the treatment of relapsed or refractory Hodgkin's lymphoma and systemic anaplastic large cell lymphoma.^{1–3} The other is trastuzumab emtansine (KADCYLA), a human epidermal growth factor receptor 2 (HER2)-targeting ADC with the tubulin polymerization inhibitor, DM1, was approved in 2013 for HER2-positive breast cancer.^{4–6} More than 50 ADC programs are currently in clinical trials, and most of them are conjugated with the same mechanism of action (MoA) of payload, a tubulin polymerization inhibitor.⁷ However, in the clinical trials of these ADCs, several dose-limiting toxicities such as thrombocytopenia and neutropenia were observed, which was considered to be mediated by the release of the drugs in the

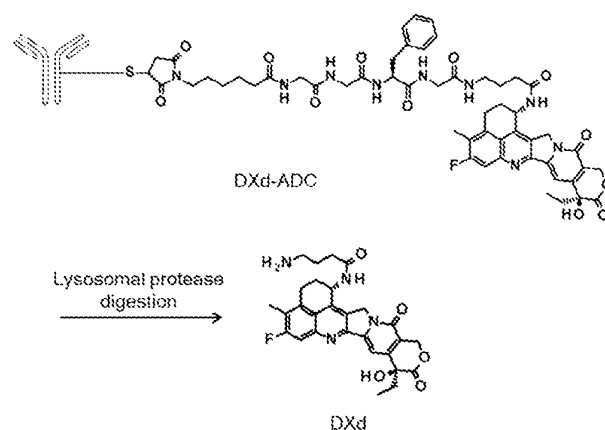


Figure 1. Structures of DXd-ADC and DXd.

^{*} Corresponding author.

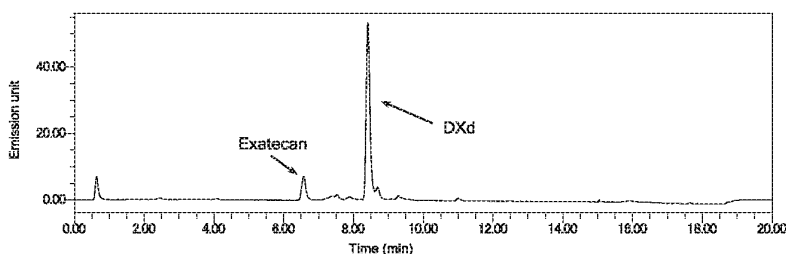
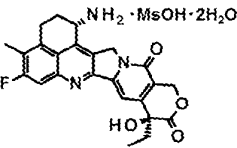
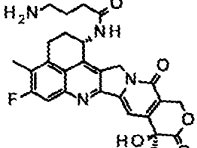


Figure 2. Metabolite profiles of anti-HER2-DXd ADC. One day after administration of 10 mg/kg of anti-HER2-DXd ADC to HER2-positive KPL-4 tumor-bearing mice, metabolites in the tumor homogenate extract were analyzed by fluorescence high performance liquid chromatography. Each metabolite was identified by retention time comparison and accurate mass analysis. ADC information; the drug to antibody ratio (DAR) was 2.6 and aggregation rate (Agg. rate) was 3.0%.

Table 1
Characteristics of exatecan mesylate and DXd

 Exatecan mesylate		 DXd	
	Exatecan mesylate	DXd	
Topoisomerase I inhibition (IC ₅₀)	0.25	0.52 (μM)	
Cytotoxicity (KPL-4 cell, IC ₅₀)	0.9	48.7 (nM)	
Log D	pH 7.4 2.5	0.2	
Permeability coefficient (PAMPA)	pH 5.0 18.3	<0.1 (10 ⁻⁶ cm/s)	
	pH 7.4 28.5	<0.1	

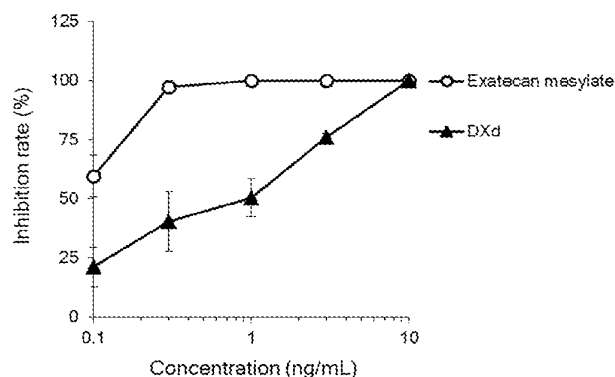


Figure 3. Concentration-dependent inhibition of CFU-GM colony formation with human umbilical cord blood cells. The human umbilical cord blood cells were suspended in MethoCult 4230 culture medium containing 50 ng/mL stem cell factor; 10 ng/mL each of GM-CSF, IL-3, IL-6, and G-CSF; 1% of an antibiotic/antimycotic solution; and a serially diluted solution of each compound. The cell suspension was seeded in 35-mm dishes, and the cultures were incubated at 37 °C for 9 days. Each plot represents mean ± SD (*n* = 3).

plasma.¹³ Currently, several linker-payload technologies focusing on different MoAs of drugs such as calicheamicin, pyrrolobenzodiazepine (PBD) dimer, duocarmycin, and SN-38 have been evaluated in clinical settings.⁷

Topoisomerase I inhibitors such as irinotecan (CPT-11) are widely used for the treatment of colorectal,¹⁴ gastric,¹⁰ and other cancers.^{13–15} However, CPT-11 causes side effects such as severe

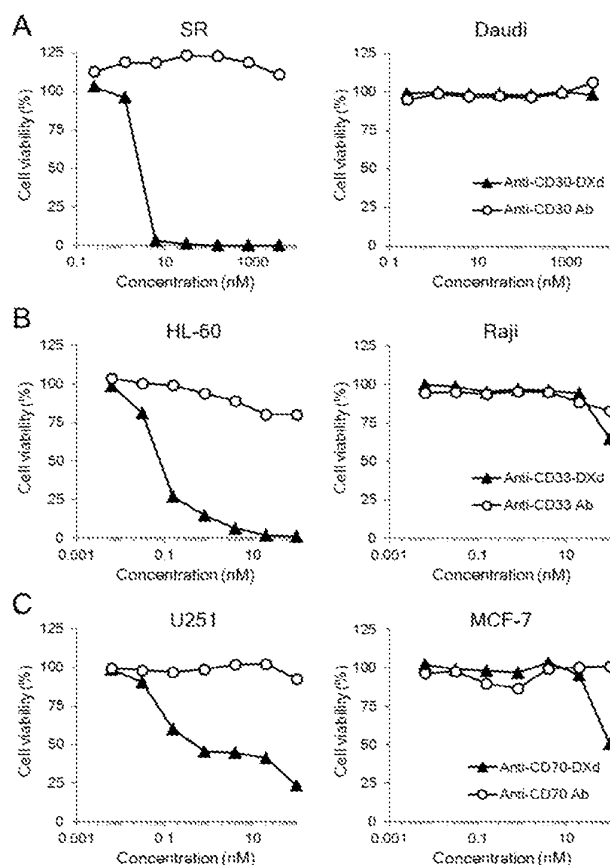


Figure 4. Target specific cytotoxicity of different DXd-ADCs. Cytotoxicity of (A) anti-CD30-DXd in CD30-positive SR cells and negative Daudi cells compared to anti-CD30 Ab, (B) anti-CD33-DXd in CD33-positive HL-60 cells and negative Raji cells compared to anti-CD33 Ab, (C) anti-CD70-DXd in CD70-positive U251 cells and negative MCF-7 cells compared to anti-CD70 Ab. Each cell line was treated with each Ab or ADC for 6 days, and then cell viability was measured. Each plot represents mean (*n* = 2). ADC information; anti-CD30-DXd (DAR 3.8, Agg. rate 5.3%), anti-CD33-DXd (DAR 3.7, Agg. rate 5.1%), and anti-CD70-DXd (DAR 3.6, Agg. rate 5.5%).

watery diarrhea and there is a risk of toxicity enhancement based on the polymorphisms of UGT1A1.^{14,15} We previously developed exatecan mesylate (DX-8951f) as a novel topoisomerase I inhibitor that has a more potent efficacy against various tumor xenograft models including CPT-11-resistant tumors in vivo.¹⁶ Although its toxicity is not affected by UGT1A1 polymorphisms,¹⁷ neutropenia was still observed as with CPT-11.

Based on this developmental experience, we have developed a novel payload-linker technology by using an anti-HER2 antibody, trastuzumab, and the exatecan derivatives (DX-8951 derivatives, DXds).¹⁸ Trastuzumab and DXds were bound together by a maleimide caproyl glycyl-glycyl-phenylalanyl-glycine (GGFG) peptide linker through cysteine residues following the reduction of the interchain disulfide bonds of the antibody. The anti-HER2-DXd ADCs showed antitumor activity in vitro and in vivo, indicating the importance of the new technology.

Among the previously reported ADC structures,¹⁸ we focused herein on one ADC shown in Figure 1. The peptide linker was designed to be cleaved by lysosomal enzymes and subsequently to release the DXd. Firstly, we examined the in vivo payload release from the anti-HER2-DXd ADC. After intravenous administration of the ADC to HER2-positive KPL-4 tumor-bearing mice, the DXd was mainly detected in the tumor as the released payload as designed (Fig. 2).

And we compared the characteristics of DXd to those of exatecan mesylate (Table 1). The LogD and permeability coefficient of DXd were lower than those of exatecan mesylate, indicating that the membrane permeability of DXd was lower than that of exatecan mesylate. Although DXd showed a comparable topoisomerase I inhibitory activity to that of exatecan mesylate in the cell-free assay, its cytotoxicity in the cell-based assay was approximately 50-fold weaker than that of exatecan mesylate, probably owing to its lower membrane permeability. Furthermore, a colony forming unit granulocyte/macrophage (CFU-GM) assay was performed to evaluate the potential myelotoxicity of each compound. The IC₅₀ and IC₉₀ concentrations of DXd were 0.62 and 6.73 ng/mL,

respectively, which were approximately 10-fold higher than the corresponding values for exatecan mesylate (Fig. 3). These data suggest that DXd had a weaker myelotoxicity potential than exatecan mesylate did, and therefore we expected that we could establish a novel ADC with a high safety profile by using DXd as an ADC payload.

In the next step, we synthesized several ADCs targeting CD30, CD33, and CD70 to confirm the ability of the DXd-linker technology to function with antibodies other than trastuzumab. All the targets were well-known, validated targets for ADC proved by both pre-clinical and clinical evaluations of the respective ADC programs.^{19–23} In addition, all the antibodies for the ADC synthesis were produced by referencing the amino acid sequences of the antibody parts of these ADC programs. The anti-CD30-DXd was effective against the CD30-positive SR but not CD30-negative Daudi cells (Fig. 4A). Similarly, the anti-CD33-DXd and anti-CD70-DXd killed CD33- and CD70-positive cells, respectively (Fig. 4B and C). Since all the unconjugated antibodies showed no cell growth inhibition, the cytotoxicity of these ADCs was derived from the released payload, DXd. This target-specific efficacy was comparable to that shown by the trastuzumab ADC and, therefore, we confirmed that the DXd-linker technology could be applied to several kinds of antibodies, indicating the wide application of the developed technology.

In addition, we evaluated the application of DXd-linker technology to B7-H3, a novel ADC target. B7-H3, which is a member of the B7 family,²⁴ is overexpressed in a variety of human clinical tumors and its expression is significantly associated with poor patient outcomes.^{25–27} It has been suggested that the high expression (over

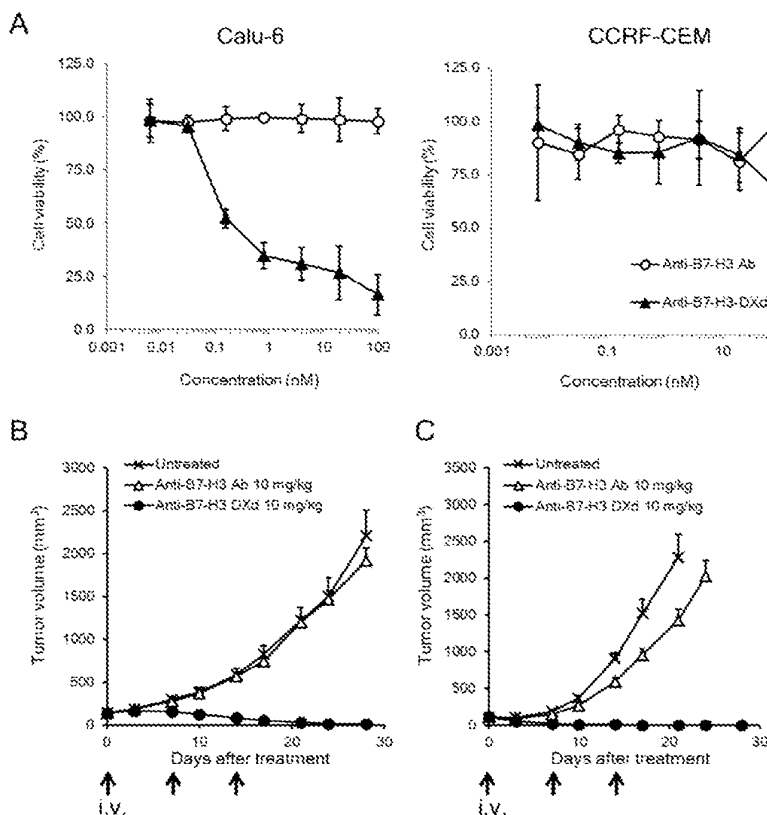


Figure 5. Efficacy of anti-B7-H3-DXd. (A) anti-B7-H3-DXd in B7-H3-positive Calu-6 cells and negative CCRF-CEM cells compared to anti-B7-H3 Ab. Each cell line was treated with each Ab or ADC for 6 days, and then cell viability was measured. Each plot represents mean $\pm$ SD ($n = 3$). Antitumor efficacy of anti-B7-H3-DXd in (B) Calu-6 and (C) A375 xenograft models. Eleven days after mice were inoculated with each cell line, they were randomized (Day 0). Each drug was administered intravenously to mice on Day 0, 7, and 14. Each plot represents mean $\pm$ SE ($n = 6$). ADC information; anti-B7-H3-DXd (DAR 3.2–3.4, Agg. rate 0.6–4.8%).

10^5 molecules per cell) of a relevant molecule is one of the critical factors for selecting the ADC target,²⁸ and B7-H3 would satisfy this criterion. We produced an anti-B7-H3 antibody in-house and then synthesized the ADC. Against B7-H3-positive Calu-6 cells, anti-B7-H3-DXd showed potent cytotoxicity but not against B7-H3-negative CCRF-CEM cells (Fig. 5A). In addition to the in vitro evaluation, we examined the antitumor activity of the anti-B7-H3-DXd against B7-H3-positive Calu-6 and A375 xenograft models. Although the unconjugated anti-B7-H3 antibody did not inhibit tumor growth in both models, the ADC strongly inhibited tumor growth and induced tumor regressions (Fig. 5B and C). Notably, all the ADC-treated mice grafted with the A375 tumor cells exhibited complete regression (Fig. 5C). These results suggest that B7-H3 is a promising ADC target for the DXd-linker technology, and the ADC has strong efficacy in vivo.

In this study, we demonstrated the versatility of a novel payload-linker technology using a topoisomerase I inhibitor, DXd, by applying the technology to various antibodies including those validated for ADCs (e.g., anti-CD30, CD33, and CD70 antibodies). Moreover, we focused on B7-H3 as a novel ADC target, and the anti-B7-H3-DXd showed potent efficacy in vitro and in vivo. The toxicity analysis revealed that DXd showed weak myelotoxicity in vitro, which may have contributed to mitigating the neutropenia that is a common dose-limiting toxicity observed in clinical trials of tubulin inhibitor ADCs. Presently, one of the linker-optimized anti-B7-H3-DXd ADCs has exhibited no adverse effects at doses of up to 30 mg/kg in preliminary safety studies in cynomolgus monkeys. As other topoisomerase I inhibitor-based ADCs, several technologies using camptothecin analogues were reported.^{29–31} Among them, sacituzumab govitecan (IMMU-132, an anti-TROP-2 ADC) is currently being evaluated in clinical trials.^{32,33} The payload used in that ADC is SN-38, which is an active metabolite of CPT-11. In a phase 1 study, similar toxicities to those associated with CPT-11 were observed clinically, such as diarrhea and neutropenia,³³ which is considered to be due to the instability of its linker part. The linker has pH-dependent cleavage site³² and a half of the conjugated SN-38 is released in human and monkey serum within only 24 h.^{32,34} By contrast, the DXd ADC has different payload and linker part compared to sacituzumab govitecan. The DXd payload has about 10-fold more potent topoisomerase inhibitory activity than SN-38 (data not shown). Moreover, the DXd ADC has a stable linker part which is designed to be cleaved by lysosomal enzymes supposed to have an intracellular specific payload release. These improvements could lead to a wider TI in DXd ADC than that in the SN-38 ADC. In conclusion, these findings suggest that this novel technology, which has a low myelotoxic potential, could be broadly used in the ADC field and would provide an additional payload-linker option to the conventional technologies.

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			97f12d04894013afe07e4609543dbc505148918e		
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29	Non Patent Literature	B211-- KRAUS_ET_AL____Demonstrati on_of_ligand_d.pdf	1031262	no	5
			58bb78568391f72136dff331952b859d01c7c665		
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30	Non Patent Literature	B212-- KRAUS_ET_AL____Isolation_and d_characteriz.pdf	1057616	no	5
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31	Non Patent Literature	B213-- KUMAZAWA_ET_AL____Antitu mor_activity_of_.pdf	1702322	no	8
			90445af36906579b7eb52d2a1a05792b60e69312		
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32	Non Patent Literature	B214-- KUMAZAWA_ET_AL____DE_31 0__a_novel_macrom.pdf	1572163	no	8
			93437c0a01e9218aa7344fbc525cd7532b9a79e1		
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33	Non Patent Literature	B215-- KUMAZAWA_ET_AL____Potent _and_broad_antit.pdf	1782116	no	11
			f1b6b8ba96a97b19b35f4e3ce6fc0a54d387840		
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34	Non Patent Literature	B216-- LINNENBACH_ET_AL____Sequ ence_investigati.pdf	1352913	no	5
			39783894bd40fe64baa0a99552be6153bdeeaab0		
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35	Non Patent Literature	B217-- LIPINSKI_ET_AL____Human_tr ophoblast_cell.pdf	1095635	no	4
			1710d3a66a9839e4a033be905e99a64f55916abf		
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36	Non Patent Literature	B218-- McDonagh_et_al____Engineer ed_antibody_dr.pdf	1419318	no	9
			f0421f627cd7576660a4916e4953999dcf96241		
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37	Non Patent Literature	B219-- MITSUI_ET_AL____A_New_Water_soluble_Camp.pdf	1291440	no	7
			9f033bf915278f843adc1417fdbf8c8d8e01c353		
Warnings:					
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38	Non Patent Literature	B220-- Moghaddas_et_al____Whether_HER2_positive.pdf	628627	no	11
			d4954871db53f819741c9f11fcc0b426e53dd488f		
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39	Non Patent Literature	B221-- M_HLMANN_ET_AL____TROP2_expression_as_pr.pdf	857442	no	7
			721fe7f2628153b82e14d879adae5bf1658918b3		
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40	Non Patent Literature	B222-- N_MASABUCHI____Pharmacokinetics_of_DE_31.pdf	372217	no	4
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41	Non Patent Literature	B223-- N_V__Sergina_et_al____Escape_from_HER_fa.pdf	520757	no	6
			63ddb5e31f80aeeb4825e099a354b8c8da328f2ac		
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42	Non Patent Literature	B224-- NAIDU_ET_AL____Expression_of_c_erbB3_pro.pdf	1230138	no	6
			312321d75eea433ac7f5e82d054e400fd151c845		
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43	Non Patent Literature	B225-- NAKADA_ET_AL____Novel_antibody_drug_conj.pdf	498572	no	4
			55792303019338b1409bbbe8a5b88447a7bf7fa0		
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44	Non Patent Literature	B226-- NING_ET_AL____TROP2_expre ssion_and_its_c.pdf	693659	no	6
			6ad6e413579b39aea637a6b29cdc4bf25a6644f9		
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45	Non Patent Literature	B227-- Non_Final_Office_Action_issue d_in_U_S__P.pdf	378759	no	10
			33830bad5621fc4dccc3af96c12940ae784953aa		
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46	Non Patent Literature	B228-- Non_Final_Office_Action_issue d_in_U_S__P.pdf	406869	no	11
			94ecc31d908adba54f73080eb3446de03d69a112		
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47	Non Patent Literature	B229-- Non_Final_Office_Action_issue d_in_U_S__P.pdf	372484	no	10
			9421d68c4144b3a27e9728cf9a9a893f0c53e628		
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48	Non Patent Literature	B230-- Non_Final_Office_Action_issue d_in_U_S__P.pdf	745751	no	31
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49	Non Patent Literature	B231-- Non_Final_Office_Action_issue d_in_U_S__P.pdf	272521	no	10
			0603d5056801c52cbcf0d3a451fd154a97ca76		
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50	Non Patent Literature	B232-- Notice_of_Allowance_issued_i n_U_S__Paten.pdf	351575	no	5
			d46bed48ce1a0639420553161799b88b785fd9dc		
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51	Non Patent Literature	B233-- Notice_of_Allowance_on_US_1 5_821662_DTD_.pdf	351884	no	5
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52	Non Patent Literature	B234-- OCHI_et_al___A_possible_mec hanism_for_th.pdf	1821800	no	10
			d41ef1906e6d7347d31ac711415ed3a9812da881		
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53	Non Patent Literature	B235-- Office_Action_dated_Novemb er_21__2017_in.pdf	855238	no	11
			9c62f64d09ff40da1551f6e8eb578052968ee56f		
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54	Non Patent Literature	B236-- Office_Action_issued_April_22 _2016__in_.pdf	359593	no	12
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55	Non Patent Literature	B237-- Office_Action_issued_in_Colo mbian_Applic.pdf	7574455	no	16
			2f3abcb246e9fbabc1c57341f968fe1d31f791d7		
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56	Non Patent Literature	B238-- Office_Action_issued_in_US_A pplication_N.pdf	374528	no	10
			0ad63a8b264f5618a6175208d6a6299bf0226b0b		
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57	Non Patent Literature	B239-- Office_Action_with_Search_Re port_dated_A.pdf	363670	no	8
			7b8a8dbe3e429c9958837a0e040b7c6663b10d24		
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58	Non Patent Literature	B240-- OGITANI_et_al____Bystander_ killing_effec.pdf	1140972 0c32fc91b85335bd3c50d265d6724a64863 ac202	no	8
Warnings:					
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59	Non Patent Literature	B241-- OGITANI_et_al____DS_8201a_ _A_Novel_HER2_.pdf	1317417 92048521844e73dbd34a6d09e4051afb19c 11330	no	13
Warnings:					
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60	Non Patent Literature	B242-- OGITANI_et_al____Wide_appli cation_of_a_n.pdf	354458 81a59dc6f4bc42e034e2f2072578662ecd05 5070	no	4
Warnings:					
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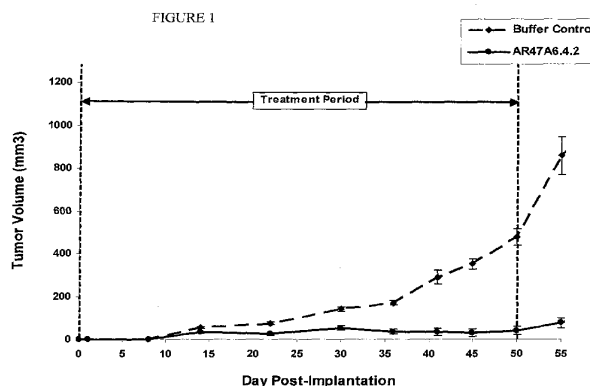
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[Continued on next page]

(54) Title: HUMANIZED AND CHIMERIC ANTI-TROP-2 ANTIBODIES THAT MEDIATE CANCER CELL CYTOTOXICITY



(57) Abstract: Expression of TROP-2, an approximately 35 kDa transmembrane protein and a substrate of protein kinase C, has been linked to several cancers. TROP-2 is also known as GA733-1, epithelial glycoprotein 1 (EGP-I) and tumor-associated calcium signal transducer-2. A monoclonal antibody against TROP-2 from the hybridoma AR47A6.4.2, deposited with the International Depository Authority of Canada (IDAC) as accession number 141205-05, was previously shown to be a cancerous disease modifying antibody (CDMAB), preventing tumour growth and reducing tumour burden in several cancer models including prostate, pancreatic and breast cancer by cytotoxicity. The variable regions of this monoclonal antibody were also isolated, sequenced and complementarity determining regions (CDRs) determined. Now, a chimeric antibody and humanized antibodies are generated that have similar TROP-2 binding activity as the parent 141205-05 monoclonal antibody. The monoclonal, chimeric and humanized antibodies can be conjugated to toxins, enzymes, radioactive compounds, cytokines, interferons, target or reporter moieties and hematogenous cells to treat cancer. These antibodies are also used in binding assays to determine TROP-2 expression on cells.

WO 2008/144891 A1

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WO 2008/144891

PCT/CA2008/000979

Humanized And Chimeric Anti-TROP-2 Antibodies That Mediate Cancer Cell Cytotoxicity

FIELD OF THE INVENTION

This invention relates to the diagnosis and treatment of cancerous diseases, particularly to the mediation of cytotoxicity of tumor cells; and most particularly to the use of cancerous disease modifying antibodies (CDMAB), optionally in combination with one or more CDMAB/chemotherapeutic agents, as a means for initiating the cytotoxic response. The invention further relates to binding assays, which utilize the CDMAB of the instant invention.

BACKGROUND OF THE INVENTION

TROP-2 is a cell surface glycoprotein expressed on most carcinomas, as well as some normal human tissues. It was initially defined as a molecule recognized by two murine monoclonal antibodies raised to a human choriocarcinoma cell line BeWo that recognized an antigen on human trophoblast cells (Faulk 1978). The same molecule was independently discovered by other investigators which led to multiple names describing the same antigen. Hence, TROP-2 was also referred to as GA733-1 and epithelial glycoprotein-1 (EGP-1) (Basu 1995, Fornaro 1995).

The TROP-2 gene is an intronless gene that was thought to have been formed through the retroposition of a homologous gene *GA733-2* (also known as *epithelial glycoprotein-2*, *EpCAM* and *Trop-1*) via an RNA intermediate. The *TROP-2* gene has been mapped to chromosome 1p32 (Calabrese 2001). The protein component of TROP-2 has a molecular mass of approximately 35 kilodaltons. Its mass may be increased by 11-13 kilodaltons with heterogeneous N-linked glycosylation of its extracellular domain. There are many cysteine residues in the extracellular domain which could form disulfide bridge sites. TROP-2 is a substrate for protein kinase C, a Ca^{2+} dependent protein kinase and the intracellular serine 303 residue has been shown to be phosphorylated (Basu 1995). It has also been shown that crossing-linking of TROP-2 with anti-TROP-2 antibodies transduced a calcium signal as shown by a rise in cytoplasmic Ca^{2+} (Ripani 1998). These data support signal transduction as a physiological function of TROP-2, although to date no physiological ligand has been identified. An association between TROP-2 expression and cancer has been shown in a report in which *TROP-2* was identified as a member of a group of genes reported

WO 2008/144891

PCT/CA2008/000979

to be the most highly overexpressed in ovarian serous papillary carcinoma compared to normal ovarian epithelium in a large-scale gene expression analysis using cDNA microarray technology (Santin 2004). A recent study, analyzing 74 colorectal human cancer samples by quantitative real-time RT-PCR and 34 of the samples by immunohistochemistry, examined

5 TROP-2 expression levels in cancer and normal patient sections. TROP-2 was found to be more highly expressed in cancer versus normal patient samples, and the study further demonstrated a correlation between TROP-2 expression levels and biological aggressiveness. High levels of TROP-2 were found to be associated with poor prognosis, a decrease in patient survival and an increase in the frequency of liver metastases (Ohmachi 2006), suggesting that

10 TROP-2 may be useful as a prognostic indicator and may be an attractive therapeutic target.

The expression profile of TROP-2 has been elucidated through immunohistochemistry (IHC) and flow cytometry studies using many different TROP-2 antibodies. Anti-TROP-2 antibodies 162-25.3 and 162-46.2 were produced through immunization of mice with the human choriocarcinoma cell line BeWo, and were investigated

15 for their reactivity to a series of tumor and lymphoid cell lines and peripheral blood mononuclear cells. In this study both antibodies appeared to be trophoblast specific, staining 3 of the 4 choriocarcinoma cell lines tested, while none of the other lymphoid or tumor cell lines (representing fibrosarcoma, cervical sarcoma, colon carcinoma, melanoma, neuroblastoma, erythroleukemia) were stained in an indirect immunofluorescence FACS

20 assay. In addition, none of the normal peripheral blood cells were stained. The antibodies were tested for staining of formalin-fixed paraffin-embedded placenta tissue sections and frozen normal sections of liver, kidney, spleen, thymus and lymph node tissues. The placenta tissue sections were stained with both antibodies, while there was no staining of the other normal tissues (Lipinski 1981). These two antibodies have strictly been reported for use in *in*

25 *vitro* diagnostic studies.

Anti-TROP-2 antibody MOv16 was generated through the immunization of mice with a crude membrane preparation of poorly differentiated ovarian carcinoma OvCa4343/83. MOv16 was tested for reactivity to a series of frozen tissue sections of benign and malignant ovarian tumors. MOv16 reacted with 31 of 54 malignant ovarian tumors and 2

30 of 16 benign ovarian tumors. Of the 5 mucinous ovarian tumors that were tested, MOv16 was completely unreactive. MOv16 was also tested for reactivity to frozen sections of non-

WO 2008/144891

PCT/CA2008/000979

ovarian malignant tumors where it was found to bind 117 of 189 breast carcinoma sections and 12 of 18 lung carcinoma sections. MOv16 was completely unreactive on 16 non-epithelial tumors that were tested (including liposarcomas, chondrosarcomas, endotheliomas, histiocytomas and dysgerminomas). When tested on frozen normal tissue sections, MOv-16
5 was reactive with breast, pancreas, kidney and prostate sections. MOv16 reactivity was reported to be negative on lung, spleen, skin, ovary, thyroid, parotid gland, stomach, larynx, uterus and colon sections, though the number of tissue sections that were used was not reported. The authors noted that frozen tissue sections were used because MOv16 was unreactive to paraffin embedded tissues (Miotti 1987). This antibody has also only been
10 reported for use in *in vitro* diagnostic studies.

Anti-TROP-2 antibody Rs7-3G11 (RS7) was generated through the immunization of mice with a crude membrane preparation derived from a surgically removed human primary squamous cell carcinoma of the lung. IHC was used to examine the staining of RS7 on frozen sections of human tumor and normal tissues. RS7 bound to 33 of the 40
15 sections representing tumors of the breast, colon, kidney, lung, prostate and squamous cell cancer. Of the normal tissues RS7 bound to 16 of 20 sections of breast, colon, kidney, liver, lung and prostate tissues while none of the five sections of spleen tissue were stained. In this study the authors noted that it appeared that antigen density in tumors was higher than in normal epithelial tissues (Stein 1990).

Additional studies of the tissue specificity of RS7 were carried out on both tumor and normal tissues. RS7 was tested on a panel of frozen tumor sections and bound to 65 of the 77 sections representing tumors of the lung, stomach, kidney, bladder, colon, breast, ovary, uterus and prostate. There was no binding to the 5 lymphomas tested. RS7 was tested on a panel of 85 frozen human normal tissue sections composed of a total of 24 tissue types.
25 39 sections of 13 normal tissues (lung, bronchus, trachea, esophagus, colon, liver, pancreas, kidney, bladder, skin, thyroid, breast and prostate) were stained by RS7. The authors of this study noted that in the tissues in which positive staining was observed, the reactivity was generally restricted to epithelial cells, primarily in ducts or glands. It was also noted that this study was limited to frozen sections since it was observed that RS7 was not reactive on
30 formalin-fixed paraffin-embedded sections (Stein 1993).

WO 2008/144891

PCT/CA2008/000979

Polyclonal anti-TROP-2 antibodies were prepared by immunizing mice with a synthetic peptide corresponding to amino acid positions between 169 and 182 of the cytoplasmic domain of human TROP-2. The polyclonal antibodies were tested on a tissue array slide that contained formalin-fixed human esophageal hyperplasia and carcinoma tissues. Ten of the 55 carcinoma specimens displayed heavy staining with the polyclonal antibodies, while the mild hyperplasia tissue stained very weakly, indicating expression levels may be related to malignant transformation (Nakashima 2004).

Overall, IHC reactivity patterns obtained with different anti-TROP-2 antibodies were consistent. Expression in cancer was seen primarily in carcinomas, and most carcinomas were reactive. In normal tissues, expression appeared to be limited to cells of epithelial origin, and there was some evidence that staining of carcinomas was stronger than staining of corresponding normal epithelial tissues.

In addition to being used in IHC studies, antibody RS7 was tested in *in vivo* models with initial experiments consisting of tumor targeting studies in nude mouse xenograft models. Radiolabeled RS7 injected *i.v.* was shown to accumulate specifically in the tumor of mice bearing either Calu-3 (lung adenocarcinoma) or GW-39 (colon carcinoma) tumors (Stein 1990). Further studies were done to investigate the biodistribution of radiolabeled RS7 in a xenograft system and to study the therapeutic potential of RS7 as an immunoconjugate. In this study the therapeutic efficacy of ^{131}I -labeled RS7 F(ab')₂ was investigated in nude mice bearing Calu-3 human lung adenocarcinoma xenografts. Three weeks following inoculation of the mice with Calu-3 cells, when the tumors had reached a size of approximately 0.3-0.9 grams, groups of 6-7 mice were treated with a single dose *i.v.* of either 1.0 mCi ^{131}I -RS7-F(ab')₂ or 1.5 mCi ^{131}I -RS7-F(ab')₂ and compared to a similar group of untreated control mice. The single dose of 1.0 mCi ^{131}I -RS7-F(ab')₂ resulted in tumor growth suppression for approximately 5 weeks, while the single dose of 1.5 mCi ^{131}I -RS7-F(ab')₂ resulted in tumor regression, and the mean tumor size did not exceed the pre-therapy size until the eighth week after radioantibody injection. Mice receiving the 1.5 mCi ^{131}I -RS7-F(ab')₂ dose experienced a mean body weight loss of 18.7 percent, indicating there was toxicity associated with the treatment. In this study, effects of treatment with naked RS7 or the F(ab')₂ fragment of RS7 were not tested (Stein 1994a). Another study was done to test the efficacy of ^{131}I -RS7 in a MDA-MB-468 breast cancer xenograft model. Groups of ten mice bearing MDA-MB-468

WO 2008/144891

PCT/CA2008/000979

tumors of approximately 0.1 cm^3 were treated with a single dose *i.v.* of either 250 microcuries ^{131}I -RS7 or 250 microcuries ^{131}I -Ag8 (an isotype matched control antibody). Groups of six mice were treated with a single dose *i.v.* of 30 micrograms of either unlabeled RS7 or Ag8. Complete regression of the tumors (except for one animal that had a transient reappearance of tumor) was seen in the animals treated with ^{131}I -RS7, which lasted for the duration of the 11 week observation period. Tumor regression was also seen in ^{131}I -Ag8 treated mice, though was only observed between 2 weeks and 5 weeks with tumors either persisting or continuing to grow for the remainder of the study. Tumor growth of mice that received unlabeled RS7 or Ag8 was not inhibited and there did not appear to be any differences in the mean tumor volume of RS7 treated mice compared to the Ag8 treated mice. Two additional groups of 10 mice bearing larger MDA-MB-468 tumors of approximately $0.2\text{-}0.3 \text{ cm}^3$ were treated with a slightly higher single dose of either 275 microcuries ^{131}I -Rs7 or 275 microcuries ^{131}I -Ag8 and compared to a similar group of untreated mice. Tumor volume was measured weekly for 15 weeks. Although in this case there was a significant difference in tumor growth between the ^{131}I -RS7 treated mice compared to the untreated mice, there was no significant difference in the tumor growth of the ^{131}I -RS7 compared to the ^{131}I -Ag8 treated mice, indicating a portion of the efficacy may have been due to non-specific effects of the radiation. Unlabeled antibodies were not tested in mice containing $0.2\text{-}0.3 \text{ cm}^3$ tumors (Shih 1995).

There have been numerous additional studies examining the efficacy of RS7 as an immunoconjugate with an attempt to select the optimal radiolabel for radioimmunotherapy (Stein 2001a, Stein 2001b, Stein 2003). A humanized version of RS7 has also been generated; however it has only been tested in preclinical xenograft models as a radioconjugate (Govindan 2004). These studies show similar positive effects as the previously described studies with RS7, however in one study, even when radiolabeled RS7 was delivered at a previously determined maximum tolerable dose, toxicity occurred leading to death in some mice (Stein 2001a). Although effective treatment of xenograft tumors in mice was achieved with radiolabeled RS7 in these studies, naked RS7 was not evaluated.

Immunizing mice with neuraminidase pre-treated H3922 human breast carcinoma cells produced the anti-TROP-2 monoclonal antibody BR110 (as disclosed in US patent No. 5,840,854, refer to Prior Patents section). By immunohistology, using human frozen tissue specimens, BR110 was shown to react with a wide range of human carcinoma

WO 2008/144891

PCT/CA2008/000979

specimens including those of the lung, colon, breast, ovarian, kidney, esophagus, pancreas, skin, lung and tonsil. No human normal tissue sections were tested. *In vitro* studies demonstrated that BR110 had no ADCC or CDC activity on the human carcinoma cell lines H3396 or H3922. *In vitro* studies analyzing the cytotoxicity of BR110-immunotoxins was performed on the human cancer cell lines H3619, H2987, MCF-7, H3396 and H2981. The EC₅₀ for the cell lines tested was 0.06, 0.001, 0.05, 0.09 and >5 micrograms/mL respectively. No cytotoxicity data was disclosed for the naked BR110 antibody. No *in vivo* data was disclosed for the naked or immunoconjugated BR110.

A number of additional antibodies have been generated that target TROP-2, such as MR54, MR6 and MR23 which were generated from immunization of mice with the ovarian cancer cell line Colo 316 (Stein 1994b) and antibody T16 which was generated by immunization of mice with the T24 bladder cancer cell line (Fradet 1984). The use of these antibodies has been limited to biochemical characterization of the TROP-2 antigen and cell line and tissue expression studies. There have been no reports of anti-cancer efficacy of these antibodies, either *in vitro* or *in vivo*. RS7 was the only antibody that was tested for therapeutic efficacy in preclinical cancer models, with its use being limited to a carrier of radioisotope. There are no reports of any naked TROP-2 antibodies exhibiting therapeutic efficacy in clinical studies or in preclinical cancer models either *in vitro* or *in vivo*.

Monoclonal Antibodies as Cancer Therapy: Each individual who presents with cancer is unique and has a cancer that is as different from other cancers as that person's identity. Despite this, current therapy treats all patients with the same type of cancer, at the same stage, in the same way. At least 30 percent of these patients will fail the first line therapy, thus leading to further rounds of treatment and the increased probability of treatment failure, metastases, and ultimately, death. A superior approach to treatment would be the customization of therapy for the particular individual. The only current therapy which lends itself to customization is surgery. Chemotherapy and radiation treatment cannot be tailored to the patient, and surgery by itself, in most cases is inadequate for producing cures.

With the advent of monoclonal antibodies, the possibility of developing methods for customized therapy became more realistic since each antibody can be directed to a single epitope. Furthermore, it is possible to produce a combination of antibodies that are directed to the constellation of epitopes that uniquely define a particular individual's tumor.

WO 2008/144891

PCT/CA2008/000979

Having recognized that a significant difference between cancerous and normal cells is that cancerous cells contain antigens that are specific to transformed cells, the scientific community has long held that monoclonal antibodies can be designed to specifically target transformed cells by binding specifically to these cancer antigens; thus giving rise to the belief that monoclonal antibodies can serve as "Magic Bullets" to eliminate cancer cells. However, it is now widely recognized that no single monoclonal antibody can serve in all instances of cancer, and that monoclonal antibodies can be deployed, as a class, as targeted cancer treatments. Monoclonal antibodies isolated in accordance with the teachings of the instantly disclosed invention have been shown to modify the cancerous disease process in a manner which is beneficial to the patient, for example by reducing the tumor burden, and will variously be referred to herein as cancerous disease modifying antibodies (CDMAB) or "anti-cancer" antibodies.

At the present time, the cancer patient usually has few options of treatment. The regimented approach to cancer therapy has produced improvements in global survival and morbidity rates. However, to the particular individual, these improved statistics do not necessarily correlate with an improvement in their personal situation.

Thus, if a methodology was put forth which enabled the practitioner to treat each tumor independently of other patients in the same cohort, this would permit the unique approach of tailoring therapy to just that one person. Such a course of therapy would, ideally, increase the rate of cures, and produce better outcomes, thereby satisfying a long-felt need.

Historically, the use of polyclonal antibodies has been used with limited success in the treatment of human cancers. Lymphomas and leukemias have been treated with human plasma, but there were few prolonged remission or responses. Furthermore, there was a lack of reproducibility and there was no additional benefit compared to chemotherapy. Solid tumors such as breast cancers, melanomas and renal cell carcinomas have also been treated with human blood, chimpanzee serum, human plasma and horse serum with correspondingly unpredictable and ineffective results.

There have been many clinical trials of monoclonal antibodies for solid tumors. In the 1980s there were at least four clinical trials for human breast cancer which produced only one responder from at least 47 patients using antibodies against specific antigens or based on tissue selectivity. It was not until 1998 that there was a successful

WO 2008/144891

PCT/CA2008/000979

clinical trial using a humanized anti-Her2/neu antibody (Herceptin®) in combination with CISPLATIN. In this trial 37 patients were assessed for responses of which about a quarter had a partial response rate and an additional quarter had minor or stable disease progression. The median time to progression among the responders was 8.4 months with median response
5 duration of 5.3 months.

Herceptin® was approved in 1998 for first line use in combination with Taxol®. Clinical study results showed an increase in the median time to disease progression for those who received antibody therapy plus Taxol® (6.9 months) in comparison to the group that received Taxol® alone (3.0 months). There was also a slight increase in median survival;
10 22 versus 18 months for the Herceptin® plus Taxol® treatment arm versus the Taxol® treatment alone arm. In addition, there was an increase in the number of both complete (8 versus 2 percent) and partial responders (34 versus 15 percent) in the antibody plus Taxol® combination group in comparison to Taxol® alone. However, treatment with Herceptin® and Taxol® led to a higher incidence of cardiotoxicity in comparison to Taxol® treatment alone
15 (13 versus 1 percent respectively). Also, Herceptin® therapy was only effective for patients who over express (as determined through immunohistochemistry (IHC) analysis) the human epidermal growth factor receptor 2 (Her2/neu), a receptor, which currently has no known function or biologically important ligand; approximately 25 percent of patients who have metastatic breast cancer. Therefore, there is still a large unmet need for patients with breast
20 cancer. Even those who can benefit from Herceptin® treatment would still require chemotherapy and consequently would still have to deal with, at least to some degree, the side effects of this kind of treatment.

The clinical trials investigating colorectal cancer involve antibodies against both glycoprotein and glycolipid targets. Antibodies such as 17-1A, which has some
25 specificity for adenocarcinomas, has undergone Phase 2 clinical trials in over 60 patients with only 1 patient having a partial response. In other trials, use of 17-1A produced only 1 complete response and 2 minor responses among 52 patients in protocols using additional cyclophosphamide. To date, Phase III clinical trials of 17-1A have not demonstrated improved efficacy as adjuvant therapy for stage III colon cancer. The use of a humanized
30 murine monoclonal antibody initially approved for imaging also did not produce tumor regression.

WO 2008/144891

PCT/CA2008/000979

Only recently have there been any positive results from colorectal cancer clinical studies with the use of monoclonal antibodies. In 2004, ERBITUX® was approved for the second line treatment of patients with EGFR-expressing metastatic colorectal cancer who are refractory to irinotecan-based chemotherapy. Results from both a two-arm Phase II clinical study and a single arm study showed that ERBITUX® in combination with irinotecan had a response rate of 23 and 15 percent respectively with a median time to disease progression of 4.1 and 6.5 months respectively. Results from the same two-arm Phase II clinical study and another single arm study showed that treatment with ERBITUX® alone resulted in an 11 and 9 percent response rate respectively with a median time to disease progression of 1.5 and 4.2 months respectively.

Consequently in both Switzerland and the United States, ERBITUX® treatment in combination with irinotecan, and in the United States, ERBITUX® treatment alone, has been approved as a second line treatment of colon cancer patients who have failed first line irinotecan therapy. Therefore, like Herceptin®, treatment in Switzerland is only approved as a combination of monoclonal antibody and chemotherapy. In addition, treatment in both Switzerland and the US is only approved for patients as a second line therapy. Also, in 2004, AVASTIN® was approved for use in combination with intravenous 5-fluorouracil-based chemotherapy as a first line treatment of metastatic colorectal cancer. Phase III clinical study results demonstrated a prolongation in the median survival of patients treated with AVASTIN® plus 5-fluorouracil compared to patients treated with 5-fluorouracil alone (20 months versus 16 months respectively). However, again like Herceptin® and ERBITUX®, treatment is only approved as a combination of monoclonal antibody and chemotherapy.

There also continues to be poor results for lung, brain, ovarian, pancreatic, prostate, and stomach cancer. The most promising recent results for non-small cell lung cancer came from a Phase II clinical trial where treatment involved a monoclonal antibody (SGN-15; dox-BR96, anti-Sialyl-LeX) conjugated to the cell-killing drug doxorubicin in combination with the chemotherapeutic agent TAXOTERE®. TAXOTERE® is the only FDA approved chemotherapy for the second line treatment of lung cancer. Initial data indicate an improved overall survival compared to TAXOTERE® alone. Out of the 62 patients who were recruited for the study, two-thirds received SGN-15 in combination with TAXOTERE® while the remaining one-third received TAXOTERE® alone. For the patients receiving SGN-

WO 2008/144891

PCT/CA2008/000979

15 in combination with TAXOTERE®, median overall survival was 7.3 months in comparison to 5.9 months for patients receiving TAXOTERE® alone. Overall survival at 1 year and 18 months was 29 and 18 percent respectively for patients receiving SNG-15 plus TAXOTERE® compared to 24 and 8 percent respectively for patients receiving

5 TAXOTERE® alone. Further clinical trials are planned.

Preclinically, there has been some limited success in the use of monoclonal antibodies for melanoma. Very few of these antibodies have reached clinical trials and to date none have been approved or demonstrated favorable results in Phase III clinical trials.

10 The discovery of new drugs to treat disease is hindered by the lack of identification of relevant targets among the products of 30,000 known genes that could contribute to disease pathogenesis. In oncology research, potential drug targets are often selected simply due to the fact that they are over-expressed in tumor cells. Targets thus identified are then screened for interaction with a multitude of compounds. In the case of potential antibody therapies, these candidate compounds are usually derived from traditional
15 methods of monoclonal antibody generation according to the fundamental principles laid down by Kohler and Milstein (1975, Nature, 256, 495-497, Kohler and Milstein). Spleen cells are collected from mice immunized with antigen (e.g. whole cells, cell fractions, purified antigen) and fused with immortalized hybridoma partners. The resulting hybridomas are screened and selected for secretion of antibodies which bind most avidly to the target. Many
20 therapeutic and diagnostic antibodies directed against cancer cells, including Herceptin® and RITUXIMAB, have been produced using these methods and selected on the basis of their affinity. The flaws in this strategy are two-fold. Firstly, the choice of appropriate targets for therapeutic or diagnostic antibody binding is limited by the paucity of knowledge surrounding tissue specific carcinogenic processes and the resulting simplistic methods, such as selection
25 by overexpression, by which these targets are identified. Secondly, the assumption that the drug molecule that binds to the receptor with the greatest affinity usually has the highest probability for initiating or inhibiting a signal may not always be the case.

Despite some progress with the treatment of breast and colon cancer, the identification and development of efficacious antibody therapies, either as single agents or co-
30 treatments, have been inadequate for all types of cancer.

WO 2008/144891

PCT/CA2008/000979

Prior Patents:

U.S. Patent No. 5,750,102 discloses a process wherein cells from a patient's tumor are transfected with MHC genes which may be cloned from cells or tissue from the patient. These transfected cells are then used to vaccinate the patient.

5 U.S. Patent No. 4,861,581 discloses a process comprising the steps of obtaining monoclonal antibodies that are specific to an internal cellular component of neoplastic and normal cells of the mammal but not to external components, labeling the monoclonal antibody, contacting the labeled antibody with tissue of a mammal that has received therapy to kill neoplastic cells, and determining the effectiveness of therapy by
10 measuring the binding of the labeled antibody to the internal cellular component of the degenerating neoplastic cells. In preparing antibodies directed to human intracellular antigens, the patentee recognizes that malignant cells represent a convenient source of such antigens.

U.S. Patent No. 5,171,665 provides a novel antibody and method for its
15 production. Specifically, the patent teaches formation of a monoclonal antibody which has the property of binding strongly to a protein antigen associated with human tumors, e.g. those of the colon and lung, while binding to normal cells to a much lesser degree.

U.S. Patent No. 5,484,596 provides a method of cancer therapy comprising surgically removing tumor tissue from a human cancer patient, treating the tumor tissue to
20 obtain tumor cells, irradiating the tumor cells to be viable but non-tumorigenic, and using these cells to prepare a vaccine for the patient capable of inhibiting recurrence of the primary tumor while simultaneously inhibiting metastases. The patent teaches the development of monoclonal antibodies which are reactive with surface antigens of tumor cells. As set forth at col. 4, lines 45 et seq., the patentees utilize autochthonous tumor cells in the development of
25 monoclonal antibodies expressing active specific immunotherapy in human neoplasia.

U.S. Patent No. 5,693,763 teaches a glycoprotein antigen characteristic of human carcinomas and not dependent upon the epithelial tissue of origin.

U.S. Patent No. 5,783,186 is drawn to Anti-Her2 antibodies which induce apoptosis in Her2 expressing cells, hybridoma cell lines producing the antibodies, methods of
30 treating cancer using the antibodies and pharmaceutical compositions including said antibodies.

WO 2008/144891

PCT/CA2008/000979

U.S. Patent No. 5,849,876 describes new hybridoma cell lines for the production of monoclonal antibodies to mucin antigens purified from tumor and non-tumor tissue sources.

5 U.S. Patent No. 5,869,268 is drawn to a method for generating a human lymphocyte producing an antibody specific to a desired antigen, a method for producing a monoclonal antibody, as well as monoclonal antibodies produced by the method. The patent is particularly drawn to the production of an anti-HD human monoclonal antibody useful for the diagnosis and treatment of cancers.

10 U.S. Patent No. 5,869,045 relates to antibodies, antibody fragments, antibody conjugates and single-chain immunotoxins reactive with human carcinoma cells. The mechanism by which these antibodies function is two-fold, in that the molecules are reactive with cell membrane antigens present on the surface of human carcinomas, and further in that the antibodies have the ability to internalize within the carcinoma cells, subsequent to binding, making them especially useful for forming antibody-drug and antibody-toxin conjugates. In
15 their unmodified form the antibodies also manifest cytotoxic properties at specific concentrations.

U.S. Patent No. 5,780,033 discloses the use of autoantibodies for tumor therapy and prophylaxis. However, this antibody is an antinuclear autoantibody from an aged mammal. In this case, the autoantibody is said to be one type of natural antibody found in the
20 immune system. Because the autoantibody comes from "an aged mammal", there is no requirement that the autoantibody actually comes from the patient being treated. In addition the patent discloses natural and monoclonal antinuclear autoantibody from an aged mammal, and a hybridoma cell line producing a monoclonal antinuclear autoantibody.

U.S. Patent No. 5,840,854 discloses a specific antibody, BR110 directed
25 against GA733-1. This patent discloses *in vitro* function for BR110 as an immunotoxin conjugate. There was no *in vitro* function as a naked antibody disclosed for this antibody. There was also no *in vivo* function disclosed for this antibody.

U.S. Patent No. 6,653,104 claims immunotoxin-conjugated antibodies, including but not limited to RS7, directed against a host of antigens, including but not limited
30 to EGP-1. The immunotoxin is limited to those possessing ribonucleolytic activity. However,

WO 2008/144891

PCT/CA2008/000979

the examples disclose only a specific immunotoxin-conjugated antibody, LL2, directed against CD22. There was no *in vitro* or *in vivo* function for RS7 disclosed in this application.

U.S. Application No. 20040001825A1 discloses a specific antibody, RS7 directed against EGP-1. This application discloses *in vitro* function for RS7 as a radiolabeled conjugate. There was no *in vitro* function as a naked antibody disclosed for this antibody. This application also discloses *in vivo* function for RS7 resulting from radiolabeled and unlabeled conjugate administered sequentially. However, this study was limited to one patient and it is unknown whether any of the observed function was due to the unlabeled antibody. There was no *in vivo* function for RS7 resulting from the administration of the naked antibody.

SUMMARY OF THE INVENTION

This application utilizes methodology for producing patient specific anti-cancer antibodies taught in the U.S. 6,180,357 patent for isolating hybridoma cell lines which encode for cancerous disease modifying monoclonal antibodies. These antibodies can be made specifically for one tumor and thus make possible the customization of cancer therapy. Within the context of this application, anti-cancer antibodies having either cell-killing (cytotoxic) or cell-growth inhibiting (cytostatic) properties will hereafter be referred to as cytotoxic. These antibodies can be used in aid of staging and diagnosis of a cancer, and can be used to treat tumor metastases. These antibodies can also be used for the prevention of cancer by way of prophylactic treatment. Unlike antibodies generated according to traditional drug discovery paradigms, antibodies generated in this way may target molecules and pathways not previously shown to be integral to the growth and/or survival of malignant tissue. Furthermore, the binding affinities of these antibodies are suited to requirements for initiation of the cytotoxic events that may not be amenable to stronger affinity interactions. Also, it is within the purview of this invention to conjugate standard chemotherapeutic modalities, e.g. radionuclides, with the CDMAB of the instant invention, thereby focusing the use of said chemotherapeutics. The CDMAB can also be conjugated to toxins, cytotoxic moieties, enzymes e.g. biotin conjugated enzymes, cytokines, interferons, target or reporter moieties or hematogenous cells, thereby forming an antibody conjugate. The CDMAB can be used alone or in combination with one or more CDMAB/chemotherapeutic agents.

WO 2008/144891

PCT/CA2008/000979

The prospect of individualized anti-cancer treatment will bring about a change in the way a patient is managed. A likely clinical scenario is that a tumor sample is obtained at the time of presentation, and banked. From this sample, the tumor can be typed from a panel of pre-existing cancerous disease modifying antibodies. The patient will be conventionally staged but the available antibodies can be of use in further staging the patient. The patient can be treated immediately with the existing antibodies, and a panel of antibodies specific to the tumor can be produced either using the methods outlined herein or through the use of phage display libraries in conjunction with the screening methods herein disclosed. All the antibodies generated will be added to the library of anti-cancer antibodies since there is a possibility that other tumors can bear some of the same epitopes as the one that is being treated. The antibodies produced according to this method may be useful to treat cancerous disease in any number of patients who have cancers that bind to these antibodies.

In addition to anti-cancer antibodies, the patient can elect to receive the currently recommended therapies as part of a multi-modal regimen of treatment. The fact that the antibodies isolated via the present methodology are relatively non-toxic to non-cancerous cells allows for combinations of antibodies at high doses to be used, either alone, or in conjunction with conventional therapy. The high therapeutic index will also permit re-treatment on a short time scale that should decrease the likelihood of emergence of treatment resistant cells.

If the patient is refractory to the initial course of therapy or metastases develop, the process of generating specific antibodies to the tumor can be repeated for re-treatment. Furthermore, the anti-cancer antibodies can be conjugated to red blood cells obtained from that patient and re-infused for treatment of metastases. There have been few effective treatments for metastatic cancer and metastases usually portend a poor outcome resulting in death. However, metastatic cancers are usually well vascularized and the delivery of anti-cancer antibodies by red blood cells can have the effect of concentrating the antibodies at the site of the tumor. Even prior to metastases, most cancer cells are dependent on the host's blood supply for their survival and an anti-cancer antibody conjugated to red blood cells can be effective against *in situ* tumors as well. Alternatively, the antibodies may be conjugated to other hematogenous cells, e.g. lymphocytes, macrophages, monocytes, natural killer cells, etc.

WO 2008/144891

PCT/CA2008/000979

There are five classes of antibodies and each is associated with a function that is conferred by its heavy chain. It is generally thought that cancer cell killing by naked antibodies are mediated either through antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). For example murine IgM and IgG2a antibodies can activate human complement by binding the C-1 component of the complement system thereby activating the classical pathway of complement activation which can lead to tumor lysis. For human antibodies the most effective complement activating antibodies are generally IgM and IgG1. Murine antibodies of the IgG2a and IgG3 isotype are effective at recruiting cytotoxic cells that have Fc receptors which will lead to cell killing by monocytes, macrophages, granulocytes and certain lymphocytes. Human antibodies of both the IgG1 and IgG3 isotype mediate ADCC.

The cytotoxicity mediated through the Fc region requires the presence of effector cells, their corresponding receptors, or proteins e.g. NK cells, T-cells and complement. In the absence of these effector mechanisms, the Fc portion of an antibody is inert. The Fc portion of an antibody may confer properties that affect the pharmacokinetics of an antibody *in vivo*, but *in vitro* this is not operative.

Another possible mechanism of antibody mediated cancer killing may be through the use of antibodies that function to catalyze the hydrolysis of various chemical bonds in the cell membrane and its associated glycoproteins or glycolipids, so-called catalytic antibodies.

There are three additional mechanisms of antibody-mediated cancer cell killing. The first is the use of antibodies as a vaccine to induce the body to produce an immune response against the putative antigen that resides on the cancer cell. The second is the use of antibodies to target growth receptors and interfere with their function or to down regulate that receptor so that its function is effectively lost. The third is the effect of such antibodies on direct ligation of cell surface moieties that may lead to direct cell death, such as ligation of death receptors such as TRAIL R1 or TRAIL R2, or integrin molecules such as alpha V beta 3 and the like.

The clinical utility of a cancer drug is based on the benefit of the drug under an acceptable risk profile to the patient. In cancer therapy survival has generally been the most sought after benefit, however there are a number of other well-recognized benefits in addition

WO 2008/144891

PCT/CA2008/000979

to prolonging life. These other benefits, where treatment does not adversely affect survival, include symptom palliation, protection against adverse events, prolongation in time to recurrence or disease-free survival, and prolongation in time to progression. These criteria are generally accepted and regulatory bodies such as the U.S. Food and Drug Administration (F.D.A.) approve drugs that produce these benefits (Hirschfeld *et al.* Critical Reviews in Oncology/Hematology 42:137-143 2002). In addition to these criteria it is well recognized that there are other endpoints that may presage these types of benefits. In part, the accelerated approval process granted by the U.S. F.D.A. acknowledges that there are surrogates that will likely predict patient benefit. As of year-end 2003, there have been sixteen drugs approved under this process, and of these, four have gone on to full approval, i.e., follow-up studies have demonstrated direct patient benefit as predicted by surrogate endpoints. One important endpoint for determining drug effects in solid tumors is the assessment of tumor burden by measuring response to treatment (Therasse *et al.* Journal of the National Cancer Institute 92(3):205-216 2000). The clinical criteria (RECIST criteria) for such evaluation have been promulgated by Response Evaluation Criteria in Solid Tumors Working Group, a group of international experts in cancer. Drugs with a demonstrated effect on tumor burden, as shown by objective responses according to RECIST criteria, in comparison to the appropriate control group tend to, ultimately, produce direct patient benefit. In the pre-clinical setting tumor burden is generally more straightforward to assess and document. In that pre-clinical studies can be translated to the clinical setting, drugs that produce prolonged survival in pre-clinical models have the greatest anticipated clinical utility. Analogous to producing positive responses to clinical treatment, drugs that reduce tumor burden in the pre-clinical setting may also have significant direct impact on the disease. Although prolongation of survival is the most sought after clinical outcome from cancer drug treatment, there are other benefits that have clinical utility and it is clear that tumor burden reduction, which may correlate to a delay in disease progression, extended survival or both, can also lead to direct benefits and have clinical impact (Eckhardt *et al.* Developmental Therapeutics: Successes and Failures of Clinical Trial Designs of Targeted Compounds; ASCO Educational Book, 39th Annual Meeting, 2003, pages 209-219).

Using substantially the process of U.S. 6,180,357, and as disclosed in U.S. patent application S.N. 11/709,676 the contents of each of which are herein incorporated by

WO 2008/144891

PCT/CA2008/000979

reference, the mouse monoclonal antibody, AR47A6.4.2 was obtained following immunization of mice with cells from human ovarian tumor tissue. The AR47A6.4.2 antigen was expressed on the cell surface of a wide range of human cell lines from different tissue origins. The ovarian cancer cell line OVCAR-3 was susceptible to the cytotoxic effect of

5 AR47A6.4.2 *in vitro*.

The result of AR47A6.4.2 cytotoxicity against human cancer cells *in vitro* was further extended by demonstrating its anti-tumor activity *in vivo* (as disclosed in S.N. 11/709,676). AR47A6.4.2 prevented tumor growth and reduced tumor burden in an *in vivo* prophylactic BxPC-3 model of human pancreatic cancer. On day 49 post-implantation, the

10 last day of treatment, the mean tumor volume in the AR47A6.4.2 treated group was 53 percent less than that of the buffer control-treated group ($p<0.05$). There were no clinical signs of toxicity throughout the study. In summary, AR47A6.4.2 was well-tolerated and decreased the tumor burden in this human pancreatic cancer xenograft model.

To further determine the efficacy of AR47A6.4.2 on the BxPC-3 model of

15 human pancreatic cancer, the antibody was tested on an established BxPC-3 xenograft model (as disclosed in S.N. 11/709,676). AR47A6.4.2 significantly reduced tumor burden in an established model of human pancreatic cancer. On day 54, one day after the last dose of antibody was administered, AR47A6.4.2-treated animals had a mean tumor volume that was 40 percent of the mean tumor volume in the control-treated animals ($p<0.0001$). These results

20 correspond to a mean T/C of 30 percent for AR47A6.4.2. There were no clinical signs of toxicity throughout the study. In summary, AR47A6.4.2 was well-tolerated and decreased the tumor burden in this established human pancreatic cancer xenograft model. AR47A6.4.2 has demonstrated efficacy in both a preventative and established model of human pancreatic

cancer.

25 AR47A6.4.2 has demonstrated anti-cancer effects in against a human pancreatic cancer model. To extend this finding AR47A6.4.2 was tested on a xenograft model of PL45 human pancreatic cancer (as disclosed in S.N. 11/79,676). AR47A6.4.2 completely inhibited tumor growth in the PL45 *in vivo* prophylactic model of human pancreatic cancer. Treatment with ARIUS antibody AR47A6.4.2 reduced the growth of

30 PL45 tumors by nearly 100 percent ($p=0.0005$, t-test), compared to the buffer-treated group, as determined on day 77, 20 days after the last dose of antibody when almost all mice in

WO 2008/144891

PCT/CA2008/000979

control and antibody-treated group were living. At day 102, 45 days after last dose, all mice in the control group had been removed from the study due to tumor volume. However AR47A6.4.2 still demonstrated almost complete inhibition of tumor growth and 4 mice (some mice had been lost due to non-cancer related incidents) in that group were still alive. There were no obvious clinical signs of toxicity throughout the study. In summary, AR47A6.4.2 was well-tolerated and almost completely inhibited the tumor growth in this human pancreatic cancer xenograft model. AR47A6.4.2 treatment also demonstrated increased survival in comparison to buffer treatment. AR47A6.4.2 therefore has demonstrated efficacy in two different models of human pancreatic cancer.

AR47A6.4.2 has demonstrated anti-cancer properties against two different human pancreatic cancer xenograft models. To determine the efficacy of AR47A6.4.2 against a different human cancer xenograft model, the antibody was tested on a PC-3 prostate cancer xenograft model (as disclosed in S.N. 11/709,676). AR47A6.4.2 inhibited tumor growth in the PC-3 *in vivo* prophylactic model of human prostate adenocarcinoma cells. Treatment with ARIUS antibody AR47A6.4.2 reduced the growth of PC-3 tumors by 60.9 percent ($p=0.00037$, t-test), compared to the buffer treated group, as determined on day 32 after 5 doses of treatment with antibody when almost all mice in control and antibody-treated group were still alive. All mice in the control group had been removed from the study by day 47, 3 days before the last dose of antibody, due to tumor volume/lesions. At day 77, 27 days after last dose of antibody, 40 percent of the mice in the AR47A6.4.2-treated group still were still alive. There were no obvious clinical signs of toxicity throughout the study. In summary, AR47A6.4.2 was well-tolerated and significantly inhibited the tumor growth in this human prostate cancer xenograft model. Treatment with antibody also demonstrated a survival benefit in comparison to the control group. AR47A6.4.2 has demonstrated efficacy against two different human cancer indications: pancreatic and prostate.

AR47A6.4.2 has demonstrated anti-cancer properties against two different human pancreatic and a prostate cancer xenograft model. To determine the efficacy of AR47A6.4.2 against another human cancer xenograft model, the antibody was tested on a MCF-7 cancer xenograft model (as disclosed in S.N. 11/709,676). AR47A6.4.2 reduced tumor growth in the MCF-7 *in vivo* prophylactic model of human breast cancer. Treatment with ARIUS antibody AR47A6.4.2 resulted in a marked tumor growth delay. AR47A6.4.2

WO 2008/144891

PCT/CA2008/000979

induced T/C percent values that were lower than 42 percent from day 18 to day 35 of treatment and close to 42 percent up to day 49 (optimal T/C percent value of 10.9 percent at day 18). At day 53, after treatment was terminated, efficacy with treatment of AR47A6.4.2 was still observed with a T/C of 57 percent. At the end of the study (day 91), 2 mice from the AR47A6.4.2 treatment group remained tumor-free. A post-treatment survival benefit was associated with AR47A6.4.2 administration. The buffer control group reached 100 percent mortality by day 85 post-treatment while 33.3 percent of the AR47A6.4.2 mice were still alive at day 91 post-treatment. There were no clinical signs of toxicity throughout the study. In summary, AR47A6.4.2 was well-tolerated, reduced tumor growth and provided a survival benefit in this human breast cancer xenograft model. AR47A6.4.2 has demonstrated efficacy against three different human cancer indications; pancreatic, prostate and breast.

AR47A6.4.2 has demonstrated anti-cancer properties against two different human pancreatic, a prostate and a breast cancer xenograft model. To determine the efficacy of AR47A6.4.2 against another human cancer xenograft model, the antibody was tested on a Colo 205 colon cancer xenograft model (as disclosed in S.N. 11/709,676). AR47A6.4.2 inhibited tumor growth in the Colo 205 *in vivo* prophylactic model of human colorectal adenocarcinoma cells. Treatment with ARIUS antibody AR47A6.4.2 reduced the growth of Colo 205 tumors by 60.2 percent ($p=0.0003851$, t-test), compared to the buffer-treated group, as determined on day 27, 4 days before the last dose of antibody. There were no obvious clinical signs of toxicity throughout the study. In summary, AR47A6.4.2 was well-tolerated and significantly inhibited the tumor growth in this human colon cancer xenograft model. AR47A6.4.2 has demonstrated efficacy against four different human cancer indications; pancreatic, prostate, breast and colon. Treatment benefits were observed in several well-recognized models of human cancer disease suggesting pharmacologic and pharmaceutical benefits of this antibody for therapy in other mammals, including man. *In toto*, this data demonstrates that the AR47A6.4.2 antigen is a cancer associated antigen and is expressed on human cancer cells, and is a pathologically relevant cancer target.

As disclosed previously (S.N. 11/709,676), biochemical data indicated that the antigen recognized by AR47A6.4.2 is TROP-2. This was supported by studies that showed a monoclonal antibody (clone 77220.11, R&D Systems, Minneapolis, MN) reactive against TROP-2 identifies proteins that were bound to AR47A6.4.2 by immunoprecipitation. In

WO 2008/144891

PCT/CA2008/000979

addition, AR47A6.4.2 specifically recognized the recombinant form of human TROP-2 by Western Immunoblot. The AR47A6.4.2 epitope does not appear to be carbohydrate dependent but does appear to be conformation dependent. AR47A6.4.2 was also demonstrated to bind to a distinct epitope from another anti-TROP-2 antibody: AR52A301.5.

5 In order to determine the utility of the AR47A6.4.2 epitope, the expression of AR47A6.4.2 antigen in frozen normal human tissue sections (experiments showed no reactivity of this antibody with formalin fixed tissues) was previously determined (as disclosed in S.N. 11/709,676). Binding to 12 human normal organs, ovary, pancreas, thyroid, brain (cerebrum, cerebellum), lung, spleen, uterus, cervix, heart, skin, and skeletal muscle was
10 performed using a human normal tissue screening array (Biochain, CA, USA). The array contained 20 normal human organs; however only 12 of the organs were interpretable after staining. The AR47A6.4.2 antibody showed binding predominantly to epithelial tissues (endothelium of blood vessels, follicular epithelium of thyroid, acinar and ductal epithelium of pancreas, alveolar epithelium of lung, and epidermal keratinocytes of skin). The antibody
15 also showed equivocal binding to lymphoid tissue of the spleen and binding to neural tissue of the brain. Cellular localization was cytoplasmic and membranous with diffuse staining pattern. AR47A6.4.2 showed a similar binding pattern when compared to a research anti-TROP-2 antibody (clone 77220.11).

 To further extend the therapeutic benefit of AR47A6.4.2, the frequency and
20 localization of the antigen within various human cancer tissues and their corresponding normal tissue sections (10 colon cancers and 1 normal colon, 7 ovarian cancers and 1 normal ovary, 11 breast cancers and 3 normal breast, 14 lung cancers and 3 normal lung, 13 prostate cancers and 3 normal prostate, and 13 pancreatic cancers and 4 normal pancreas) was also previously determined (as disclosed in S.N. 11/709,676). AR47A6.4.2 showed moderate to
25 strong binding to 5/10 (50 percent), 6/7 (86 percent), 10/11 (91 percent), 11/14 (79 percent), 13/13 (100 percent) and 2/13 (15 percent) of colon, ovarian, breast, lung, prostate and pancreatic cancers, respectively. In addition, equivocal to weak binding was observed in 2/10 (20 percent), 1/11 (9 percent), 3/14 (21 percent), and 2/13 (15 percent) colon, breast, lung and pancreatic cancer sections, respectively. In all of the tested tumors, the binding was specific
30 for the tumor cells. For the corresponding normal tissues the antibody showed binding to 0/1,

WO 2008/144891

PCT/CA2008/000979

0/1, 3/3, 3/3, 3/3 and 4/4 of normal colon, ovary, breast, lung, prostate, and pancreatic tissues. However, the binding was predominantly to the epithelial tissues of the normal organs.

IHC studies were previously conducted to characterize the AR47A6.4.2 antigen cross reactivity in frozen normal tissues of various species (as disclosed in S.N. 11/709,676). AR47A6.4.2 showed no detectable binding to the tested mouse, rat, guinea pig, goat, sheep, hamster, chicken, cow, horse or pig normal tissues. For the normal rabbit and dog tissues, there was dissimilar binding to that observed in the corresponding human tissues. For the cynomolgus monkey normal tissues, AR47A6.4.2 showed similar tissue specificity as observed in the corresponding human normal tissues for all of the tested organs except for the ovary and testis in which no detectable binding was observed for the cynomolgus monkey sections. For the rhesus monkey normal tissues, AR47A6.4.2 showed similar tissue specificity as observed in the corresponding human normal tissues. It should be noted that the rhesus monkey normal tissue panel was smaller than what was tested for the cynomolgus monkey. Based on the staining profiles, both the cynomolgus and rhesus monkey have similar AR47A6.4.2 antigen distribution to human tissues.

To facilitate production of antibody chimera, the genes encoding the variable regions of both heavy and light chains were separately cloned and sequenced (as previously disclosed in S.N. 11/709,676).

The present invention describes the development and use of AR47A6.4.2, chimeric AR47A6.4.2 ((ch)AR47A6.4.2) and humanized variants, (hu)AR47A6.4.2. AR47A6.4.2 was identified by its effect in cytotoxic assays, in tumor growth models and in prolonging survival time in mammals suffering from cancerous disease. This invention represents an advance in the field of cancer treatment in that it describes, for the first time, reagents that bind specifically to an epitope or epitopes present on the target molecule, TROP-2, and that also have *in vitro* cytotoxic properties, as a naked antibody, against malignant tumor cells but not normal cells, and which also directly mediate, as a naked antibody, inhibition of tumor growth and extension of survival in *in vivo* models of human cancer. This is an advance in relation to any other previously described anti-TROP-2 antibody, since none have been shown to have similar properties. It also provides an advance in the field since it clearly demonstrates, and for the first time, the direct involvement of TROP-2 in events associated with growth and development of certain types of tumors. It also represents an

WO 2008/144891

PCT/CA2008/000979

advance in cancer therapy since it has the potential to display similar anti-cancer properties in human patients. A further advance is that inclusion of these antibodies in a library of anti-cancer antibodies will enhance the possibility of targeting tumors expressing different antigen markers by determination of the appropriate combination of different anti-cancer antibodies, to find the most effective in targeting and inhibiting growth and development of the tumors.

In all, this invention teaches the use of the AR47A6.4.2 antigen as a target for a therapeutic agent, that when administered can reduce the tumor burden of a cancer expressing the antigen in a mammal, and can also lead to a prolonged survival of the treated mammal. This invention also teaches the use of CDMAB (AR47A6.4.2, chimeric AR47A6.4.2 ((ch)AR47A6.4.2) and humanized variants, (hu)AR47A6.4.2), and its derivatives, and antigen binding fragments thereof, and cellular cytotoxicity inducing ligands thereof to target their antigen to reduce the tumor burden of a cancer expressing the antigen in a mammal, and lead to prolonged survival of the treated mammal. Furthermore, this invention also teaches the use of detecting the AR47A6.4.2 antigen in cancerous cells that can be useful for the diagnosis, prediction of therapy, and prognosis of mammals bearing tumors that express this antigen.

Accordingly, it is an objective of the invention to utilize a method for producing cancerous disease modifying antibodies (CDMAB) raised against cancerous cells derived from a particular individual, or one or more particular cancer cell lines, which CDMAB are cytotoxic with respect to cancer cells while simultaneously being relatively non-toxic to non-cancerous cells, in order to isolate hybridoma cell lines and the corresponding isolated monoclonal antibodies and antigen binding fragments thereof for which said hybridoma cell lines are encoded.

It is an additional objective of the invention to teach cancerous disease modifying antibodies, ligands and antigen binding fragments thereof.

It is a further objective of the instant invention to produce cancerous disease modifying antibodies whose cytotoxicity is mediated through antibody dependent cellular toxicity.

It is yet an additional objective of the instant invention to produce cancerous disease modifying antibodies whose cytotoxicity is mediated through complement dependent cellular toxicity.

WO 2008/144891

PCT/CA2008/000979

It is still a further objective of the instant invention to produce cancerous disease modifying antibodies whose cytotoxicity is a function of their ability to catalyze hydrolysis of cellular chemical bonds.

5 A still further objective of the instant invention is to produce cancerous disease modifying antibodies which are useful for in a binding assay for diagnosis, prognosis, and monitoring of cancer.

Other objects and advantages of this invention will become apparent from the following description wherein are set forth, by way of illustration and example, certain embodiments of this invention.

10 BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

15 Figure 1 demonstrates the effect of AR47A6.4.2 on tumor growth in a prophylactic human MDA-MB-231 breast cancer model. The vertical dashed lines indicate the period during which the antibody was intraperitoneally administered. Data points represent the mean $\pm$ SEM.

20 Figure 2 demonstrates the effect of AR47A6.4.2 on mouse survival in a prophylactic MDA-MB-231 breast cancer model. Data points represent the survival percentage.

Figure 3 demonstrates the effect of AR47A6.4.2 on mouse body weight in a prophylactic MDA-MB-231 breast adenocarcinoma model. Data points represent the mean $\pm$ SEM.

25 Figure 4 demonstrates the effect of AR47A6.4.2 on tumor growth in an established human PL45 pancreatic cancer model in a dose-response manner. The vertical dashed lines indicate the period during which the antibody was intraperitoneally administered. Data points represent the mean $\pm$ SEM.

Figure 5 demonstrates the effect of AR47A6.4.2 on mouse survival in an established PL45 pancreatic cancer model. Data points represent the survival percentage.

30 Figure 6 demonstrates the effect of AR47A6.4.2 on mouse body weight in an established PL45 pancreatic cancer model. Data points represent the mean $\pm$ SEM.

WO 2008/144891

PCT/CA2008/000979

Figure 7 tabulates an IHC comparison of AR47A6.4.2 on various human tumor and normal tissue sections from different tissue micro arrays.

Figure 8. Representative micrographs showing the binding pattern on breast tumor tissue obtained with AR47A6.4.2 (A) or the isotype control antibody (B) and on
5 prostate tumor tissue obtained with AR47A6.4.2 (C) or the isotype control antibody (D) and on pancreatic tumor tissue obtained with AR47A6.4.2 (E) or the isotype control antibody (F) from various human tumor tissue microarrays. Magnification is 400X for the breast and pancreatic tumor tissue and 200X for the prostate tumor tissue.

Figure 9. Representative micrographs showing the binding pattern obtained
10 with AR47A6.4.2 on ovarian tumor tissue (A) or ovarian normal tissue (B). AR47A6.4.2 showed strong binding to the tumor but not the corresponding normal tissue. Magnification is 200X.

Figure 10. List of kinases whose phosphorylation is affected by treatment of BxPC-3 cells treated with AR47A6.4.2 followed by serum and supplement stimulation.

Figure 11. List of secreted angiogenic factors affected by the treatment of
15 BxPC-3 cells treated with AR47A6.4.2.

Figure 12 demonstrates *in vitro* CDC activity of AR47A6.4.2 on two different human pancreatic cancer cell lines; PL45 and BxPC-3.

Figure 13. Binding of AR47A6.4.2 to CLIPS peptides (SEQ ID NOS 13-32,
20 respectively, in order of appearance) that were synthesized based on the TROP-2 amino acid sequence.

Figure 14. Amino acid sequence of TROP-2 (SEQ ID NO: 33). The discontinuous epitope recognized by AR47A6.4.2 is contained within the underlined sequences. Amino acid positions 1-274 represent the extracellular portion of TROP-2; amino
25 acid positions 275-290 represent the transmembrane portion of TROP-2 and amino acid positions 291-232 represent the intracellular portion of TROP-2.

Figure 15. Primers used in the PCR amplification of light chain (SEQ ID NOS: 34-52, respectively, in order of appearance).

Figure 16. Primers used in the PCR amplification of heavy chain (SEQ ID
30 NOS: 53-68, respectively, in order of appearance).

WO 2008/144891

PCT/CA2008/000979

Figure 17. Mouse AR47A6.4.2 VH Sequence (Nucleotide and amino acid sequences disclosed as SEQ ID NOS: 69-70, respectively).

Figure 18. Mouse AR47A6.4.2 VL Sequence (Nucleotide and amino acid sequences disclosed as SEQ ID NOS: 71-72, respectively).

5 Figure 19. Oligonucleotides used for the generation of chimeric and variant humanized AR47A6.4.2 VH sequences (SEQ ID NOS: 73-92, respectively, in order of appearance).

 Figure 20. Oligonucleotides used for the generation of chimeric and variant humanized AR47A6.4.2 VL sequences (SEQ ID NOS: 93-110, respectively, in order of
10 appearance).

 Figure 21. Light chain and heavy chain expression vectors.

 Figures 22A, 22B and 22C. Humanized AR47A6.4.2 VH variants. CDRs are underlined (SEQ ID NOS: 111-113, 10, 7 and 114, respectively, in order of appearance).

 Figures 23A, 23B and 23C. Humanized AR47A6.4.2 VL variants. CDRs are
15 underlined (SEQ ID NOS: 115, 9, 8 and 116-117, respectively, in order of appearance).

 Figure 24. Activities of humanized AR47A6.4.2 VH and VL variants.

 Figure 25. Summary of the binding affinity association rate constants (K_a) and dissociation rate constants (K_d) of murine AR47A6.4.2 and various variants of (hu)AR47A.6.4.2 to rhTROP-2.

20 DETAILED DESCRIPTION OF THE INVENTION

 In general, the following words or phrases have the indicated definition when used in the summary, description, examples, and claims.

 The term “antibody” is used in the broadest sense and specifically covers, for example, single monoclonal antibodies (including agonist, antagonist, and neutralizing
25 antibodies, de-immunized, murine, chimeric or humanized antibodies), antibody compositions with polyepitopic specificity, single-chain antibodies, diabodies, triabodies, immunoconjugates and antibody fragments (see below).

 The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies
30 comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed

WO 2008/144891

PCT/CA2008/000979

against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized
5 uncontaminated by other antibodies. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma (murine or human) method first described by Kohler *et al.*, *Nature*,
10 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No.4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example.

“Antibody fragments” comprise a portion of an intact antibody, preferably
15 comprising the antigen-binding or variable region thereof. Examples of antibody fragments include less than full length antibodies, Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; single-chain antibodies, single domain antibody molecules, fusion proteins, recombinant proteins and multispecific antibodies formed from antibody fragment(s).

20 An “intact” antibody is one which comprises an antigen-binding variable region as well as a light chain constant domain (C_L) and heavy chain constant domains, C_H1, C_H2 and C_H3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

25 Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different “classes”. There are five-major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α , δ , ϵ , γ , and
30 μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

WO 2008/144891

PCT/CA2008/000979

Antibody “effector functions” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc.

“Antibody-dependent cell-mediated cytotoxicity” and “ADCC” refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes *et al. PNAS* (USA) 95:652-656 (1998).

“Effector cells” are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source thereof, e.g. from blood or PBMCs as described herein.

The terms “Fc receptor” or “FcR” are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an

WO 2008/144891

PCT/CA2008/000979

immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see review M. in Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)).

FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991); Capel *et al.*,

5 *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995).

Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *Eur. J. Immunol.* 24:2429 (1994)).

10 “Complement dependent cytotoxicity” or “CDC” refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods*
15 202:163 (1996), may be performed.

The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three
20 segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The
25 hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit
30 various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

WO 2008/144891

PCT/CA2008/000979

The term “hypervariable region” when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a “complementarity determining region” or “CDR” (e.g. residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a “hypervariable loop” (e.g. residues 2632 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). “Framework Region” or “FR” residues are those variable domain residues other than the hypervariable region residues as herein defined. Papain digestion of antibodies produces two identical antigen-binding fragments, called “Fab” fragments, each with a single antigen-binding site, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

“Fv” is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site. The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have

WO 2008/144891

PCT/CA2008/000979

hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The “light chains” of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

“Single-chain Fv” or “scFv” antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term “diabodies” refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (V_H) connected to a variable light domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

The term “triabodies” or “trivalent trimers” refers to the combination of three single chain antibodies. Triabodies are constructed with the amino acid terminus of a V_L or V_H domain, i.e., without any linker sequence. A triabody has three Fv heads with the polypeptides arranged in a cyclic, head-to-tail fashion. A possible conformation of the triabody is planar with the three binding sites located in a plane at an angle of 120 degrees from one another. Triabodies can be monospecific, bispecific or trispecific.

An “isolated” antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

WO 2008/144891

PCT/CA2008/000979

An antibody “which binds” an antigen of interest, e.g. TROP-2 antigen, is one capable of binding that antigen with sufficient affinity such that the antibody is useful as a therapeutic or diagnostic agent in targeting a cell expressing the antigen. Where the antibody is one which binds TROP-2, it will usually preferentially bind TROP-2 as opposed to other receptors, and does not include incidental binding such as non-specific Fc contact, or binding to post-translational modifications common to other antigens and may be one which does not significantly cross-react with other proteins. Methods, for the detection of an antibody that binds an antigen of interest, are well known in the art and can include but are not limited to assays such as FACS, cell ELISA and Western blot.

As used herein, the expressions “cell”, “cell line”, and “cell culture” are used interchangeably, and all such designations include progeny. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. It will be clear from the context where distinct designations are intended.

“Treatment or treating” refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. Hence, the mammal to be treated herein may have been diagnosed as having the disorder or may be predisposed or susceptible to the disorder.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth or death. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal

WO 2008/144891

PCT/CA2008/000979

cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

A “chemotherapeutic agent” is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, carnomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziqune; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (“Ara-C”);

WO 2008/144891

PCT/CA2008/000979

- cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®, Aventis, Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16);
- 5 ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on
- 10 tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.
- 15 "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, mice, SCID or nude mice or strains of mice, domestic and farm animals, and zoo, sports, or pet animals, such as sheep, dogs, horses, cats, cows, etc. Preferably, the mammal herein is human.
- "Oligonucleotides" are short-length, single- or double-stranded
- 20 polydeoxynucleotides that are chemically synthesized by known methods (such as phosphotriester, phosphite, or phosphoramidite chemistry, using solid phase techniques such as described in EP 266,032, published 4 May 1988, or via deoxynucleoside H-phosphonate intermediates as described by Froehler *et al.*, *Nucl. Acids Res.*, 14:5399-5407, 1986. They are then purified on polyacrylamide gels.
- 25 In accordance with the present invention, "humanized" and/or "chimeric" forms of non-human (e.g. murine) immunoglobulins refer to antibodies which contain specific chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which results in the decrease of a human anti-mouse antibody (HAMA), human anti-chimeric antibody (HACA)
- 30 or a human anti-human antibody (HAHA) response, compared to the original antibody, and contain the requisite portions (e.g. CDR(s), antigen binding region(s), variable domain(s) and

WO 2008/144891

PCT/CA2008/000979

so on) derived from said non-human immunoglobulin, necessary to reproduce the desired effect, while simultaneously retaining binding characteristics which are comparable to said non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from the complementarity
5 determining regions (CDRs) of the recipient antibody are replaced by residues from the CDRs of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human FR residues. Furthermore, the humanized antibody may comprise residues which are found neither in the recipient
10 antibody nor in the imported CDR or FR sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR residues are those of a human immunoglobulin consensus
15 sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

“De-immunized” antibodies are immunoglobulins that are non-immunogenic, or less immunogenic, to a given species. De- immunization can be achieved through structural alterations to the antibody. Any de- immunization technique known to those skilled in the art
20 can be employed. One suitable technique for de- immunizing antibodies is described, for example, in WO 00/34317 published June 15, 2000.

An antibody which induces “apoptosis” is one which induces programmed cell death by any means, illustrated by but not limited to binding of annexin V, caspase activity, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation,
25 and/or formation of membrane vesicles (called apoptotic bodies).

As used herein “antibody induced cytotoxicity” is understood to mean the cytotoxic effect derived from the hybridoma supernatant or antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05, a humanized antibody
of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as
30 accession number 141205-05, a chimeric antibody of the isolated monoclonal antibody
produced by the hybridoma deposited with the IDAC as accession number 141205-05, antigen

WO 2008/144891

PCT/CA2008/000979

binding fragments, or antibody ligands thereof, which effect is not necessarily related to the degree of binding.

Throughout the instant specification, hybridoma cell lines, as well as the isolated monoclonal antibodies which are produced therefrom, are alternatively referred to by their internal designation, AR47A6.4.2 (murine), (ch)AR47A6.4.2 (chimeric), (hu)AR47A6.4.2 (humanized) or Depository Designation, IDAC 141205-05.

As used herein "antibody-ligand" includes a moiety which exhibits binding specificity for at least one epitope of the target antigen, and which may be an intact antibody molecule, antibody fragments, and any molecule having at least an antigen-binding region or portion thereof (i.e., the variable portion of an antibody molecule), e.g., an Fv molecule, Fab molecule, Fab' molecule, F(ab')₂ molecule, a bispecific antibody, a fusion protein, or any genetically engineered molecule which specifically recognizes and binds at least one epitope of the antigen bound by the isolated monoclonal antibody produced by the hybridoma cell line designated as IDAC 141205-05 (the IDAC 141205-05 antigen), a humanized antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05, a chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 and antigen binding fragments.

As used herein "cancerous disease modifying antibodies" (CDMAB) refers to monoclonal antibodies which modify the cancerous disease process in a manner which is beneficial to the patient, for example by reducing tumor burden or prolonging survival of tumor bearing individuals, and antibody-ligands thereof.

A "CDMAB related binding agent", in its broadest sense, is understood to include, but is not limited to, any form of human or non-human antibodies, antibody fragments, antibody ligands, or the like, which competitively bind to at least one CDMAB target epitope.

A "competitive binder" is understood to include any form of human or non-human antibodies, antibody fragments, antibody ligands, or the like which has binding affinity for at least one CDMAB target epitope.

Tumors to be treated include primary tumors and metastatic tumors, as well as refractory tumors. Refractory tumors include tumors that fail to respond or are resistant to

WO 2008/144891

PCT/CA2008/000979

treatment with chemotherapeutic agents alone, antibodies alone, radiation alone or combinations thereof. Refractory tumors also encompass tumors that appear to be inhibited by treatment with such agents but recur up to five years, sometimes up to ten years or longer after treatment is discontinued.

- 5 Tumors that can be treated include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. Examples of solid tumors, which can be accordingly treated, include breast carcinoma, lung carcinoma, colorectal carcinoma, pancreatic carcinoma, glioma and lymphoma. Some examples of such tumors include epidermoid tumors, squamous tumors, such as head and neck tumors, colorectal tumors,
- 10 prostate tumors, breast tumors, lung tumors, including small cell and non-small cell lung tumors, pancreatic tumors, thyroid tumors, ovarian tumors, and liver tumors. Other examples include Kaposi's sarcoma, CNS neoplasms, neuroblastomas, capillary hemangioblastomas, meningiomas and cerebral metastases, melanoma, gastrointestinal and renal carcinomas and sarcomas, rhabdomyosarcoma, glioblastoma, preferably glioblastoma multiforme, and
- 15 leiomyosarcoma.

As used herein "antigen-binding region" means a portion of the molecule which recognizes the target antigen.

- As used herein "competitively inhibits" means being able to recognize and bind a determinant site to which the monoclonal antibody produced by the hybridoma cell line designated as IDAC 141205-05, (the IDAC 141205-05 antibody), a humanized antibody of
- 20 the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05, a chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05, antigen binding fragments, or antibody ligands thereof, is directed using conventional reciprocal
- 25 antibody competition assays. (Belanger L., Sylvestre C. and Dufour D. (1973), Enzyme linked immunoassay for alpha fetoprotein by competitive and sandwich procedures. Clinica Chimica Acta 48, 15).

As used herein "target antigen" is the IDAC 141205-05 antigen or portions thereof.

- 30 As used herein, an "immunoconjugate" means any molecule or CDMAB such as an antibody chemically or biologically linked to cytotoxins, radioactive agents, cytokines,

WO 2008/144891

PCT/CA2008/000979

interferons, target or reporter moieties, enzymes, toxins, anti-tumor drugs or therapeutic agents. The antibody or CDMAB may be linked to the cytotoxin, radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, toxin, anti-tumor drug or therapeutic agent at any location along the molecule so long as it is able to bind its target. Examples of immunconjugates include antibody toxin chemical conjugates and antibody-toxin fusion proteins.

Radioactive agents suitable for use as anti-tumor agents are known to those skilled in the art. For example, ^{131}I or ^{211}At is used. These isotopes are attached to the antibody using conventional techniques (e.g. Pedley *et al.*, Br. J. Cancer 68, 69-73 (1993)). Alternatively, the anti-tumor agent which is attached to the antibody is an enzyme which activates a prodrug. A prodrug may be administered which will remain in its inactive form until it reaches the tumor site where it is converted to its cytotoxin form once the antibody complex is administered. In practice, the antibody-enzyme conjugate is administered to the patient and allowed to localize in the region of the tissue to be treated. The prodrug is then administered to the patient so that conversion to the cytotoxic drug occurs in the region of the tissue to be treated. Alternatively, the anti-tumor agent conjugated to the antibody is a cytokine such as interleukin-2 (IL-2), interleukin-4 (IL-4) or tumor necrosis factor alpha (TNF- α). The antibody targets the cytokine to the tumor so that the cytokine mediates damage to or destruction of the tumor without affecting other tissues. The cytokine is fused to the antibody at the DNA level using conventional recombinant DNA techniques. Interferons may also be used.

As used herein, a "fusion protein" means any chimeric protein wherein an antigen binding region is connected to a biologically active molecule, e.g., toxin, enzyme, fluorescent proteins, luminescent marker, polypeptide tag, cytokine, interferon, target or reporter moiety or protein drug.

The invention further contemplates CDMAB of the present invention to which target or reporter moieties are linked. Target moieties are first members of binding pairs. Anti-tumor agents, for example, are conjugated to second members of such pairs and are thereby directed to the site where the antigen-binding protein is bound. A common example of such a binding pair is avidin and biotin. In a preferred embodiment, biotin is conjugated to the target antigen of the CDMAB of the present invention, and thereby provides a target for

WO 2008/144891

PCT/CA2008/000979

an anti-tumor agent or other moiety which is conjugated to avidin or streptavidin.

Alternatively, biotin or another such moiety is linked to the target antigen of the CDMAB of the present invention and used as a reporter, for example in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin.

5 Detectable signal-producing agents are useful *in vivo* and *in vitro* for diagnostic purposes. The signal producing agent produces a measurable signal which is detectable by external means, usually the measurement of electromagnetic radiation. For the most part, the signal producing agent is an enzyme or chromophore, or emits light by fluorescence, phosphorescence or chemiluminescence. Chromophores include dyes which
10 absorb light in the ultraviolet or visible region, and can be substrates or degradation products of enzyme catalyzed reactions.

Moreover, included within the scope of the present invention is use of the present CDMAB *in vivo* and *in vitro* for investigative or diagnostic methods, which are well known in the art. In order to carry out the diagnostic methods as contemplated herein, the
15 instant invention may further include kits, which contain CDMAB of the present invention. Such kits will be useful for identification of individuals at risk for certain type of cancers by detecting over-expression of the CDMAB's target antigen on cells of such individuals.

Diagnostic Assay Kits

It is contemplated to utilize the CDMAB of the present invention in the form
20 of a diagnostic assay kit for determining the presence of a tumor. The tumor will generally be detected in a patient based on the presence of one or more tumor-specific antigens, e.g. proteins and/or polynucleotides which encode such proteins in a biological sample, such as blood, sera, urine and/or tumor biopsies, which samples will have been obtained from the patient.

25 The proteins function as markers which indicate the presence or absence of a particular tumor, for example a colon, breast, lung or prostate tumor. It is further contemplated that the antigen will have utility for the detection of other cancerous tumors. Inclusion in the diagnostic assay kits of binding agents comprised of CDMABs of the present invention, or CDMAB related binding agents, enables detection of the level of antigen that
30 binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level of mRNA encoding a tumor protein, which is also indicative of the presence

WO 2008/144891

PCT/CA2008/000979

or absence of a cancer. In order for the binding assay to be diagnostic, data will have been generated which correlates statistically significant levels of antigen, in relation to that present in normal tissue, so as to render the recognition of binding definitively diagnostic for the presence of a cancerous tumor. It is contemplated that a plurality of formats will be useful for the diagnostic assay of the present invention, as are known to those of ordinary skill in the art, for using a binding agent to detect polypeptide markers in a sample. For example, as illustrated in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. Further contemplated are any and all combinations, permutations or modifications of the afore-described diagnostic assay formats.

The presence or absence of a cancer in a patient will typically be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

In an illustrative embodiment, it is contemplated that the assay will involve the use of a CDMAB based binding agent immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Illustrative detection reagents may include a CDMAB based binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. In an alternative embodiment, it is contemplated that a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. Indicative of the reactivity of the sample with the immobilized binding agent, is the extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent. Suitable polypeptides for use within such assays include full length tumor-specific proteins and/or portions thereof, to which the binding agent has binding affinity.

The diagnostic kit will be provided with a solid support which may be in the form of any material known to those of ordinary skill in the art to which the protein may be attached. Suitable examples may include a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass,

WO 2008/144891

PCT/CA2008/000979

fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Pat. No. 5,359,681.

It is contemplated that the binding agent will be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. The term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment, which, in the context of the present invention, may be a direct linkage between the agent and functional groups on the support, or may be a linkage by way of a cross-linking agent. In a preferred, albeit non-limiting embodiment, immobilization by adsorption to a well in a microtiter plate or to a membrane is preferable. Adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time may vary with temperature, and will generally be within a range of between about 1 hour and about 1 day.

Covalent attachment of binding agent to a solid support would ordinarily be accomplished by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12 A13).

It is further contemplated that the diagnostic assay kit will take the form of a two-antibody sandwich assay. This assay may be performed by first contacting an antibody, e.g. the instantly disclosed CDMAB that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

WO 2008/144891

PCT/CA2008/000979

In a specific embodiment, it is contemplated that once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support will be blocked, via the use of any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20TM (Sigma Chemical Co., St. Louis, Mo.). The immobilized antibody would then be incubated with the sample, and polypeptide would be allowed to bind to the antibody. The sample could be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (i.e., incubation time) would be selected to correspond to a period of time sufficient to detect the presence of polypeptide within a sample obtained from an individual with the specifically selected tumor. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95 percent of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time.

It is further contemplated that unbound sample would then be removed by washing the solid support with an appropriate buffer. The second antibody, which contains a reporter group, would then be added to the solid support. Incubation of the detection reagent with the immobilized antibody-polypeptide complex would then be carried out for an amount of time sufficient to detect the bound polypeptide. Subsequently, unbound detection reagent would then be removed and bound detection reagent would be detected using the reporter group. The method employed for detecting the reporter group is necessarily specific to the type of reporter group selected, for example for radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

In order to utilize the diagnostic assay kit of the present invention to determine the presence or absence of a cancer, such as prostate cancer, the signal detected from the reporter group that remains bound to the solid support would generally be compared to a

WO 2008/144891

PCT/CA2008/000979

signal that corresponds to a predetermined cut-off value. For example, an illustrative cut-off value for the detection of a cancer may be the average mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is about three standard deviations above the predetermined cut-off value would be considered positive for the cancer. In an alternate embodiment, the cut-off value might be determined by using a Receiver Operator Curve, according to the method of Sackett *et al.*, Clinical Epidemiology. A Basic Science for Clinical Medicine, Little Brown and Co., 1985, p. 106-7. In such an embodiment, the cut-off value could be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100 percent-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (i.e., the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

It is contemplated that the diagnostic assay enabled by the kit will be performed in either a flow-through or strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the binding agent-polypeptide complex as a solution containing the second binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound will be immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent. Concentration of the second binding agent at the area of immobilized antibody indicates the presence of a cancer. Generation of a pattern, such as a line, at the binding site, which can be read visually, will be indicative of a positive test. The absence of such a pattern indicates a negative result. In general, the amount of binding agent immobilized on the membrane is

WO 2008/144891

PCT/CA2008/000979

selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferred binding agents for use in the instant diagnostic assay are the instantly disclosed antibodies, antigen-binding fragments thereof, and
5 any CDMAB related binding agents as herein described. The amount of antibody immobilized on the membrane will be any amount effective to produce a diagnostic assay, and may range from about 25 nanograms to about 1 microgram. Typically such tests may be performed with a very small amount of biological sample.

Additionally, the CDMAB of the present invention may be used in the
10 laboratory for research due to its ability to identify its target antigen.

In order that the invention herein described may be more fully understood, the following description is set forth.

The present invention provides CDMAB (i.e., IDAC 141205-05 CDMAB, a humanized antibody of the isolated monoclonal antibody produced by the hybridoma
15 deposited with the IDAC as accession number 141205-05, a chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05, antigen binding fragments, or antibody ligands thereof) which specifically recognize and bind the IDAC 141205-05 antigen.

The CDMAB of the isolated monoclonal antibody produced by the hybridoma
20 deposited with the IDAC as accession number 141205-05 may be in any form as long as it has an antigen-binding region which competitively inhibits the immunospecific binding of the isolated monoclonal antibody produced by hybridoma IDAC 141205-05 to its target antigen. Thus, any recombinant proteins (e.g., fusion proteins wherein the antibody is combined with a second protein such as a lymphokine or a tumor inhibitory growth factor) having the same
25 binding specificity as the IDAC 141205-05 antibody fall within the scope of this invention.

In one embodiment of the invention, the CDMAB is the IDAC 141205-05 antibody.

In other embodiments, the CDMAB is an antigen binding fragment which may be a Fv molecule (such as a single-chain Fv molecule), a Fab molecule, a Fab' molecule, a
30 F(ab')₂ molecule, a fusion protein, a bispecific antibody, a heteroantibody or any recombinant molecule having the antigen-binding region of the IDAC 141205-05 antibody. The CDMAB

WO 2008/144891

PCT/CA2008/000979

of the invention is directed to the epitope to which the IDAC 141205-05 monoclonal antibody is directed.

The CDMAB of the invention may be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Chemical modification may also be possible. Modification by direct mutation, methods of affinity maturation, phage display or chain shuffling may also be possible.

Affinity and specificity can be modified or improved by mutating CDR and/or phenylalanine tryptophan (FW) residues and screening for antigen binding sites having the desired characteristics (e.g., Yang *et al.*, J. Mol. Biol., (1995) 254: 392-403). One way is to randomize individual residues or combinations of residues so that in a population of otherwise identical antigen binding sites, subsets of from two to twenty amino acids are found at particular positions. Alternatively, mutations can be induced over a range of residues by error prone PCR methods (e.g., Hawkins *et al.*, J. Mol. Biol., (1992) 226: 889-96). In another example, phage display vectors containing heavy and light chain variable region genes can be propagated in mutator strains of *E. coli* (e.g., Low *et al.*, J. Mol. Biol., (1996) 250: 359-68). These methods of mutagenesis are illustrative of the many methods known to one of skill in the art.

Another manner for increasing affinity of the antibodies of the present invention is to carry out chain shuffling, where the heavy or light chain are randomly paired with other heavy or light chains to prepare an antibody with higher affinity. The various CDRs of the antibodies may also be shuffled with the corresponding CDRs in other antibodies.

Derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the IDAC 141205-05 antigen or portions thereof.

These amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

For example, it is a well-established principle of protein chemistry that certain amino acid substitutions, entitled "conservative amino acid substitutions," can frequently be made in a protein without altering either the conformation or the function of the protein.

WO 2008/144891

PCT/CA2008/000979

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

EXAMPLE 1

In vivo Tumor Experiment with human MDA-MB-231 Breast Cancer Cells

AR47A6.4.2 had previously demonstrated (as disclosed in S.N. 11/709,676) efficacy in a MCF-7 human breast cancer xenograft model. To extend this finding AR47A6.4.2 was tested in a MDA-MB-231 human breast cancer xenograft model which differs from the MCF-7 model and is Her2/neu negative, estrogen and progesterone receptor negative. With reference to Figures 1, 2 and 3, 8 to 10 week old female SCID mice were implanted with 5 million human breast cancer cells (MDA-MB-231) in 100 microliters PBS solution injected subcutaneously in the right flank of each mouse. The mice were randomly divided into 2 treatment groups of 10. One day after implantation, 20 mg/kg of AR47A6.4.2 test antibody or buffer control was administered intraperitoneally to each cohort in a volume of 300 microliters after dilution from the stock concentration with a diluent that contained 2.7 mM KCl, 1 mM KH₂PO₄, 137 mM NaCl and 20 mM Na₂HPO₄. The antibody and control samples were then administered once per week for the first two weeks and twice per week for another 3 weeks. Tumor growth was measured once per week with calipers. The treatment was completed after 8 doses of antibody. Body weights of the animals were recorded at the same time as tumor measurement. All animals were euthanized according to CCAC guidelines at the end of the study once they had reached endpoint.

AR47A6.4.2 significantly inhibited tumor growth in the MDA-MB-231 *in vivo* prophylactic model of human breast cancer. Treatment with ARIUS antibody AR47A6.4.2

WO 2008/144891

PCT/CA2008/000979

reduced the growth of MDA-MB-231 tumors by 91.9 percent ($p < 0.00001$, t-test), compared to the buffer treated group, as determined on day 55, 5 days after the last dose of antibody (Figure 1). All mice in the control group were removed from the study, due to reaching endpoint, at day 108, 58 days after the last dose of antibody. However, 90 percent of the mice in AR47A6.4.2-treated group were still alive at that time (Figure 2).

There were no obvious clinical signs of toxicity throughout the study. Body weight measured at weekly intervals was a surrogate for well-being and failure to thrive. The mean body weight increased in all groups over the duration of the study (Figure 3). The mean weight gain between day 0 and day 55 was 1.3 g (6.9 percent) in the control group and 1.8 g (9.3 percent) in the AR47A6.4.2-treated group. There were no significant differences between the groups during the treatment period. In summary, AR47A6.4.2 was well-tolerated and significantly inhibited the tumor growth in a human breast cancer xenograft model.

EXAMPLE 2

In vivo Tumor Experiment with human PL45 Pancreatic Cancer Cells

AR47A6.4.2 had previously demonstrated (as disclosed in S.N. 11/709,676) efficacy in a preventative PL45 human pancreatic cancer xenograft model. To determine effective dose levels AR47A6.4.2 was tested in an established PL45 model at various doses. With reference to Figures 4, 5, and 6, 8 to 10 week old female SCID mice were implanted with 4 million human pancreatic cancer cells (PL45) in 100 microliters PBS solution injected subcutaneously in the scruff of the neck. The mice were randomly divided into 5 treatment groups of 10 when the average mouse tumor volume reached approximately 100 mm^3 . On day 32 after implantation, 20, 10, 2, or 0.2 mg/kg of AR47A6.4.2 test antibody or buffer control was administered intraperitoneally to each cohort in a volume of 300 microliters after dilution from the stock concentration with a diluent that contained 2.7 mM KCl, 1 mM KH_2PO_4 , 137 mM NaCl and 20 mM Na_2HPO_4 . The antibody and control samples were then administered three times per week for the duration of the study. Tumor growth was measured about every 4-7 days with calipers. The study was completed after 10 doses of antibody. Body weights of the animals were recorded once per week for the duration of the study. All animals were euthanized according to CCAC guidelines at the end of the study once they had reached endpoint.

WO 2008/144891

PCT/CA2008/000979

AR47A6.4.2 demonstrated dose-dependent tumor growth inhibition in the PL45 *in vivo* established model of human pancreatic cancer. Treatment with ARIUS antibody AR47A6.4.2 reduced the growth of PL45 tumors by 48.9 percent ($p=0.0001$, t-test), 34.6 percent ($p=0.0011$, t-test), 17.4 percent ($p=0.1938$, t-test) and 4.7 percent ($p=0.7065$, t-test) at a dose of 20, 10, 2 and 0.2 mg/kg respectively, compared to the buffer treated group, as determined at day 67, 14 days after last dose of treatment (Figure 4). This was when almost all mice in the control and antibody-treated groups were still alive. The survival for all groups was monitored until day 88, 35 days after the last dose of treatment. At this time point, only 20 percent (2/10) of the mice in the control group were still alive while 60 percent (6/10), 40 percent (4/10) and 90 percent (9/10) of the mice in the AR47A6.4.2-treated group at doses of 20, 10, and 2 mg/kg respectively were still alive (Figure 5).

There were no obvious clinical signs of toxicity throughout the study. Body weight measured at weekly intervals was a surrogate for well-being and failure to thrive. The mean body weight increased in all groups over the duration of the study (Figure 6). The mean weight gain between day 32 and day 67 was 0.8 g (4.1 percent) in the control group and 1.5 g (7.6 percent), 1.5 g (7.6 percent), 1.2 g (6.3 percent) or 1.9 g (9.5 percent) in the AR47A6.4.2-treated group at doses of 20, 10, 2 and 0.2 mg/kg, respectively. There was no significant difference between the groups during the treatment period.

In summary, AR47A6.4.2 was well-tolerated and significantly inhibited the tumor growth in a dose dependent manner in this established human pancreatic cancer xenograft model at 20 and 10 mg/kg. Mice in the AR47A6.4.2-treated group at doses greater than 2 mg/kg also demonstrated a significant survival benefit. *In toto*, this data demonstrates that AR47A6.4.2 is effective in the treatment of human cancer in a dose dependent manner.

EXAMPLE 3

Human Normal and Multi-Tumor Tissue Staining

Additional IHC studies (previous studies were disclosed in S.N.11/709,676) were conducted to further characterize the AR47A6.4.2 antigen prevalence in human cancers. Slides were transferred from -80°C to -20°C . After one hour, the slides were postfixfixed for 10 minutes in cold (-20°C) acetone and then allowed to come to room temperature. Slides were rinsed in 4°C cold phosphate buffered saline (PBS) 3 times for 2 minutes each followed by blocking endogenous peroxidase activity with washing in 3 percent hydrogen peroxide for

WO 2008/144891

PCT/CA2008/000979

10 minutes. Slides were then rinsed in PBS 3 times for 5 minutes followed by incubation in Universal blocking solution (Dako, Toronto, Ontario) for 5 minutes at room temperature. AR47A6.4.2, anti-human muscle actin (Clone HHF35, Dako, Toronto, Ontario), anti-cytokeratin 7 clone OV-TL 12/30 (Dako, Toronto, Ontario), anti-TROP-2 clone 77220.11 (R&D System Inc., MN, USA) or isotype control antibody (directed towards *Aspergillus niger* glucose oxidase, an enzyme which is neither present nor inducible in mammalian tissues; Dako, Toronto, Ontario) were diluted in antibody dilution buffer (Dako, Toronto, Ontario) to its working concentration of 5 micrograms/mL for each antibody, except for anti-actin which was 0.5 microgram/mL, anti-cytokeratin 7 was ready to use and commercial anti-TROP-2 was 1 microgram/mL, and incubated for 1 hour at room temperature. The slides were washed with PBS 3 times for 2 minutes each. Immunoreactivity of the primary antibodies was detected/visualized with HRP conjugated secondary antibodies as supplied (Dako Envision System, Toronto, Ontario) for 30 minutes at room temperature. Following this step the slides were washed with PBS 3 times for 5 minutes each and a color reaction developed by adding DAB (3,3'-diaminobenzidine tetrahydrachloride, Dako, Toronto, Ontario) chromogen substrate solution for immunoperoxidase staining for 10 minutes at room temperature. Washing the slides in tap water terminated the chromogenic reaction. Following counterstaining with Meyer's Hematoxylin (Sigma Diagnostics, Oakville, ON), the slides were dehydrated with graded ethanols (75-100 percent) and cleared with xylene. Using mounting media (Dako Faramount, Toronto, Ontario) the slides were coverslipped. For the pancreatic array (Tri Star, Rockville, MD) the same protocol was followed except for the following modifications. The tissue sections were initially air dried at room temperature for 2 hours and air dried again for 30 minutes after fixation with acetone. The endogenous hydrogen peroxide was blocked using 3 percent hydrogen peroxide in methanol for 15 minutes; this step was done after the primary antibody incubation.

Slides were microscopically examined using an Axiovert 200 (Zeiss Canada, Toronto, ON) and digital images acquired and stored using Northern Eclipse Imaging Software (Mississauga, ON). Results were read, scored and interpreted by a histopathologist.

Figure 7 presents a summary of the results of AR47A6.4.2 staining of panels of human tumors and corresponding normal tissues (11 colon cancers and 2 normal colon, 8 ovarian cancers and 2 normal ovary, 12 breast cancers and 4 normal breast, 15 lung cancers

WO 2008/144891

PCT/CA2008/000979

and 4 normal lung, 14 prostate cancers and 4 normal prostate and 14 pancreatic cancers and 5 normal pancreas). These tissues were distributed on four tissue microarrays (Tri Star, Rockville, MD). The antibody showed moderate to strong binding to 6/11 (55 percent), 6/8 (75 percent), 11/12 (92 percent), 12/15 (80 percent), 14/14 (100 percent) and 3/14 (21 percent) of colon, ovarian, breast, lung, prostate and pancreatic cancers, respectively (Figures 8 and 9). In addition, equivocal to weak binding was observed in 2/11 (18 percent), 1/12 (8 percent), 3/15 (20 percent) and 2/14 (14 percent) to colon, breast, lung and pancreatic cancers, respectively. The binding was specific to tumor cells in all tested tumors with over expression in tumors versus normal in some tissues. For corresponding normal tissues the antibody showed binding to 0/2, 0/2, 4/4, 4/4, 4/4 and 5/5 of normal colon, ovary, breast, lung, prostate and pancreatic tissues (Figure 9). The binding was predominantly to epithelial tissues of those organs. Anti-cytokeratin-7 or anti-actin, used as a positive antibody control, showed the expected positive binding to epithelial tissues and muscular tissues, respectively. The IgG isotype negative control showed negative binding to the tested tissues.

EXAMPLE 4

Phospho-MAPK (Mitogen-Activated Protein Kinase) Proteome Profiler Blots

To identify intracellular signaling molecules affected by AR47A6.4.2 treatment, lysates from cells treated with AR47A6.4.2 were screened using a proteome profiler human phospho-MAPK antibody array (ARY002, R&D Systems Inc., Minneapolis, MN).

Treatment and preparation of cells

Previous work (as disclosed in S.N. 11/709,676) demonstrated *in vivo* efficacy of AR47A6.4.2 in a pancreatic cancer xenograft model using BxPC-3 cells grown in severe combined immunodeficient (SCID) mice. Accordingly, screening for activation of intracellular signaling molecules was done using the BxPC-3 cell line. BxPC-3 cells were grown to near confluence, washed with phosphate buffered saline (PBS) and then starved in serum and supplement-deficient media for 4 hours at 37°C. After this, AR47A6.4.2 (20 micrograms/mL) or 8A3B.6 (isotype control; IgG2a) (20 micrograms/mL) was added to the cells and allowed to bind for 20 minutes at 4°C. Cells were then stimulated by adding fetal bovine serum (FBS), L-glutamine and sodium pyruvate to the cells to give a final

WO 2008/144891

PCT/CA2008/000979

concentration of 10 percent FBS, 1 percent L-glutamine, and 1 percent sodium pyruvate. The cells were placed in an incubator at 37°C and the cell lysate was collected 1 hour after stimulation. Lysates were collected by washing the cells twice with PBS and harvesting in lysis buffer 6 (Part no. 895561: R&D Systems antibody array ARY002). The cells were
5 resuspended by pipetting, transferred to a 1.5 mL microfuge tube and mixed by rotation at 4°C for 30 minutes. Lysates were then centrifuged at 14000xg for five minutes and the supernatant was transferred to a clean tube. Protein concentration was determined by the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL).

Human phospho-MAPK antibody array

10 The human phospho-MAPK antibody array was screened against BxPC-3 cell lysates according to the protocol described by the manufacturer (Fourth Revision, May 2006, R&D Systems antibody array ARY002). Briefly, each human phospho-MAPK profiler membrane was prepared by incubating in 1.5 mL of array buffer 1 (Part no. 895477: R&D Systems antibody array ARY002) for 1 hour on a rocking platform shaker. For each
15 treatment, 150 micrograms of total protein was diluted with lysis buffer 6 to give a final volume of 250 microliters and mixed with 1.25 mL of array buffer 1. This mixture was added to the prepared profiler membranes and incubated at 4°C overnight on a rocking platform shaker. Each membrane was then washed 3 times in 1X wash buffer (diluted in purified distilled water from a 25X stock, (Part no. 895003: R&D Systems antibody array ARY002))
20 and incubated for 2 hours with 1.5 mL of anti-phospho-MAPK detection antibody cocktail (containing biotinylated phospho-specific antibodies) (Part no. 893051: R&D Systems antibody array ARY002) prepared in 1X array buffer 2/3 (5X array buffer 2, Part no. 895478: R&D Systems antibody array ARY002; array buffer 3, Part no. 895008: R&D Systems antibody array ARY002). The membranes were washed 3 times in 1X wash buffer and
25 incubated for 30 minutes with 1.5 mL of Streptavidin-HRP (Part no. 890803: R&D Systems antibody array ARY002) diluted 1:2000 in 1X array buffer 2/3. The membranes were washed 3 times in 1X wash buffer and exposed to ECL plus Western detection reagents (GE Healthcare, Life Sciences, Piscataway, NJ) for developing. Membranes were exposed to chemiluminescent film (Kodak, Rochester, NY) and developed using an X-ray medical
30 processor. Phospho-MAPK array data on developed X-ray films were quantitated by scanning the film on a transmission-mode scanner and analyzing the array image file using

WO 2008/144891

PCT/CA2008/000979

Image J analysis software (Image J 1.37v, NIH). For each kinase, the average pixel density for corresponding duplicate spots was calculated and subtracted from background signal using the pixel density of a clear area on the membrane. The average normalized pixel density of AR47A6.4.2-treated samples was divided by the average normalized pixel density of 8A3B.6-treated samples for each corresponding phospho-protein target to obtain a ratio of relative change. The percent reduction of phospho-protein signal was determined by subtracting the ratio of relative change from 1 and multiplying by 100.

The result from phospho-MAPK array membranes incubated with AR47A6.4.2 or 8A3B.6 is shown in Figure 10. Compared with 8A3B.6, AR47A6.4.2 suppressed the phosphorylation of p42/p44 MAPK/extracellular signal-regulated kinases (ERK) (ERK1 (32 percent) and ERK2 (20 percent), Akt/ protein kinase B (PKB) (Akt1/PKBalpha (15 percent), Akt2/PKBbeta (18 percent) and Akt3/PKBgamma (27 percent)) in BxPC-3 cells stimulated with serum and supplements. These kinases are involved in intracellular signaling pathways that can affect cell proliferation, growth and survival. That AR47A6.4.2 can reduce the phosphorylation of these kinases upon stimulation by serum and supplements suggest that AR47A6.4.2 may block cell growth and survival of cancer cells through these kinases and their related intracellular signaling pathways.

EXAMPLE 5

TranSignal™ Angiogenesis Antibody Array of Conditioned Media

To determine whether AR47A6.4.2 treatment can affect secretion of angiogenic factors, conditioned media from cells treated with AR47A6.4.2 were screened using an angiogenesis array (MA6310, Panomics Inc., Redwood City, CA).

Treatment and preparation of cells

As disclosed in S.N. 11/709,676, *in vivo* efficacy of AR47A6.4.2 was demonstrated in a pancreatic cancer xenograft model using BxPC-3 cells grown in severe combined immunodeficient (SCID) mice. Accordingly, screening for secretion of angiogenic factors was performed using the BxPC-3 cell line. BxPC-3 cells were grown to near confluence, washed with phosphate buffered saline (PBS) and then replenished with 2 mL of serum-deficient media. AR47A6.4.2 (20 micrograms/mL) or 8A3B.6 (isotype control; IgG2a) (20 micrograms/mL) was added to the cells and allowed to bind for 20 minutes at 4°C. The cells were placed in an incubator at 37°C for 24 hours. After 24 hours, the conditioned media

WO 2008/144891

PCT/CA2008/000979

from each culture was collected and centrifuged at 1200 revolutions per minute (rpm) for 5 minutes to remove cells or cell debris.

TranSignal™ Angiogenesis Antibody Array

TranSignal™ angiogenesis antibody arrays were screened with BxPC-3 cell
5 conditioned media according to the protocol described by the manufacturer (Released
10/07/03, Revised 08/03/05; MA6310, Panomics Inc., Redwood City, CA). Briefly, each
TranSignal™ angiogenesis antibody array membrane was prepared by incubating in 3 mL of
1X Blocking Buffer (MA6310, Panomics Inc., Redwood City, CA) for 1 hour at room
temperature on a rocking platform shaker. The membranes were then washed twice with 4 mL
10 of 1X Wash Buffer II (20X Wash Buffer II diluted to 1X with distilled water (dH₂O),
MA6310, Panomics Inc., Redwood City, CA). After washing, the entire conditioned media
collected (2 mL) was added to a membrane and incubated overnight at 4°C on a rocking
platform shaker. The membranes were then washed 3X using 4 mL of 1X Wash Buffer I (20X
wash Buffer diluted to 1X in dH₂O, MA6310, Panomics Inc., Redwood City, CA). This was
15 followed by 3 washes with 4 mL of 1X Wash Buffer II (MA6310, Panomics Inc., Redwood
City, CA). The membranes were then incubated for 1 hour in 1.5 mL of Biotin-Conjugated
Anti-Angiogenesis Mix (MA6310, Panomics Inc., Redwood City, CA) on a rocking platform
shaker, washed 3X using 4 mL of 1X Wash Buffer I (MA6310, Panomics Inc., Redwood
City, CA) followed by 3 washes with 4 mL of 1X Wash Buffer II (MA6310, Panomics Inc.,
20 Redwood City, CA). Streptavidin-HRP, diluted 1:1000 in 1X Wash Buffer II, was added to
the membranes and incubated for 1 hour at room temperature, washed again 3X using 4 mL of
1X Wash Buffer I (MA6310, Panomics Inc., Redwood City, CA) followed by 3 washes with
4 mL of 1X Wash Buffer II (MA6310, Panomics Inc., Redwood City, CA) and developed
using Hyperfilm™ ECL reagent (RPN3114K, GE Healthcare, Life Sciences, Piscataway, NJ).
25 The membranes were exposed to chemiluminescent film (Kodak, Rochester, NY) and
developed using an X-ray medical processor. Angiogenesis array data on developed X-ray
films were quantitated by scanning the film on a transmission-mode scanner and analyzing the
array image file using Image J analysis software (Image J 1.37v, NIH). For each secreted
factor, the average pixel density for corresponding duplicate spots was calculated and
30 subtracted from background signal using the pixel density of a clear area on the membrane.
The average normalized pixel density of AR47A6.4.2-treated samples was divided by the

WO 2008/144891

PCT/CA2008/000979

average normalized pixel density of 8A3B.6-treated samples for each corresponding target to obtain a ratio of relative change. The percent reduction of signal was determined by subtracting the ratio of relative change from 1 and multiplying by 100.

The results from TranSignal™ angiogenesis antibody array membranes incubated with AR47A6.4.2 or 8A3B.6 are shown in Figure 11. Compared with 8A3B.6, AR47A6.4.2 suppressed the secretion of the potent angiogenic factors vascular endothelial growth factor (VEGF) and placental growth factor (PLGF). This observation suggests that treatment of the BxPC-3 pancreatic cancer cell line with AR47A6.4.2 may result in the inhibition of tumor growth and survival of the cancer cells by reducing the secretion of factors by the cells that promote blood vessel growth in solid tumors. This finding demonstrates a possible mechanism of action for AR47A6.4.2.

EXAMPLE 6

Demonstration of *in vitro* Complement-Dependent Cytotoxicity (CDC) activity of the Anti-TROP-2 antibody AR47A6.4.2

Therapeutic efficacy of murine AR47A6.4.2 has been previously demonstrated in xenograft tumor models of human pancreatic cancer (as disclosed in S.N. 11/709,676 and in Example 2 above). In order to elucidate its mechanisms of action, AR47A6.4.2 was evaluated *in vitro* for CDC activity on two pancreatic cancer cell lines, PL45 and BxPC-3. Established monolayers of PL45 and BxPC-3 cells, two days post plating, were treated with antibody (2, 0.2 and 0.02 micrograms/mL) and allowed to bind for one hour (37° C; 4 percent CO₂). Rabbit complement was then added to yield a final concentration of 10 percent (v/v) and cells were allowed to incubate for an additional 3 hours at 37 °C, 4 percent CO₂. CDC activity was evaluated by measuring the residual lactate dehydrogenase present in uncompromised cells using the Cytotox 96™ kit (Promega Corporation, Madison, WI, USA). Each test antibody was evaluated in triplicate and the results were expressed as percent cytotoxicity, as compared to rabbit complement only treated wells, using the following equation: percent Cytotoxicity = 100-[Test Antibody_(492nm)-Background_(492nm)]/Complement Only_(492nm) - Background_(492nm)]*100.

The results from this experiment (Figure 12) demonstrate that the anti-TROP-2 antibody AR47A6.4.2 was capable of recruiting rabbit complement in a dose-dependent manner in both pancreatic cancer target cell lines (PL45 and BxPC-3). CDC activity was not

WO 2008/144891

PCT/CA2008/000979

observed in these cell lines when treated with isotype-matched control at the highest concentration (20 micrograms/mL). This data demonstrates that AR47A6.4.2 is capable of complement recruitment *in vitro* and may be one of the mechanisms by which this antibody is exerting its effects *in vivo*.

5 EXAMPLE 7

Epitope Mapping

Epitope mapping experiments were carried out in order to determine the region(s) of the TROP-2 molecule that are recognized by AR47A6.4.2. Overlapping 15-mer peptides were synthesized based on the amino acid sequence of TROP-2 using standard

10 Fmoc-chemistry and deprotected using trifluoric acid with scavengers. Additionally, up to 30-mer double-looped, triple-looped and sheet-like peptides were synthesized on chemical scaffolds in order to reconstruct discontinuous epitopes of the TROP-2 molecule, using Chemically Linked Peptides on Scaffolds (CLIPS) technology. The looped peptides were synthesized containing a dicysteine, which was cyclized by treating with alpha,alpha'-

15 dibromoxylene and the size of the loop was varied by introducing cysteine residues at variable spacing. If other cysteines besides the newly introduced cysteines were present, they were replaced by an alanine. The side-chains of the multiple cysteines in the peptides were coupled to CLIPS templates by reacting onto credit-card format polypropylene PEPSCAN cards (455 peptide formats/card) with an 0.5 mM solution of CLIPS template such as 1,3-bis

20 (bromomethyl) benzene in ammonium bicarbonate (20 mM, pH 7.9)/acetonitrile (1:1 (v/v)). The cards were gently shaken in the solution for 30 to 60 minutes while completely covered in solution. Finally, the cards were washed extensively with excess H₂O and sonicated in disrupt-buffer containing 1 percent SDS/0.1 percent beta-mercaptoethanol in PBS (pH 7.2) at 70°C for 30 minutes, followed by sonication in H₂O for another 45 minutes. In total, 3579

25 different peptides were synthesized. The binding of antibody to each peptide was tested in a PEPSCAN-based ELISA. The 455-well credit card format polypropylene cards containing the covalently linked peptides were incubated with primary antibody solution consisting of 10 micrograms/mL of AR47A6.4.2 diluted in blocking solution (5% horse-serum (v/v), 5% ovalbumin (w/v) and 1% Tween 80 in PBS) overnight. After washing with PBS containing

30 1% Tween 80, the peptides were incubated with a 1/1000 dilution of rabbit anti-mouse

WO 2008/144891

PCT/CA2008/000979

antibody peroxidase in blocking solution (5 percent horse-serum (v/v), 5 percent ovalbumin (w/v) and Tween 80 in PBS) for one hour at 25°C. After washing with PBS containing 1 percent Tween 80, the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 2 microliters of 3 percent H₂O₂ were added. After one hour, the color development was measured. The color development was quantified on a logarithmic scale of 0 to 4000 with a charge coupled device (CCD)-camera and an image processing system.

The twenty peptides (out of 3579) to which AR47A6.4.2 bound most strongly are listed in Figure 13. Two amino acid hotspots were identified by analyzing the composition of the peptides to which AR47A6.4.2 bound. The hotspot amino acid sequence LFRERYRLH (SEQ ID NO: 11) is present in peptide numbers 1, 2, 7, 8, 12, 16, 17 and 18 and the hotspot amino acid sequence QVERTLIYY (SEQ ID NO: 12) is present in peptide numbers 11 and 20. Peptides 3-6, 10, 14, 15 and 19 most likely represent an epitope mimic, as the sequence of these peptides falls within the intracellular portion of the TROP-2 molecule. Overall these results indicate that AR47A6.4.2 recognizes a discontinuous epitope consisting of sequences around LFRERYRLH (SEQ ID NO: 11) and QVERTLIYY (SEQ ID NO: 12). The position of these amino acid sequences within the entire TROP-2 molecule amino acid sequence is presented in Figure 14.

EXAMPLE 8

Humanization of AR47A6.4.2

Recombinant DNA techniques were performed using methods well known in the art and, as appropriate, supplier instructions for use of enzymes used in these methods. Detailed laboratory methods are also described below.

mRNA was extracted from the hybridoma AR47A6.4.2 cells using a Poly A Tract System 1000 mRNA extraction kit: (Promega Corp., Madison, WI) according to manufacturer's instructions. mRNA was reverse transcribed as follows: For the kappa light chain, 5.0 microliters of mRNA was mixed with 1.0 microliter of 20 pmol/ microliter MuIgGkV_L-3' primer OL040 (Figure 15) and 5.5 microliters nuclease free water (Promega Corp., Madison, WI). For the lambda light chain, 5.0 microliters of mRNA was mixed with 1.0 microliter of 20 pmol/ microliter MuIgGλV_L-3' primer OL042 (Figure 15) and 5.5 microliters nuclease free water (Promega Corp., Madison, WI). For the gamma heavy chain,

WO 2008/144891

PCT/CA2008/000979

5 microliters of mRNA was mixed with 1.0 microliter of 20 pmol/ microliter MuIgGV_H-3' primer OL023 (Figure 16) and 5.5 microliter nuclease free water (Promega Corp., Madison, WI). All three reaction mixes were placed in the pre-heated block of the thermal cycler set at 70°C for 5 minutes. These were chilled on ice for 5 minutes before adding to each 4.0
5 microliters ImPromII 5x reaction buffer (Promega Corp., Madison, WI), 0.5 microliters RNasin ribonuclease inhibitor (Promega Corp., Madison, WI), 2.0 microliters 25mM MgCl₂ (Promega Corp., Madison, WI), 1.0 microliter 10mM dNTP mix (Invitrogen, Paisley, UK) and 1.0 microliter Improm II reverse transcriptase (Promega Corp., Madison, WI). The reaction mixes were incubated at room temperature for 5 minutes before being transferred to a
10 pre-heated PCR block set at 42°C for 1 hour. After this time the reverse transcriptase was heat inactivated by incubating at 70°C in a PCR block for fifteen minutes.

Heavy and light chain sequences were amplified from cDNA as follows: A PCR master mix was prepared by adding 37.5 microliters 10x Hi-Fi Expand PCR buffer: (Roche, Mannheim, Germany), 7.5 microliters 10mM dNTP mix (Invitrogen, Paisley, UK)
15 and 3.75 microliters Hi-Fi Expand DNA polymerase (Roche, Mannheim, Germany) to 273.75 microliters nuclease free water. This master mix was dispensed in 21.5 microliter aliquots into 15 thin walled PCR reaction tubes on ice. Into six of these tubes was added 2.5 microliters of MuIgVH-3' reverse transcription reaction mix and 1.0 microliter of heavy chain 5' primer pools HA to HF (see Figure 16 for primer sequences and primer pool constituents). To
20 another seven tubes was added 2.5 microliters of MuIgKVL-3' reverse transcripton reaction and 1.0 microliter of light chain 5' primer pools LA to LG (Figure 15). Into the final tube was added 2.5 microliters of MuIgKVL-3' reverse transcripton reaction and 1.0 microliter of lambda light chain primer MuIgλVL5'-LI. Reactions were placed in the block of the thermal cycler and heated to 95°C for 2 minutes. The polymerase chain reaction (PCR) reaction was
25 performed for 40 cycles of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 30 seconds. Finally the PCR products were heated at 72°C for 5 minutes, and then held at 4°C.

Amplification products were cloned into pGEM-T easy vector using the pGEM-T easy Vector System I (Promega Corp., Madison, WI) kit and sequenced. The resultant VH and VL sequences are shown in Figures 17 and 18 respectively.

30 For generation of a chimeric antibody, VH region genes were amplified by PCR using the primers OL334 and OL335 (Figure 19); these were designed in order to

WO 2008/144891

PCT/CA2008/000979

engineer in a 5' MluI and a 3' HindIII restriction enzyme site using plasmid DNA from one of the cDNA clones as a template. Into a 0.5 mL PCR tube was added 5 microliters 10x Hi-Fi Expand PCR buffer: (Roche, Mannheim, Germany), 1.0 microliter 10mM dNTP mix (Invitrogen, Paisley, UK), 0.5 microliters of Primer OL330, 0.5 microliters of primer OL331, 1.0 microliter template DNA and 0.5 microliters Hi-Fi Expand DNA polymerase (Roche, Mannheim, Germany) to 41.5 microliters nuclease free water.

VL regions were amplified in a similar method using the oligonucleotides OL336 and OL337 (Figure 20) to engineer in BssHII and BamHI restriction enzyme sites. Reactions were placed in the block of the thermal cycler and heated to 95°C for 2 minutes.

The polymerase chain reaction (PCR) reaction was performed for 30 cycles of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 30 seconds. Finally the PCR products were heated at 72°C for 5 minutes, and then held at 4°C. VH and VL region PCR products were then cloned into the vectors pANT15 and pANT13 respectively (Figure 21) at the MluI/HindIII and BssHII/BamHI sites respectively. Both pANT15 and pANT13 are pAT153-based plasmids containing a human Ig expression cassette. The heavy chain cassette in pANT15 consists of a human genomic IgG1 constant region gene driven by the hCMVie promoter, with a downstream human IgG polyA region. pANT15 also contains a hamster *dhfr* gene driven by the SV40 promoter with a downstream SV40 polyA region. The light chain cassette of pANT13 is comprised of the genomic human kappa constant region driven by the hCMVie promoter with downstream light chain polyA region. Cloning sites between a human Ig leader sequence and the constant regions allow for the insertion of the variable region genes.

NS0 cells (ECACC 85110503, Porton, UK) were co-transfected with these two plasmids via electroporation and selected in DMEM (Invitrogen, Paisley, UK) plus 5 percent FBS (Ultra low IgG Cat No. 16250-078 Invitrogen, Paisley, UK) plus Penicillin/Streptomycin (Invitrogen, Paisley, UK) plus 100 nM Methotrexate (Sigma, Poole, UK). Methotrexate resistant colonies were isolated and antibody was purified by Protein A affinity chromatography using a 1 mL HiTrap MabSelect SuRe column (GE Healthcare, Amersham, UK) following the manufacturers recommended conditions.

The chimeric antibody was tested in an ELISA-based competition assay using AR47A6.4.2 mouse antibody, biotinylated using Biotintag micro biotinylation kit (Sigma, Poole, UK). Biotinylated mouse AR47A6.4.2 was used to bind OVCAR-3 cells in the

WO 2008/144891

PCT/CA2008/000979

presence of varying concentrations of competing antibody. OVCAR-3 cells were cultured to near confluence in tissue culture treated, flat bottomed, 96 well plates and then fixed.

Biotinylated mouse AR47A6.4.2 antibody was diluted to 1 microgram/mL and mixed with an equal volume of competing antibody at concentrations ranging from 0-5 micrograms/mL. 100

5 microliters of the antibody mixes were transferred into the wells of the OVCAR-3 coated plate and incubated at room temperature for 1 hour. The plate was washed, and bound biotinylated mouse AR47A6.4.2 was detected by adding a strepavidin-HRP conjugate (Sigma, Poole, UK) (diluted at 1:500) and OPD substrate (Sigma, Poole, UK). The assay was developed in the dark for 5 minutes before being stopped by the addition of 3 M HCl. The
10 assay plate was then read in a MRX TCII plate reader (Dynex Technologies, Worthing, UK) at an absorbance of 490nm. The chimeric antibody ((ch)AR47A6.4.2) was shown to be equivalent to the mouse AR47A6.4.2 antibody in competing with biotinylated AR47A6.4.2 antibody for binding to OVCAR-3 cells.

Humanized VH and VL sequences were designed by comparison of mouse
15 AR47A6.4.2 sequences and homologous human VH and VL sequences. Sequences of the VH variants are given in Figure 22 and of the VL variants in Figure 23. Humanized V region genes were constructed using the mouse AR47A6.4.2 VH and VL templates for PCR using long overlapping oligonucleotides to introduce amino acids from homologous human VH and VL sequences. Oligonucleotides used for the generation of variant humanized VH and VL
20 sequences are shown in Figures 19 and 20 respectively. Variant genes were cloned directly into the expression vectors pSVgpt and pSVhyg as detailed in US2004260069 (Hellendoorn, Carr and Baker).

All combinations of variant humanized heavy and light chains (including the chimeric constructs) were transiently transfected into CHO-K1 cells (ECACC 85051005,
25 Porton, UK) and supernatants harvested after 48 hours. The supernatants were quantified for antibody expression in a IgG Fc/Kappa ELISA using purified human IgG1/Kappa (Sigma, Poole, UK) as standards. Immunosorb 96 well plates (Nalge nunc, Hereford, UK) were coated with mouse anti-human IgG Fc-specific antibody (I6260 Sigma, Poole, UK) diluted at 1:1500 in 1X PBS (pH 7.4) at 37°C for 1 hour. Plates were washed three times in PBS + 0.05 percent
30 Tween 20 before adding samples and standards, diluted in 2 percent BSA/PBS. Plates were incubated at room temperature for 1 hour before washing three times in PBS/Tween and

WO 2008/144891

PCT/CA2008/000979

adding 100 microliters/well of detecting antibody goat anti-human kappa light chain peroxidase conjugate (A7164 Sigma, Poole, UK) diluted 1:1000 in 2 percent BSA/PBS. Plates were incubated at room temperature for 1 hour before washing five times with PBS/Tween. Bound antibody was detected using OPD substrate (Sigma, Poole, UK). The assay was

5 developed in the dark for 5 minutes before being stopped by the addition of 3 M HCl. The assay plate was then read in a MRX TCII plate reader (Dynex Technologies, Worthing, UK) at 490 nm.

Binding of the humanized variants was assayed in the competition binding ELISA described above. A standard curve was generated with varying concentrations (156.25

10 ng/mL to 5 micrograms/mL) of purified chimeric antibody ((ch)AR47A6.4.2) competing for binding with mouse AR47A6.4.2 to fixed OVCAR-3 cells on a 96-well microtitre plate. Binding of mouse AR47A6.4.2 to OVCAR-3 cells was detected with goat anti-mouse IgG:HRP conjugate (A2179 Sigma, Poole, UK) and developed using TMB substrate (Sigma, Poole, UK) Using the chimeric standard curve, the percentage inhibition expected at the

15 concentrations tested was calculated for each variant and compared to that actually observed. The results were then normalized by dividing the observed inhibition of the test sample by the expected inhibition for each of the various heavy/light chain combinations. Thus a sample with an observed/expected ratio = 1.0 has the same binding affinity as the chimeric antibody whereas a value >1.0 has reduced binding to TROP-2 and a sample with a ratio <1.0 has

20 improved binding to TROP-2. The results are shown in Figure 24.

Combinations of VH and VL genes were cloned into the dual vector pANT18 (pANT 18 vector is based on the plasmid pANT15 described previously, with the light chain cassette from pANT13 cloned into the SpeI/PciI restriction enzyme sites) and transfected into CHO/dhfr- cells (ECACC, 94060607) by electroporation and selected in media (high glucose

25 DMEM with L-glutamine and Na pyruvate (Invitrogen, Paisley UK) plus 5 percent dialysed FBS (Cat No. 26400-044 Invitrogen, Paisley, UK), Proline (Sigma, Poole, UK) and Penicillin/Streptomycin (Invitrogen, Paisley, UK) depleted of Hypoxanthine and Thymidine. Antibodies were purified by Protein A affinity chromatography as above. The purified antibodies were filter sterilized before storing (in PBS pH 7.4) at +4°C. The concentrations of

30 the antibodies were calculated by human IgG1/kappa capture ELISA as above.

WO 2008/144891

PCT/CA2008/000979

Four of the purified antibody samples were tested for binding to OVCAR-3 cells expressing human TROP-2 via a competition ELISA as above. Varying concentrations of each antibody (156 ng/mL to 5 micrograms/mL) were mixed with purified mouse AR47A6.4.2 and added to microtiter plates coated with fixed OVCAR-3 cells. Binding of mouse AR47A6.4.2 was detected with goat anti-mouse IgG (Fc):HRP conjugate as above. Absorbance at 450 nm was measured on a plate reader and this was plotted against the test antibody concentration. The concentration of selected variants required to inhibit mouse AR47A6.4.2 binding to OVCAR-3 cells by 50 percent (IC_{50}) was calculated and compared to the chimeric antibody.

The IC_{50} for the four variant humanized antibodies and the chimeric antibody are as follows:

(ch)AR47A6.4.2	= 1.71 micrograms/mL
(hu)AR47A6.4.2 variant HV2/KV3	= 2.24 micrograms/mL
(hu)AR47A6.4.2 variant HV2/KV4	= 3.04 micrograms/mL
(hu)AR47A6.4.2 variant HV3/KV3	= 2.04 micrograms/mL
(hu)AR47A6.4.2 variant HV3/KV4	= 1.02 micrograms/mL

EXAMPLE 9

Determination of the binding affinity of AR47A6.4.2 and (hu)AR47A.6.4.2 to rhTROP-2

The binding affinity of AR47A6.4.2, (hu)AR47A6.4.2 variant HV2/KV3, (hu)AR47A6.4.2 variant HV2/KV4, (hu)AR47A6.4.2 variant HV3/KV3 and (hu)AR47A6.4.2 variant HV3/KV4 was compared by the determination of the respective dissociation constants (K_D) subsequent to binding to recombinant human TROP-2 (rhTrop-2).

An anti-polyHistidine monoclonal antibody (R&D Systems, Minneapolis, MN, USA) was immobilized using the standard amine coupling procedure. The surface of a CM5 sensor chip (GE Healthcare, Piscataway, NJ USA formerly Biacore) was activated by injection of 104 microliters of a 1:1 mixture of 0.4M EDC and 0.1M NHS (flow rate 10 microliters/minute). The anti-polyHistidine antibody was injected at a concentration of 20 micrograms/mL (diluted in 10 mM sodium acetate pH 5.5) to reach approximately 2000 RU. Finally, 119 microliters of 1.0 M ethanolamine-HCL pH 8.5 was injected over the surface to block any unoccupied activated sites on the sensor chip surface. HIS-tagged rhTROP-2 (R&D Systems, Minneapolis, MN, USA) was injected at 1 microgram/mL and captured by

WO 2008/144891

PCT/CA2008/000979

the HIS tag on the chip surface, followed by injection of AR47A6.4.2, (hu)AR47A6.4.2 variant HV2/KV3, (hu)AR47A6.4.2 variant HV2/KV4, (hu)AR47A6.4.2 variant HV3/KV3 or (hu)AR47A6.4.2 variant HV3/KV4. Regeneration of the sensor chip surface for subsequent injections was accomplished by injection of 10mM Glycine-HCl pH 2.0 for 60 seconds at a flow rate of 50 microliters/minute. Antibodies were diluted in running buffer (HBS-EP+, GE Healthcare, Piscataway, NJ USA formerly Biacore) and serially injected at concentrations ranging from 0.67 to 333 nM, and the surface was regenerated between each cycle. As a control, each antibody concentration was also injected over a reference surface, which had immobilized anti-polyHistidine antibody but did not have captured rhTROP-2 on the surface. Using Biacore T100 Evaluation Software Version 1.1, kinetic analysis was performed on the obtained sensograms using a simple 1:1 interaction model. The association and dissociation rate constants measured were used to calculate the KD of the antibodies. The experiments were conducted using a Biacore T100 system (GE Healthcare, Piscataway, NJ USA formerly Biacore). The results of these experiments yielded values of 3.03 nM for murine AR47A6.4.2 while all four (hu)AR47A6.4.2 were between 0.613 to 0.697 nM. (Figure 25), indicating that all of the antibodies are in the nanomolar to subnanomolar range, and that the affinities of the humanized antibodies are higher than that of the parental murine AR47A6.4.2. The association rate constants (Ka) and dissociation rate constants (Kd) were also tabulated (Figure 25).

EXAMPLE 10

Isolation of Competitive Binders

Given an antibody, an individual ordinarily skilled in the art can generate a competitively inhibiting CDMAB, for example a competing antibody, which is one that recognizes the same epitope (Belanger L *et al. Clinica Chimica Acta* 48:15-18 (1973)). One method entails immunizing with an immunogen that expresses the antigen recognized by the antibody. The sample may include but is not limited to tissues, isolated protein(s) or cell line(s). Resulting hybridomas could be screened using a competition assay, which is one that identifies antibodies that inhibit the binding of the test antibody, such as ELISA, FACS or Western blotting. Another method could make use of phage display antibody libraries and panning for antibodies that recognize at least one epitope of said antigen (Rubinstein JL *et al. Anal Biochem* 314:294-300 (2003)). In either case, antibodies are selected based on their

WO 2008/144891

PCT/CA2008/000979

ability to displace the binding of the original labeled antibody to at least one epitope of its target antigen. Such antibodies would therefore possess the characteristic of recognizing at least one epitope of the antigen as the original antibody.

EXAMPLE 11

5 Cloning of the Variable Regions of the AR47A6.4.2 Monoclonal Antibody

The sequences of the variable regions from the heavy (V_H) and light (V_L) chains of monoclonal antibody produced by the AR47A6.4.2 hybridoma cell line were previously determined (as disclosed in S.N. 11/709,676). To generate chimeric and humanized IgG, the variable light and variable heavy domains can be subcloned into an appropriate vector for expression (as disclosed in Example 8 above).

10 In another embodiment, AR47A6.4.2 or its de-immunized, chimeric or humanized version is produced by expressing a nucleic acid encoding the antibody in a transgenic animal, such that the antibody is expressed and can be recovered. For example, the antibody can be expressed in a tissue specific manner that facilitates recovery and purification. In one such embodiment, an antibody of the invention is expressed in the mammary gland for secretion during lactation. Transgenic animals include but are not limited to mice, goat and rabbit.

(i) Monoclonal Antibody

DNA encoding the monoclonal antibody (as disclosed in S.N. 11/709,676) is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cell serves as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences. Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a

WO 2008/144891

PCT/CA2008/000979

disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

(ii) Humanized Antibody

A humanized antibody has one or more amino acid residues introduced into it
5 from a non-human source. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be performed using the method of Winter and co-workers by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody (Jones *et al.*, Nature 321:522-525 (1986); Riechmann *et al.*, Nature 332:323-327 (1988); Verhoeyen *et al.*, Science
10 239:1534-1536 (1988); reviewed in Clark, Immunol. Today 21:397-402 (2000)).

A humanized antibody can be prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are
15 available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e. the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and
20 import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

(iii) Antibody Fragments

Various techniques have been developed for the production of antibody
25 fragments. These fragments can be produced by recombinant host cells (reviewed in Hudson, Curr. Opin. Immunol. 11:548-557 (1999); Little *et al.*, Immunol. Today 21:364-370 (2000)). For example, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter *et al.*, Biotechnology 10:163-167 (1992)). In another embodiment, the F(ab')₂ is formed using the leucine zipper GCN4 to promote
30 assembly of the F(ab')₂ molecule. According to another approach, Fv, Fab or F(ab')₂ fragments can be isolated directly from recombinant host cell culture.

WO 2008/144891

PCT/CA2008/000979

EXAMPLE 12

A Composition Comprising the Antibody of the Present Invention

The antibody of the present invention can be used as a composition for preventing/treating cancer. The composition for preventing/treating cancer, which comprises the antibody of the present invention, can be administered as they are in the form of liquid preparations, or as pharmaceutical compositions of suitable preparations to human or mammals (e.g., rats, rabbits, sheep, swine, bovine, feline, canine, simian, etc.) orally or parenterally (e.g., intravascularly, intraperitoneally, subcutaneously, etc.). The antibody of the present invention may be administered in itself, or may be administered as an appropriate composition. The composition used for the administration may contain a pharmacologically acceptable carrier with the antibody of the present invention or its salt, a diluent or excipient. Such a composition is provided in the form of pharmaceutical preparations suitable for oral or parenteral administration.

Examples of the composition for parenteral administration are injectable preparations, suppositories, etc. The injectable preparations may include dosage forms such as intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, intraarticular injections, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared by dissolving, suspending or emulsifying the antibody of the present invention or its salt in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant (e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mols) adduct of hydrogenated castor oil)), etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is usually filled in an appropriate ampoule. The suppository used for rectal administration may be prepared by blending the antibody of the present invention or its salt with conventional bases for suppositories. The composition for oral administration includes solid or liquid preparations, specifically, tablets (including dragees and film-coated tablets), pills, granules, powdery

WO 2008/144891

PCT/CA2008/000979

preparations, capsules (including soft capsules), syrup, emulsions, suspensions, etc. Such a composition is manufactured by publicly known methods and may contain a vehicle, a diluent or excipient conventionally used in the field of pharmaceutical preparations. Examples of the vehicle or excipient for tablets are lactose, starch, sucrose, magnesium stearate, etc.

5 Advantageously, the compositions for oral or parenteral use described above are prepared into pharmaceutical preparations with a unit dose suited to fit a dose of the active ingredients. Such unit dose preparations include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid compound contained is generally 5 to 500 mg per dosage unit form; it is preferred that the antibody described above
10 is contained in about 5 to about 100 mg especially in the form of injection, and in 10 to 250 mg for the other forms.

 The dose of the aforesaid prophylactic/therapeutic agent or regulator comprising the antibody of the present invention may vary depending upon subject to be administered, target disease, conditions, route of administration, etc. For example, when used
15 for the purpose of treating/preventing, e.g., breast cancer in an adult, it is advantageous to administer the antibody of the present invention intravenously in a dose of about 0.01 to about 20 mg/kg body weight, preferably about 0.1 to about 10 mg/kg body weight and more preferably about 0.1 to about 5 mg/kg body weight, about 1 to 5 times/day, preferably about 1 to 3 times/day. In other parenteral and oral administration, the agent can be administered in a
20 dose corresponding to the dose given above. When the condition is especially severe, the dose may be increased according to the condition.

 The antibody of the present invention may be administered as it stands or in the form of an appropriate composition. The composition used for the administration may contain a pharmacologically acceptable carrier with the aforesaid antibody or its salts, a
25 diluent or excipient. Such a composition is provided in the form of pharmaceutical preparations suitable for oral or parenteral administration (e.g., intravascular injection, subcutaneous injection, etc.). Each composition described above may further contain other active ingredients. Furthermore, the antibody of the present invention may be used in combination with other drugs, for example, alkylating agents (e.g., cyclophosphamide,
30 ifosfamide, etc.), metabolic antagonists (e.g., methotrexate, 5-fluorouracil, etc.), anti-tumor antibiotics (e.g., mitomycin, adriamycin, etc.), plant-derived anti-tumor agents (e.g.,

WO 2008/144891

PCT/CA2008/000979

vincristine, vindesine, Taxol, etc.), cisplatin, carboplatin, etoposide, irinotecan, etc. The antibody of the present invention and the drugs described above may be administered simultaneously or at staggered times to the patient.

The method of treatment described herein, particularly for cancers, may also be carried out with administration of other antibodies or chemotherapeutic agents. For example, an antibody against EGFR, such as ERBITUX® (cetuximab), may also be administered, particularly when treating colon cancer. ERBITUX® has also been shown to be effective for treatment of psoriasis. Other antibodies for combination use include Herceptin® (trastuzumab) particularly when treating breast cancer, AVASTIN® particularly when treating colon cancer and SGN-15 particularly when treating non-small cell lung cancer. The administration of the antibody of the present invention with other antibodies/chemotherapeutic agents may occur simultaneously, or separately, via the same or different route.

The chemotherapeutic agent/other antibody regimens utilized include any regimen believed to be optimally suitable for the treatment of the patient's condition. Different malignancies can require use of specific anti-tumor antibodies and specific chemotherapeutic agents, which will be determined on a patient to patient basis. In a preferred embodiment of the invention, chemotherapy is administered concurrently with or, more preferably, subsequent to antibody therapy. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

The preponderance of evidence shows that AR47A6.4.2 mediates anti-cancer effects and prolongs survival through ligation of epitopes present on TROP-2. It has previously been shown (as disclosed in S.N. 11/709,676) that AR47A6.4.2 antibodies can be used to immunoprecipitate the cognate antigen from expressing cells such as MDA-MB-231 cells. Further it could be shown that AR47A6.4.2, chimeric AR47A6.4.2 ((ch)AR47A6.4.2) or humanized variants, (hu)AR47A6.4.2 can be used in the detection of cells and/or tissues which express a TROP-2 antigenic moiety which specifically binds thereto, utilizing techniques illustrated by, but not limited to FACS, cell ELISA or IHC.

As with the AR47A6.4.2 antibody, other anti-TROP-2 antibodies could be used to immunoprecipitate and isolate other forms of the TROP-2 antigen, and the antigen can

WO 2008/144891

PCT/CA2008/000979

also be used to inhibit the binding of those antibodies to the cells or tissues that express the antigen using the same types of assays.

SEQ ID NOs

	SEQ ID NO:	Sequence
Heavy CDR1	1	NYGMN
Heavy CDR2	2	WINTKTGEPTYAEEFKG
Heavy CDR3	3	GGYGSSYWFVDV
Light CDR1	4	KASQDVSIAVA
Light CDR2	5	SASYRYT
Light CDR3	6	QQHYITPLT
HV2	7	Q I Q L V Q S G H E V K K P G A S V K V S C K A S G Y T F T N Y G M N W V R Q A P G Q G L E W M G W I N T K T G E P T Y A E E F K G R F V F S L E T S A S T A Y L Q I S S L K A E D T A M Y F C G R G G Y G S S Y W Y F D V W G Q G T T V T V S S
KV3	8	D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q D V S I A V A W Y Q Q K P G K A P K V L I Y S A S Y R Y T G V P D R F S G S G S G T D F T F T I S S L Q P E D I A V Y Y C Q Q H Y I T P L T F G G G T K V E I K
KV4	9	D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q D V S I A V A W Y Q Q K P G K A P K V L I Y S A S Y R Y T G V P S R F S G S G S G T D F T F T I S S L Q P E D I A V Y Y C Q Q H Y I T P L T F G G G T K V E I K
HV3	10	Q I Q L V Q S G H E V K K P G A S V K V S C K A S G Y T F T N Y G M N W V R Q A P G Q G L E W M G W I N T K T G E P T Y A E E F K G R F V F S L E T S A S T A Y L Q I S S L K A E D M A M Y F C G R G G Y G S S Y W Y F D V W G Q G T T V T V S S

WO 2008/144891

PCT/CA2008/000979

All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

5 It is to be understood that while a certain form of the invention is illustrated, it is not to be limited to the specific form or arrangement of parts herein described and shown. It will be apparent to those skilled in the art that various changes may be made without departing from the scope of the invention and the invention is not to be considered limited to what is shown and described in the specification. One skilled in the art will readily
10 appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. Any oligonucleotides, peptides, polypeptides, biologically related compounds, methods, procedures and techniques described herein are presently representative of the preferred embodiments, are intended to be exemplary and are not intended as limitations on the scope. Changes therein and other uses
15 will occur to those skilled in the art which are encompassed within the spirit of the invention and are defined by the scope of the appended claims. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious
20 to those skilled in the art are intended to be within the scope of the following claims.

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Winnipeg, Manitoba Canada R3E 3R2

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Tel: (204) 789-6030
Fax: (204) 789-2018

International Form IDAC/BP/4

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
(issued pursuant to Rule 7.1 of the *Budapest Treaty Regulations*)

ATTACH COPIES OF THE ORIGINAL DEPOSIT CONTRACT AND VIABILITY STATEMENT

This International Depository Authority accepts the deposit of the microorganism specified below, which was received by it on December 14, 2005

To (Name of Depositor): Valerie Harris

Address: ARIUS Research Inc., 55 York Street, Suite 1600, Toronto, ON, M5J 1R7

Identification of Deposit

Reference assigned by depositor: AR47A6.4.2

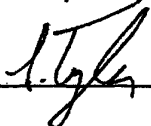
Accession Number assigned by this IDA: 141205-05

The deposit identified above was accompanied by:

☐ a scientific description (specify): _____

☐ a proposed taxonomic designation (specify): _____

Signature of person(s) authorized to represent IDAC:



Date: December 14, 2005

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National Microbiology Laboratory, Public Health Agency of Canada
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Winnipeg, Manitoba Canada R3E 3R2

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Fax: (204) 789-2018

International Form IDAC/BP/9

STATEMENT OF VIABILITY

(Issued pursuant to Rule 10.2 of the *Budapest Treaty* Regulations)

Party to Whom the Viability Statement is Issued

Name: Ferris Lander

Address: 2855 PGA Boulevard, Palm Beach Gardens, Florida, USA 33410

Depositor

Name: Valerie Harris

Address: ARIUS Research Inc., 55 York Street, Suite 1600, Toronto, ON, M5J 1R7

Identification of the Deposit

Accession Number given by the International Depository Authority: 141205-05

Date of the original deposit (or most recent relevant date): December 14, 2005

Viability Test

The viability of the deposit identified above was tested on (most recent test date)

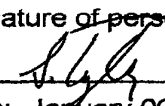
On the date indicated above, the culture was:

☒ viable

☐ no longer viable

Conditions under which the Viability Test were performed (to be filled in if the information has been requested and the results of the test were negative): _____

Signature of person(s) authorized to represent IDAC


Date: January 09, 2006

Statement of Viability 1/1

File number: 084 (05)

WO 2008/144891

PCT/CA2008/000979

CLAIMS

What is claimed is:

Claim 1. A method of reduction of a human pancreatic, breast, prostate, ovarian or colon tumor in a mammal, wherein said human pancreatic, breast, prostate, ovarian or colon tumor expresses at least one epitope of an antigen which specifically binds to the isolated monoclonal antibody produced by the hybridoma cell line deposited with the IDAC as accession number 141205-05 or a CDMAB thereof, which CDMAB is characterized by an ability to competitively inhibit binding of said isolated monoclonal antibody to its target antigen, comprising administering to said mammal said monoclonal antibody or CDMAB thereof in an amount effective to result in a reduction of said mammal's pancreatic, breast, prostate, ovarian or colon tumor burden.

Claim 2. The method of claim 1 wherein said isolated monoclonal antibody is conjugated to a cytotoxic moiety.

Claim 3. The method of claim 2 wherein said cytotoxic moiety is a radioactive isotope.

Claim 4. The method of claim 1 wherein said isolated monoclonal antibody or CDMAB thereof activates complement.

Claim 5. The method of claim 1 wherein said isolated monoclonal antibody or CDMAB thereof mediates antibody dependent cellular cytotoxicity.

Claim 6. The method of claim 1 wherein said isolated monoclonal antibody is a humanized antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or an antigen binding fragment produced from said humanized antibody.

Claim 7. The method of claim 1 wherein said isolated monoclonal antibody is a chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or an antigen binding fragment produced from said chimeric antibody.

WO 2008/144891

PCT/CA2008/000979

Claim 8. A method of reduction of a human pancreatic, breast, prostate, ovarian or colon tumor susceptible to antibody induced cellular cytotoxicity in a mammal, wherein said human pancreatic, breast, prostate, ovarian or colon tumor expresses at least one epitope of an antigen which specifically binds to the isolated monoclonal antibody produced by the hybridoma cell line deposited with the IDAC as accession number 141205-05 or a CDMAB thereof, which CDMAB is characterized by an ability to competitively inhibit binding of said isolated monoclonal antibody to its target antigen, comprising administering to said mammal said monoclonal antibody or said CDMAB thereof in an amount effective to result in a reduction of said mammal's pancreatic, breast, prostate, ovarian or colon tumor burden.

Claim 9. The method of claim 8 wherein said isolated monoclonal antibody is conjugated to a cytotoxic moiety.

Claim 10. The method of claim 9 wherein said cytotoxic moiety is a radioactive isotope.

Claim 11. The method of claim 8 wherein said isolated monoclonal antibody or CDMAB thereof activates complement.

Claim 12. The method of claim 8 wherein said isolated monoclonal antibody or CDMAB thereof mediates antibody dependent cellular cytotoxicity.

Claim 13. The method of claim 8 wherein said isolated monoclonal antibody is a humanized antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or an antigen binding fragment produced from said humanized antibody.

Claim 14. The method of claim 8 wherein said isolated monoclonal antibody is a chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or an antigen binding fragment produced from said chimeric antibody.

Claim 15. A method of reduction of a human pancreatic, breast, prostate, ovarian or colon tumor in a mammal, wherein said human pancreatic, breast, prostate, ovarian or colon tumor expresses at least one epitope of an antigen which specifically binds to the isolated

WO 2008/144891

PCT/CA2008/000979

monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or a CDMAB thereof, which CDMAB is characterized by an ability to competitively inhibit binding of said isolated monoclonal antibody to its target antigen, comprising administering to said mammal said monoclonal antibody or CDMAB thereof in conjunction with at least one chemotherapeutic agent in an amount effective to result in a reduction of said mammal's pancreatic, breast, prostate, ovarian or colon tumor burden.

Claim 16. The method of claim 15 wherein said isolated monoclonal antibody is conjugated to a cytotoxic moiety.

Claim 17. The method of claim 16 wherein said cytotoxic moiety is a radioactive isotope.

Claim 18. The method of claim 15 wherein said isolated monoclonal antibody or CDMAB thereof activates complement.

Claim 19. The method of claim 15 wherein said isolated monoclonal antibody or CDMAB thereof mediates antibody dependent cellular cytotoxicity.

Claim 20. The method of claim 15 wherein said isolated monoclonal antibody is a humanized antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or an antigen binding fragment produced from said humanized antibody.

Claim 21. The method of claim 15 wherein said isolated monoclonal antibody is a chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or an antigen binding fragment produced from said chimeric antibody.

Claim 22. Use of monoclonal antibodies for reduction of human breast, pancreatic, ovarian, prostate or colon tumor burden, wherein said human breast, pancreatic, ovarian, prostate or colon tumor expresses at least one epitope of an antigen which specifically binds to the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or a CDMAB thereof, which CDMAB is characterized by an ability to competitively inhibit binding of said isolated monoclonal antibody to its target

WO 2008/144891

PCT/CA2008/000979

antigen, comprising administering to said mammal said monoclonal antibody or CDMAB thereof in an amount effective to result in a reduction of said mammal's human breast, pancreatic, ovarian, prostate or colon tumor burden.

Claim 23. The method of claim 22 wherein said isolated monoclonal antibody is conjugated to a cytotoxic moiety.

Claim 24. The method of claim 23 wherein said cytotoxic moiety is a radioactive isotope.

Claim 25. The method of claim 22 wherein said isolated monoclonal antibody or CDMAB thereof activates complement.

Claim 26. The method of claim 22 wherein said isolated monoclonal antibody or CDMAB thereof mediates antibody dependent cellular cytotoxicity.

Claim 27. The method of claim 22 wherein said isolated monoclonal antibody is a humanized antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05.

Claim 28. The method of claim 22 wherein said isolated monoclonal antibody is a chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05.

Claim 29. Use of monoclonal antibodies for reduction of human breast, pancreatic, ovarian, prostate or colon tumor burden, wherein said human breast, pancreatic, ovarian, prostate or colon tumor expresses at least one epitope of an antigen which specifically binds to the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or a CDMAB thereof, which CDMAB is characterized by an ability to competitively inhibit binding of said isolated monoclonal antibody to its target antigen, comprising administering to said mammal said monoclonal antibody or CDMAB thereof; in conjunction with at least one chemotherapeutic agent in an amount effective to result in a reduction of said mammal's human breast, pancreatic, ovarian, prostate or colon tumor burden.

WO 2008/144891

PCT/CA2008/000979

- Claim 30. The method of claim 29 wherein said isolated monoclonal antibody is conjugated to a cytotoxic moiety.
- Claim 31. The method of claim 30 wherein said cytotoxic moiety is a radioactive isotope.
- Claim 32. The method of claim 29 wherein said isolated monoclonal antibody or CDMAB thereof activates complement.
- Claim 33. The method of claim 29 wherein said isolated monoclonal antibody or CDMAB thereof mediates antibody dependent cellular cytotoxicity.
- Claim 34. The method of claim 29 wherein said isolated monoclonal antibody is a humanized antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05.
- Claim 35. The method of claim 29 wherein said isolated monoclonal antibody is a chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05.
- Claim 36. A process for reduction of a human breast, pancreatic, ovarian, prostate or colon tumor which expresses at least one epitope of human TROP-2 antigen which is specifically bound by the isolated monoclonal antibody produced by hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05, comprising:
administering to an individual suffering from said human tumor, at least one isolated monoclonal antibody or CDMAB thereof that binds the same epitope or epitopes as those bound by the isolated monoclonal antibody produced by the hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05;
wherein binding of said epitope or epitopes results in a reduction of breast, pancreatic, ovarian, prostate or colon tumor burden.
- Claim 37. A process for reduction of a human breast, pancreatic, ovarian, prostate or colon tumor which expresses at least one epitope of human TROP-2 antigen which is specifically bound by the isolated monoclonal antibody produced by hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05, comprising:

WO 2008/144891

PCT/CA2008/000979

administering to an individual suffering from said human tumor, at least one isolated monoclonal antibody or CDMAB thereof, that binds the same epitope or epitopes as those bound by the isolated monoclonal antibody produced by the hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05; in conjunction with at least one chemotherapeutic agent;

wherein said administration results in a reduction of tumor burden.

Claim 38. A binding assay to determine a presence of cancerous cells in a tissue sample selected from a human tumor, which is specifically bound by the isolated monoclonal antibody produced by hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05, the humanized antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or the chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05, comprising:

providing a tissue sample from said human tumor;

providing at least one of said isolated monoclonal antibody, said humanized antibody, said chimeric antibody or CDMAB thereof that recognizes the same epitope or epitopes as those recognized by the isolated monoclonal antibody produced by a hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05;

contacting at least one said provided antibodies or CDMAB thereof with said tissue sample; and determining binding of said at least one provided antibody or CDMAB thereof with said tissue sample;

whereby the presence of said cancerous cells in said tissue sample is indicated.

Claim 39. A binding assay to determine the presence of cells which express TROP-2 which is specifically recognized by the isolated monoclonal antibody produced by the hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05, the humanized antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or the chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05, comprising:

providing a cell sample;

WO 2008/144891

PCT/CA2008/000979

providing the isolated monoclonal antibody produced by the hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05, said humanized antibody, said chimeric antibody or CDMAB thereof;

contacting said isolated monoclonal antibody or said antigen binding fragment with said cell sample; and

determining binding of said isolated monoclonal antibody or CDMAB thereof with said cell sample;

whereby the presence of cells which express an antigen of TROP-2 which is specifically bound by said isolated monoclonal antibody or said CDMBA thereof is determined.

Claim 40. A method for inducing complement dependent cytotoxicity of cancerous cells, which express at least one epitope of TROP-2 on the cell's surface, which at least one epitope, when bound by the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as 141205-05 or an antigen binding fragment produced from said isolated monoclonal antibody results in cell cytotoxicity, comprising:

providing the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as 141205-05 or an antigen binding fragment produced from said isolated monoclonal antibody, and

contacting said cancerous cells with said isolated monoclonal antibody or said antigen binding fragment;

whereby cytotoxicity occurs as a result of binding of said isolated monoclonal antibody or said antigen binding fragment with said at least one epitope of TROP-2.

Claim 41. The method of claim 40 wherein said isolated monoclonal antibody is conjugated to a cytotoxic moiety.

Claim 42. The method of claim 41 wherein said cytotoxic moiety is a radioactive isotope.

Claim 43. The method of claim 40 wherein said isolated monoclonal antibody activates complement.

WO 2008/144891

PCT/CA2008/000979

Claim 44. The method of claim 40 wherein said isolated monoclonal antibody mediates cellular cytotoxicity.

Claim 45. The method of claim 40 wherein said monoclonal antibody is a humanized antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as 141205-05 or an antigen binding fragment produced from said humanized antibody.

Claim 46. The method of claim 40 wherein said monoclonal antibody is a chimeric antibody of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as 141205-05 or an antigen binding fragment produced from said chimeric antibody.

Claim 47. A method for inducing complement dependent cytotoxicity of cancerous cells, which express at least one epitope of TROP-2 on the cell's surface, which at least one epitope, when bound by the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as 141205-05 or an antigen binding fragment produced from said isolated monoclonal antibody results in cell cytotoxicity, comprising:

providing an isolated monoclonal antibody which competitively inhibits binding of the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as 141205-05 or of an antigen binding fragment produced from said isolated monoclonal antibody, and which when bound by said at least one epitope of TROP-2, results in cell cytotoxicity; and

contacting said cancerous cells with said isolated monoclonal antibody or said antigen binding fragment;

whereby cytotoxicity occurs as a result of binding of said isolated monoclonal antibody or said antigen binding fragment with said at least one epitope of TROP-2.

Claim 48. A monoclonal antibody which specifically binds to the same epitope or epitopes as the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05.

Claim 49. An isolated monoclonal antibody or CDMAB thereof, which specifically binds to human TROP-2, in which the isolated monoclonal antibody or CDMAB thereof reacts with the same epitope or epitopes of human TROP-2 as the isolated monoclonal antibody produced

WO 2008/144891

PCT/CA2008/000979

by a hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05; said isolated monoclonal antibody or CDMAB thereof being characterized by an ability to competitively inhibit binding of said isolated monoclonal antibody to its target human TROP-2 antigen.

Claim 50. An isolated monoclonal antibody or CDMAB thereof that recognizes the same epitope or epitopes as those recognized by the isolated monoclonal antibody produced by the hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05; said monoclonal antibody or CDMAB thereof being characterized by an ability to competitively inhibit binding of said isolated monoclonal antibody to its target epitope or epitopes.

Claim 51. A humanized antibody that specifically binds the same epitope or epitopes of human TROP-2 as the isolated monoclonal antibody produced by the hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05, comprising:

a heavy chain variable region comprising the complementarity determining region amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and a light chain variable region comprising the complementarity determining region amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6;

or a human TROP-2 binding fragment thereof.

Claim 52. A humanized antibody that specifically binds the same epitope or epitopes of human TROP-2 as the isolated monoclonal antibody produced by the hybridoma cell line AR47A6.4.2 having IDAC Accession No. 141205-05, comprising:

a heavy chain variable region comprising the complementarity determining region amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and a light chain variable region comprising the complementarity determining region amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6; and variable domain framework regions from the heavy and light chains of a human antibody or human antibody consensus framework;

or a human TROP-2 binding fragment thereof.

Claim 53. A humanized antibody that specifically binds human TROP-2, wherein said monoclonal antibody comprises a heavy chain variable region amino acid sequence of SEQ ID NO:7; and a light chain variable region amino acid sequence selected of SEQ ID NO:8;

or a human TROP-2 binding fragment thereof.

WO 2008/144891

PCT/CA2008/000979

Claim 54. A humanized antibody that specifically binds human TROP-2, wherein said monoclonal antibody comprises a heavy chain variable region amino acid sequence of SEQ ID NO:7; and a light chain variable region amino acid sequence selected of SEQ ID NO:9; or a human TROP-2 binding fragment thereof.

Claim 55. A humanized antibody that specifically binds human TROP-2, wherein said monoclonal antibody comprises a heavy chain variable region amino acid sequence of SEQ ID NO:10; and a light chain variable region amino acid sequence selected of SEQ ID NO:8; or a human TROP-2 binding fragment thereof.

Claim 56. A humanized antibody that specifically binds human TROP-2, wherein said monoclonal antibody comprises a heavy chain variable region amino acid sequence of SEQ ID NO:10; and a light chain variable region amino acid sequence selected of SEQ ID NO:9; or a human TROP-2 binding fragment thereof.

Claim 57. A composition effective for treating a human pancreatic, prostate, ovarian, breast or colon tumor comprising in combination:

- an antibody or CDMAB of any one of claims 1, 2, 3, 6, 7, 8, 17, 49, 50, 54, 55, or 56;
- a conjugate of said antibody or an antigen binding fragment thereof with a member selected from the group consisting of cytotoxic moieties, enzymes, radioactive compounds, cytokines, interferons, target or reporter moieties and hematogenous cells; and
- a requisite amount of a pharmacologically acceptable carrier;

wherein said composition is effective for treating said human pancreatic, breast, prostate, ovarian or colon tumor.

Claim 58. A composition effective for treating a human pancreatic, breast, prostate, ovarian or colon tumor comprising in combination:

- an antibody or CDMAB of any one of claims 1, 2, 3, 6, 7, 8, 17, 49, 50, 54, 55, or 56;
- and a requisite amount of a pharmacologically acceptable carrier;

wherein said composition is effective for treating said human pancreatic, breast, prostate, ovarian or colon tumor.

WO 2008/144891

PCT/CA2008/000979

Claim 59. A composition effective for treating a human pancreatic, breast, prostate, ovarian or colon tumor comprising in combination:

a conjugate of an antibody, antigen binding fragment, or CDMAB of any one of claims 1, 2, 3, 6, 7, 8, 17, 49, 50, 54, 55, or 56; with a member selected from the group consisting of cytotoxic moieties, enzymes, radioactive compounds, cytokines, interferons, target or reporter moieties and hematogenous cells; and

a requisite amount of a pharmacologically acceptable carrier;

wherein said composition is effective for treating said human pancreatic, breast, prostate, ovarian or colon tumor.

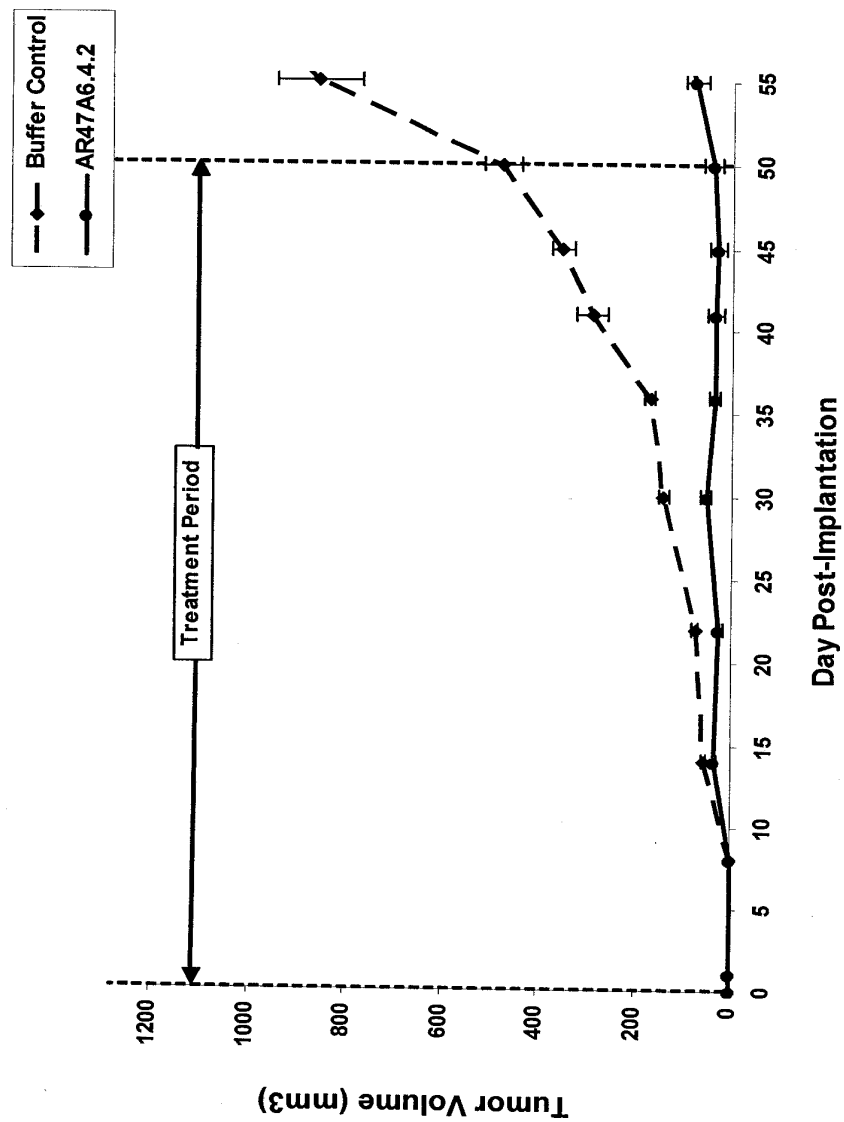
Claim 60. An assay kit for detecting the presence of a human cancerous tumor, wherein said human cancerous tumor expresses at least one epitope of an antigen which specifically binds to the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or a CDMAB thereof, which CDMAB is characterized by an ability to competitively inhibit binding of said isolated monoclonal antibody to its target antigen, the kit comprising the isolated monoclonal antibody produced by the hybridoma deposited with the IDAC as accession number 141205-05 or a CDMAB thereof, and means for detecting whether the monoclonal antibody, or a CDMAB thereof, is bound to a polypeptide whose presence, at a particular cut-off level, is diagnostic of said presence of said human cancerous tumor.

WO 2008/144891

PCT/CA2008/000979

1/29

FIGURE 1

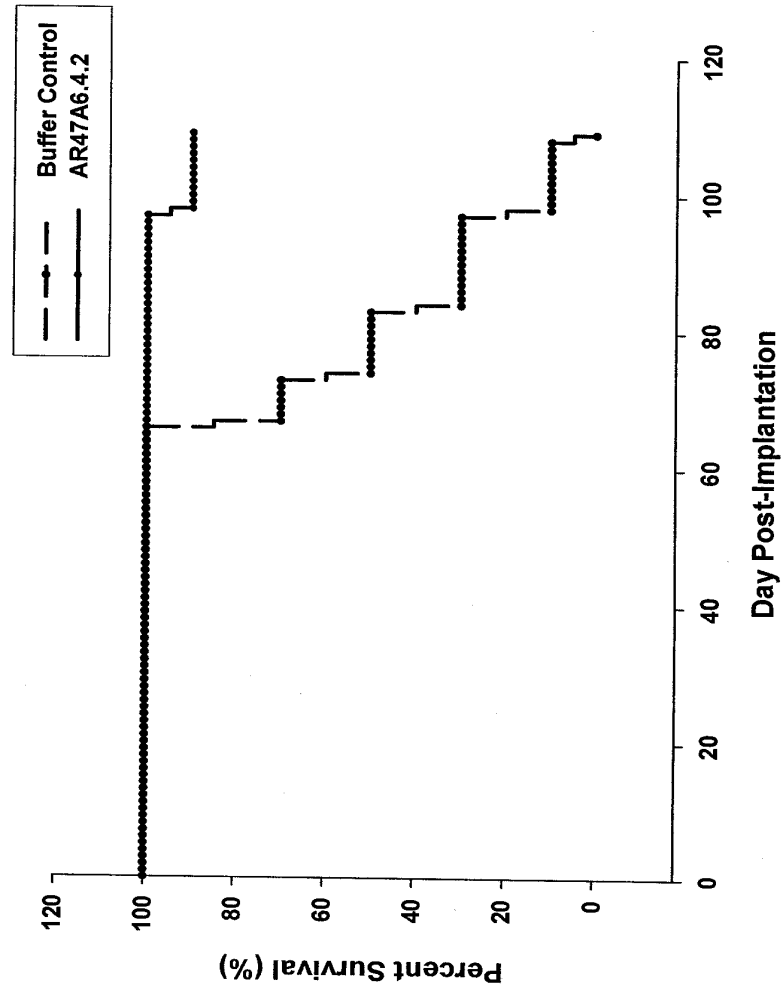


WO 2008/144891

PCT/CA2008/000979

2/29

FIGURE 2

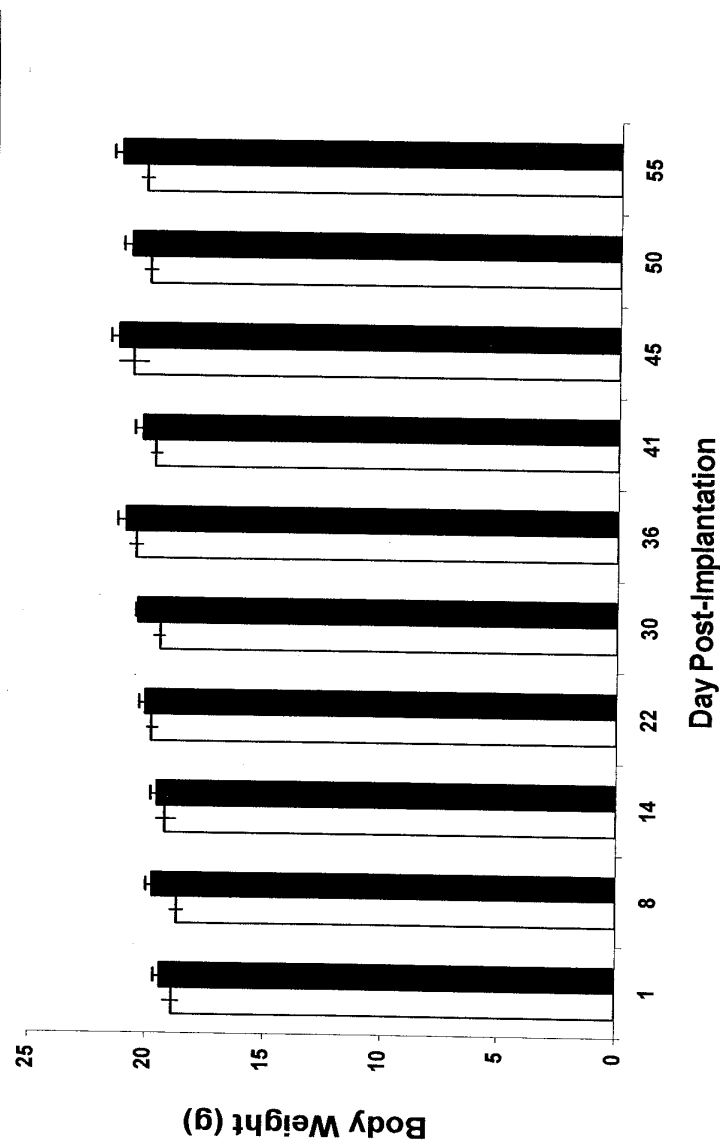


WO 2008/144891

PCT/CA2008/000979

3/29

FIGURE 3

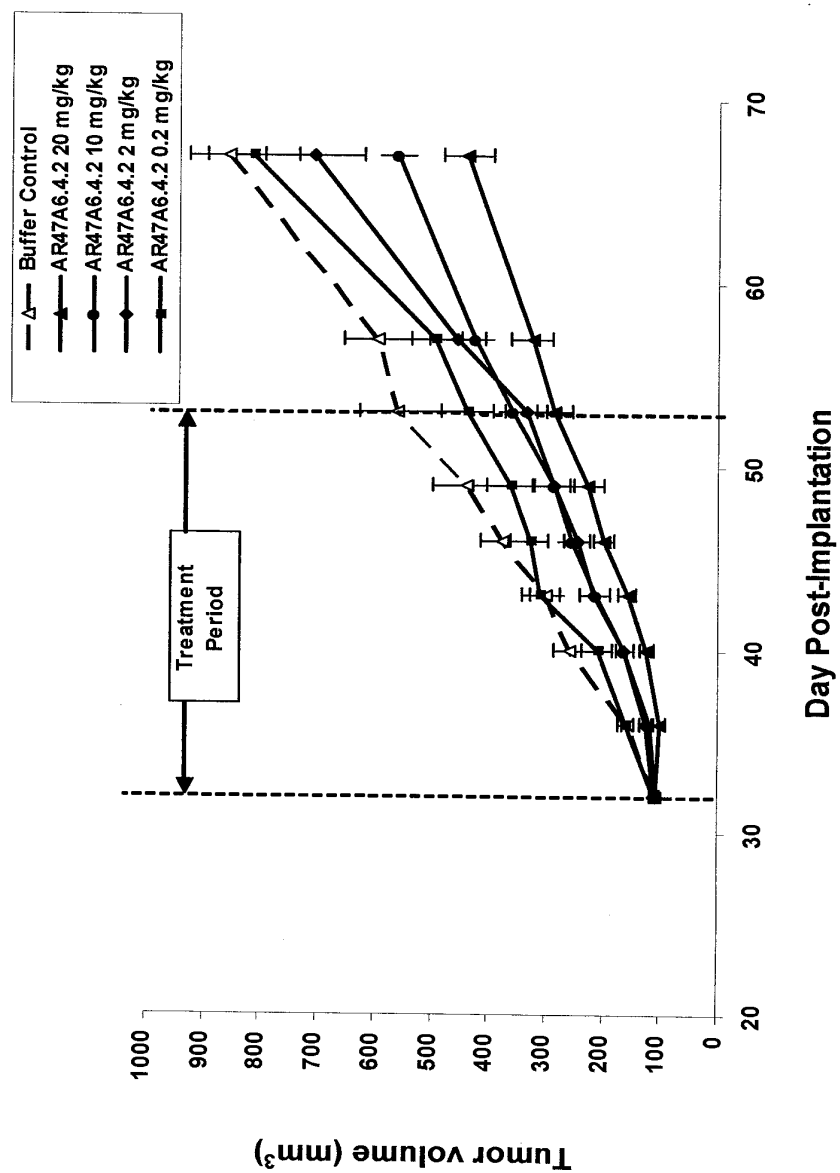


WO 2008/144891

PCT/CA2008/000979

4/29

FIGURE 4

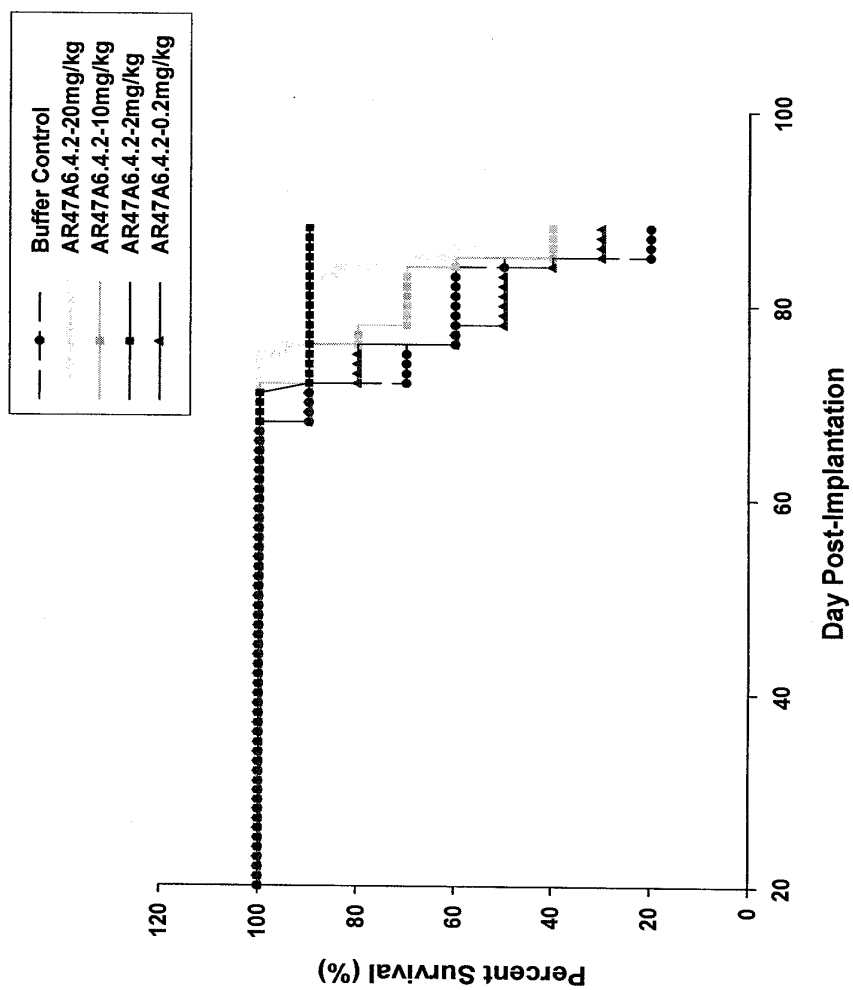


WO 2008/144891

PCT/CA2008/000979

5/29

FIGURE 5

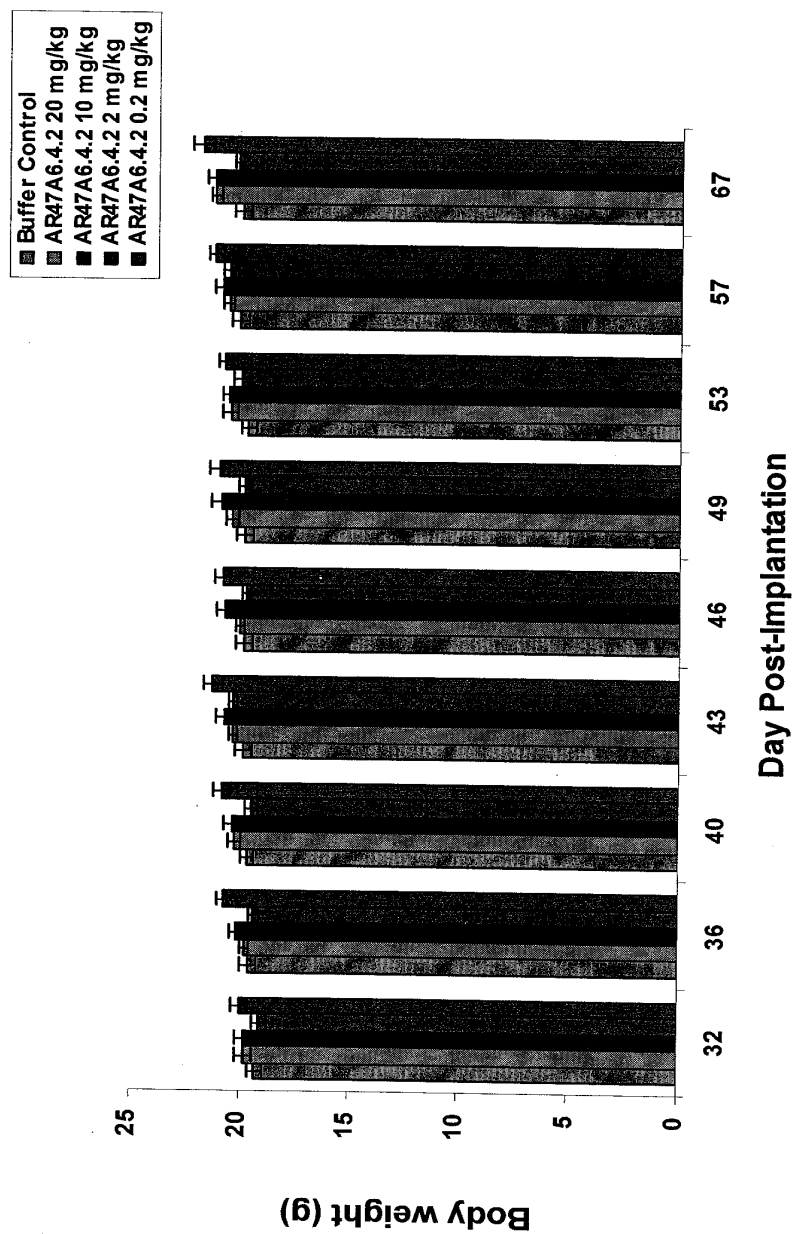


WO 2008/144891

PCT/CA2008/000979

6/29

FIGURE 6



WO 2008/144891

PCT/CA2008/000979

7/29

FIGURE 7

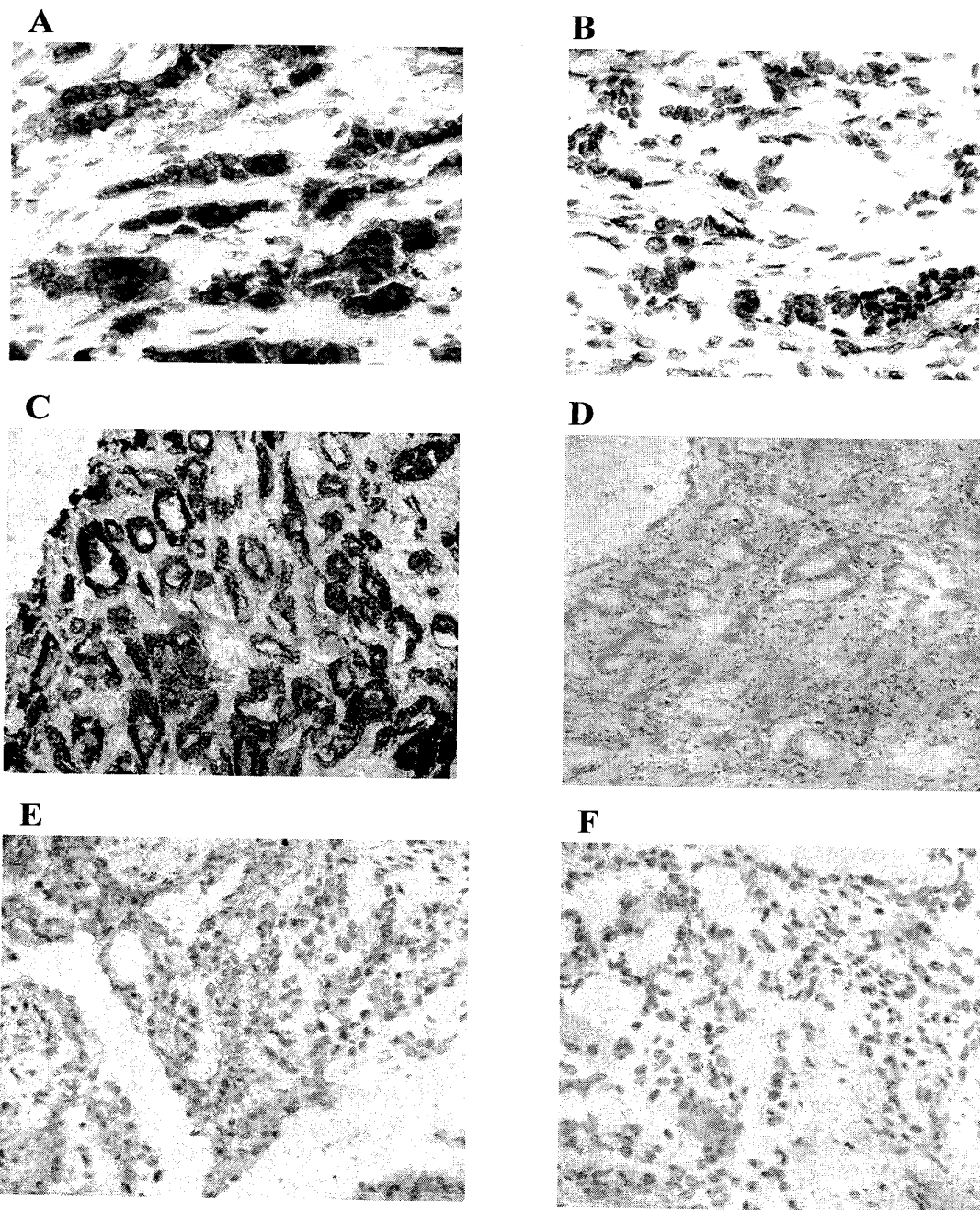
Tissue	Total	IHC Score		
		-	Moderate-Strong (++,+++) Positive	Weak (+/-,+) Positive
Colon Cancer	11	3	6/11 (55%)	2/11 (18%)
Normal Colon	2	2	0	0
Ovarian Cancer	8	2	6/8 (75%)	0
Normal Ovary	2	2	0	0
Breast Cancer	12	0	11/12 (92%)	1/12 (8%)
Normal Breast	4	0	4/4 (100%)	0
Lung Cancer	15	0	12/15 (80%)	3/15 (20%)
Normal Lung	4	0	3/4 (75%)	1/4 (25%)
Prostate Cancer	14	0	14/14 (100%)	0
Normal Prostate	4	0	3/4 (75%)	1/4 (25%)
Pancreatic Cancer	14	9	3/14 (21%)	2/14 (14%)
Normal Pancreas	5	0	5/5 (100%)	0
				5/5 (100%)

WO 2008/144891

8/29

PCT/CA2008/000979

FIGURE 8

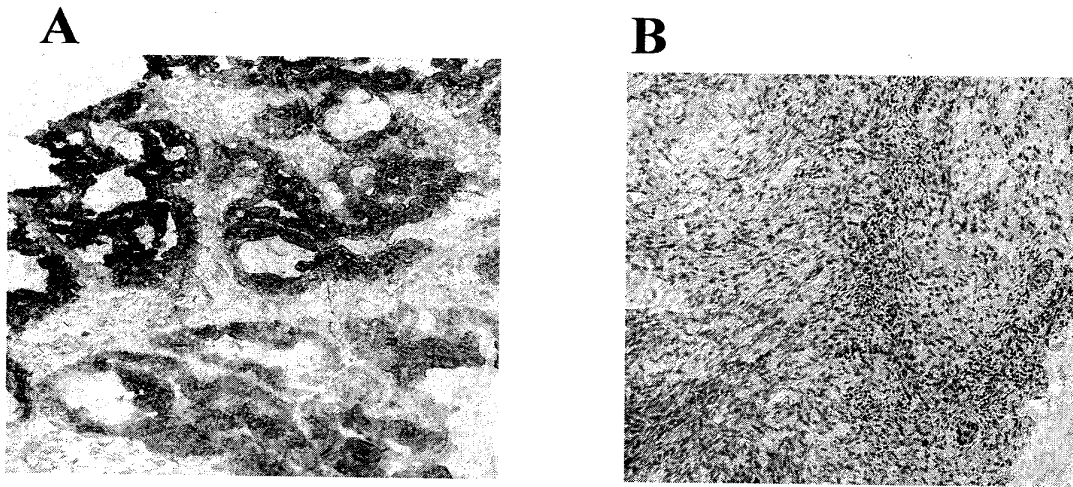


WO 2008/144891

PCT/CA2008/000979

9/29

FIGURE 9



WO 2008/144891

PCT/CA2008/000979

10/29

FIGURE 10

Phosphorylated Protein (Target)	Percent Suppression With AR47A6.4.2
ERK1 (p44MAPK)	32
ERK2 (p42MAPK)	20
Akt1 (PKBalpha)	15
Akt2 (PKBbeta)	18
Akt3 (PKBgamma)	27

WO 2008/144891

PCT/CA2008/000979

11/29

FIGURE 11

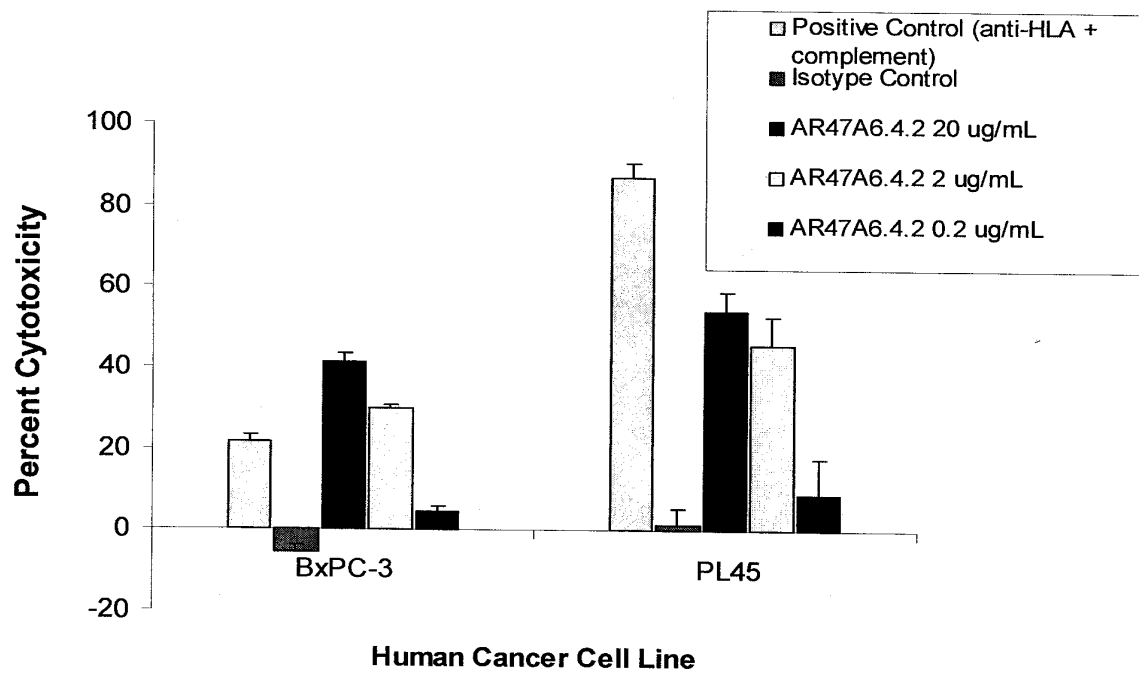
Secreted Factor	Percent Suppression
VEGF	21
PLGF	77

WO 2008/144891

PCT/CA2008/000979

12/29

FIGURE 12



WO 2008/144891

PCT/CA2008/000979

13/29

FIGURE 13

Number	Peptide	Binding of AR47A6.4.2
1	RRLFRERYRLHPKF	518
2	RLFRERYRLHPKFV	387
3	GMAVLVITNRRKSG	349
4	MAVLVITNRRKSGK	335
5	LVAGMAVLVITNRR	317
6	AVLVITNRRKSGKY	308
7	AELRRLFRERYRLH	306
8	LFRERYRLHPKFVA	303
9	LFQGRGGLDLRVRG	302
10	VAGMAVLVITNRRK	296
11	LQVERTLIYYLDEI	293
12	ELRRLFRERYRLHP	291
13	LVRTHHILIDLRHR	291
14	AGMAVLVITNRRKS	285
15	LVITNRRKSGKYKK	283
16	DAELRRLFRERYRL	280
17	LRRLFRERYRLHPK	277
18	LDALRRLFRERYR	276
19	VLVITNRRKSGKYK	273
20	PLQVERTLIYYLDE	262

WO 2008/144891

14/29

PCT/CA2008/000979

FIGURE 14

```
1  MARGPGLAPP  PLRLPLLLLV  LAAVTGHTAA  QDNCTCPTNK  MTVCSPDGPG
51 GRCQCRA LGS  GMAVDCSTLT  SKCLLLKARM  SAPKNARTLV  RPSEHALVDN
101 DGLYDPDCDP  EGRFKARQCN  QTSVCWCVNS  VGVRRTDKGD  LSLRCDDLVR
151 THHILIDLRH  RPTAGAFNHS  DLDAELRRLF RERYRLHPKF  VAAVHYEQPT
201 IQIELRQNTS  QKAAGEVDIG  DAAYYFERDI  KGESLFQGRG  GLDLRVRGEP
251 LQVERTLIYY  LDEIPPKFSM  KRLTAGLIAV  IVVVVVALVA  GMAVLVITNR
310 RKSGKYKKVE  IKELGELRKE  PSL
```

WO 2008/144891

PCT/CA2008/000979

15/29

FIGURE 15

Primer Code	Sequence	Length	Name-Pool
OL024	ATGRAGWCACAKWCYCAGGTCTTT	24	MuIgkV _L 5'-LA
OL025	ATGGAGACAGACACACTCCTGCTAT	25	MuIgkV _L 5'-LB
OL026	ATGGAGWCAGACACACTSCTGYTATGGGT	29	MuIgkV _L 5'-LC
OL027	ATGAGGRCCCCCTGCTCAGWTTYTTGGIWTCTT	32	MuIgkV _L 5'-LD
OL028	ATGGGCWTC AAGATGRAGTCACAKWYYCWGG	31	MuIgkV _L 5'-LD
OL029	ATGAGTGTGCYCACTCAGGTCCCTGSGTT	29	MuIgkV _L 5'-LE
OL030	ATGTGGGGAYCGKTTTYAMMCTTTTCAATTG	31	MuIgkV _L 5'-LE
OL031	ATGGAAGCCCCAGCTCAGCTTCTCTTCC	28	MuIgkV _L 5'-LE
OL032	ATGAGIMMKT C IMTTCAITTCYTGGG	26	MuIgkV _L 5'-LF
OL033	ATGAKGTHCYCIGCTCAGYTYCTIRG	26	MuIgkV _L 5'-LF
OL034	ATGGTRTCCWCASCTCAGTTCCTTG	25	MuIgkV _L 5'-LF
OL035	ATGTATATATGTTTGTTGTCTATTTCT	27	MuIgkV _L 5'-LF
OL036	ATGAAGTTGCCTGTTAGGCTGTTGGTGCT	29	MuIgkV _L 5'-LG
OL037	ATGGATTTWCARGTGCAGATTWTCAGCTT	29	MuIgkV _L 5'-LG
OL038	ATGGTYCTYATVTCCTTGCTGTTCTGG	27	MuIgkV _L 5'-LG
OL039	ATGGTYCTYATVTTRCTGCTGCTATGG	27	MuIgkV _L 5'-LG
OL040	ACTGGATGGTGGAAGATGGA	21	MuIgkV _L 3'-1
OL041	ATGGCCTGGAYTYCWCTYWTMYTCT	25	MuIgλV _L 5'-LI
OL042	AGCTCYTCWG WGGAIGGYGGRAA	23	MuIgλV _L 3'-1

WO 2008/144891

PCT/CA2008/000979

16/29

FIGURE 16

Primer Code	Sequence	Length	Name-Pool
OL007	ATGRASTTSKGGYTMARCTKGRTTT	25	MuIgV _H 5'-HA
OL008	ATGRAATGSASCTGGGTYWYCTCTT	26	MuIgV _H 5'-HB
OL009	ATGGACTCCAGGCTCAATTTAGTTTTTCCT	29	MuIgV _H 5'-HC
OL010	ATGGCTGTCYTRGBGCTGYTCYTCTG	26	MuIgV _H 5'-HC
OL011	ATGGVTTGGSTGTGGAMCTTGCTATTCCT	29	MuIgV _H 5'-HC
OL012	ATGAAATGCAGCTGGRTYATSTTCTT	26	MuIgV _H 5'-HD
OL013	ATGGRCAGRCTTACWYTYTCATTCCT	26	MuIgV _H 5'-H-D
OL014	ATGATGGTGTTAAGTCTTCTGTACCT	26	MuIgV _H 5'-HD
OL015	ATGGGATGGAGCTRTATCATSYTCTT	26	MuIgV _H 5'-HE
OL016	ATGAAGWTGTGGBTRAACCTGGRT	23	MuIgV _H 5'-HE
OL017	ATGGRATGGASCKKIRTCTTTMTCT	25	MuIgV _H 5'-HE
OL018	ATGAACCTTYGGGYTSAGMTTGRTTT	25	MuIgV _H 5'-HF
OL019	ATGTACTTGGGACTGAGCTGTGTAT	25	MuIgV _H 5'-HF
OL020	ATGAGAGTGCTGATTCTTTTG	23	MuIgV _H 5'-HF
OL021	ATGGATTTTGGGCTGATTTTTTTTATTG	28	MuIgV _H 5'-HF
OL023	CCAGGGGCCARKGGATARACIGRTGG	26	MuIgGV _H 3'-2

WO 2008/144891

PCT/CA2008/000979

17/29

FIGURE 17

90
CAGATCCAGTTGGTGCAGTCTGGACCTGAGCTGAAGAA GCCTGGAGAGACAGTCAAGATCTCTGCAAGGCTTCTGGGTATACCTTCACA
GTCTAGGTCAACCACGTCAGACCTGGACTCGACTTCTTCGACCTCTCTGTGAGGACGTTCCGAA GACCCATATGGAAGTGT
Q I Q L V Q S G P E L K K P G E T V K I S C K A S G Y T F T
18
AACATGGAATGA ACTGGGTGAAGCAGGCTCCAGGAA GGTTTAAAGTGGAT GGGCTGGATAAACACCAAACTGGAGAGCCCAACATAT
TTGATACCTTACTTGACCCACTT CGTCCGAGGT CCTTCCCAA ATTTACCTACCCGACCTATTGTGGTTTTGACCTCTCGGTTGTATA
N Y G M N W V K Q A P G K G L K W M G W I N T K T G E P T Y
27
GCTGAAGAGTCAAGGGACGGTTTGCCTTCTCTTTGGA AACCTCTGCCAGCACTGCCTATTTGCAGATCAACAACCTCAAAAAGAGGAC
CGACTTCTCAAGTTCCCTGCCAAACGGAAGAGA AACCTTTGGA GAOGTCTGACGGATAAACGTCTAGTTGTGGAGTTTTTCTCCTG
A E E F K G R F A F S L E T S A S T A Y L Q I N N L K K E D
36
ACGGCTACATATTCTGTGGAAGAGGGGGCTACGGTAGTAGCTACTGGTACTT CGATGTCTGGGGCGAGGGACACGGTCACCGTCTCC
TGCCGATGTATAAAGACACCTTCTCCCCCGATGCCATCATCGA TGACCATGAAGCTACAGACCCCGCTCCCTGGTCCAGTGGCAGAGG
T A T Y F C G R G G Y G S S Y W Y F D V W G A G T T V T V S

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WO 2008/144891

PCT/CA2008/000979

FIGURE 18

90
GACATCCA GATGACTCAGTCTCCAGCCTCCCTATCTGCACTCTGTGGGAGAAACTGTCAACCATCACATGTCGAGCAACTGAGAAATTATAC
CTGTAGGTCTACTGAGTCAGAGGTCGGAGGATAGACGTAGACACCCTCTTTGACAGTGGTAGTGACAGCTCGTTCACTCTTTATAAATG
D I Q M T Q S P A S L S A S V G E T V T I T C R A S E N I Y
18
AGTTATTTAGCATGGTATCAGCAGAAACAGGAAAATCTCCTCAGTCTCGTCTATAATGCAAAAACCTTAGCAGAA GGTGTGCCATCA
TCAATAAA TCGTACCATAGTCGTCCTTTGTCCCTTTTAGAGGAGTCGAGGACCAGATATACGTTTTTGGGAATCGTCTCCACACGGTAGT
S Y L A W Y Q Q K Q G K S P Q L L V Y N A K T L A E G V P S
27
AGGTTCAGTGGCAGTGGTTCAGGCACACAGTTTTTCTCTGAAGA TCAACAGCCTGCGGCCTGAAGATTTTGGGAGTTATTACTGTCAACAT
TCCAAGTCACCGTCACCCAGTCCGTGTGTCAAAAGAGACTTCTAGTTGTGGGACGCGGGACTTCTAAAAACCCCTCAATAATGACAGTTGTA
R F S G S G S G T Q F S L K I N S L R P E D F G S Y Y C Q H
321
CATATAGGTTCTCCGCTCAGGTT CGGTGCTGGGACCACCAAGCTGGAGCTGAGA
GTAATACCAAGAGCGGAGTGCAAGCCACGACCC TGGTT CGACCTCGACTCT
H Y G S P L T F G A G T K L E L R

18/29

WO 2008/144891

PCT/CA2008/000979

19/29

FIGURE 19

Oligonucleotide Code	Sequence	Length
OL 334	GATCACGCGTGTCCACTCCACAGATCCAGTTGGTGCAGTC	39
OL 335	GTACAAAGCTTACCTGAGGAGACGGTGACCCGTGG	33
OL 368	GATCACGCGTGTCCACTCCACAGATCCAGTTGGTGCAGTCTGGACCTGAGCTGAA	54
OL 369	CAGTCTGGACCTGAGCTGAAGAAGCCTGGAGCGTCAGTCAAGGTCTCCTGCAA	53
OL 370	TAAACCTGTCTGGAGCCTGCGGCACCCAGTTC	34
OL 371	CAGGCTCCAGGACAGGGTTTAGAGTGGATGGGC	33
OL 372	TGCTGATCTGCAAAATAGGCAGTGTGGCAGAGGTTTCCAAAGAGAAACCGTCC	58
OL 373	CTGCCTATTTCAGATCAGCAGCCTCAAAGCAGAGGACACGGCTATGTATTCTGTG	57
OL 374	CTAGAAGCTTACCTGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAGACA	50
OL 375	GATCACGCGTGTCCACTCCACAGATCCAGTTGGTGCAGTCTGGACATGAGGTGAAGAAGCC	60
OL 376	AATACATAGCCATGTCCTCTGC	22
OL 377	GCAGAGGACATGGCTATGTATT	22
OL 378	AGAGGACATGGCTATGTATTACTGTGGAAGA	31
OL 379	CTGGCAGAGGTGTCCAAAGAGA	22
OL 380	TCTCTTTGGACACCTCTGCCAG	22
OL 381	GATCACGCGTGTCCACTCCACAGGTCCAGTTGGTG	34
OL 382	GAGGTGTCCATAGAGAAGACA	21
OL 383	TGTCCTCTCTATGGACACCTC	21
OL 384	AGCCCCCTCTTGCACAGTAATA	22
OL 385	TATTACTGTGCAAGAGGGGGCT	22

WO 2008/144891

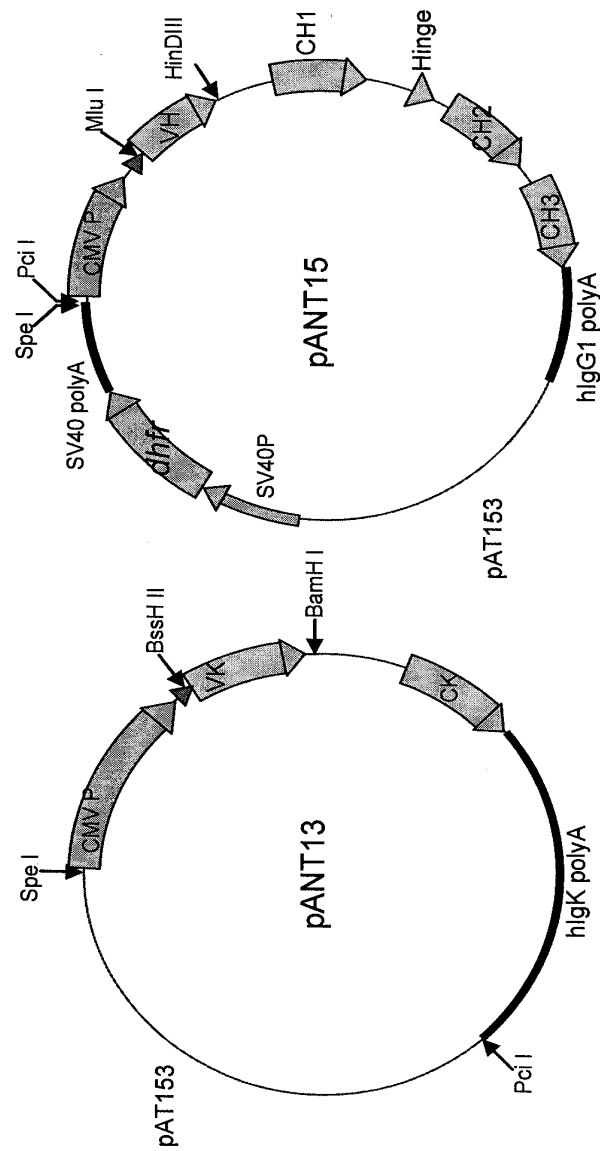
PCT/CA2008/000979

20/29

FIGURE 20

Oligonucleotide Code	Sequence	Length
OL 336	CATGGCGCGGATGTGACATTGTGATGACCCAGTC	35
OL 337	TGCGGGATCCAACTGAGGAAGCAAAGTTTAAATTCTACTCAGTTTCAGCTCCAGCTTGGTCC	63
OL 386	GATCGCGCGGATGTGACATTGTGATGACCCAGTCTCCCTCATTCCTGTCCGCATCAGTAGG	62
OL 387	ATTCTGTCCGCATCAGTAGGAGACAGGGTCACCATCACCTGC	43
OL 388	ACTTTAGGAGCTTTTCTCTGGTTTC	24
OL 389	GAAACCAGGAAAAGCTCCTAAAAGT	24
OL 390	GTCTTCAGCCTGCAGAGAACTGATGGTG	28
OL 391	GTCTCTGCAGGCTGAAGACATCGCAGTTTATT	33
OL 392	GATCGGATCCAACTGAGGAAGCAAAGTTTAAATTCTACTCAGTTTAAATCTCCACCTTGGTCCACCACCGAAGC	75
OL 393	GATCGCGCGGATGTGACATTGTGATGACCCAGTCTCCCTCATCCCTGTCCGCA	54
OL 394	CGATGTCTTCAGGCTGCAGAGAAC	24
OL 395	GTCTCTGCAGCCTGAAGACATCG	24
OL 396	GATCGCGCGGATGTGACATTGATCAGATGACCCAGT	34
OL 397	TCCACTGCCACTGAAGCGATC	21
OL 398	GATCGCTTCAGTGGCAGTGGA	21
OL 399	CACTGAAGCGACTAGGGACTCCA	23
OL 400	TGGAGTCCCTAGTCGCTTCAGTG	23
OL 401	TCTGCAGCCTGAAGACATCGCAACTTATTACTGTC	35

FIGURE 21



WO 2008/144891

PCT/CA2008/000979

22/29

FIGURE 22A

Heavy chain HV6:

Q I Q L V Q S G H E V K K P G A S V K V S C K A S G Y T F T N Y G
10 20 30
R1--
M N W V R Q A P G Q G L E W M G W I N T K T G E P T Y A E E F K G R
40 50 52A 60
F V F S M D T S A S T A Y L Q I S S L K A E D M A M Y Y C G R G G
70 80 82A B C 90
R3--
Y G S S Y W Y F D V W G Q G T T V T V S S
100 A B C D 110

Heavy Chain HV5:

Q I Q L V Q S G H E V K K P G A S V K V S C K A S G Y T F T N Y G
10 20 30
R1--
M N W V R Q A P G Q G L E W M G W I N T K T G E P T Y A E E F K G R
40 50 52A 60
F V F S L D T S A S T A Y L Q I S S L K A E D M A M Y Y C G R G G
70 80 82A B C 90
R3--
Y G S S Y W Y F D V W G Q G T T V T V S S
100 A B C D 110

WO 2008/144891

PCT/CA2008/000979

23/29

FIGURE 22B

Heavy Chain HV4:

Q I Q L V Q S G H E V K K P G A S V K V S C K A S G Y T F T N Y G
10 20 30
R1--
M N W V R Q A P G Q G L E W M G W I N T K T G E P T Y A E E F K G R
40 50 60
F V F S L E T S A S T A Y L Q I S S L K A E D M A M Y Y C G R G G
70 80 90
R3--
Y G S S Y W Y F D V W G Q G T T V T V S S
100 A B C D 110

Heavy Chain HV3:

Q I Q L V Q S G H E V K K P G A S V K V S C K A S G Y T F T N Y G
10 20 30
R1--
M N W V R Q A P G Q G L E W M G W I N T K T G E P T Y A E E F K G R
40 50 60
F V F S L E T S A S T A Y L Q I S S L K A E D M A M Y Y F C G R G G
70 80 90
R3--
Y G S S Y W Y F D V W G Q G T T V T V S S
100 A B C D 110

WO 2008/144891

PCT/CA2008/000979

24/29

FIGURE 22C

Heavy Chain HV2:

Q I Q L V Q S G H E V K K P G A S V K V S C K A S G Y T F T N Y G
10 20 30
R1--
M N W V R Q A P G Q G L E W M G W I N T K T G E P T Y A E E F K G R
40 50 60
F V F S L E T S A S T A Y L Q I S S L K A E D T A M Y F C G R G G
70 80 90
R3--
Y G S S Y W Y F D V W G Q G T T V T V S S
100 A B C D 110

Heavy Chain HV1:

Q I Q L V Q S G P E L K K P G A S V K V S C K A S G Y T F T N Y G
10 20 30
R1--
M N W V R Q A P G Q G L E W M G W I N T K T G E P T Y A E E F K G R
40 50 60
F V F S L E T S A S T A Y L Q I S S L K A E D M A M Y F C G R G G
70 80 90
R3--
Y G S S Y W Y F D V W G Q G T T V T V S S
100 A B C D 110

FIGURE 23A

Light Chain KV5:

D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	V	S	I	A	V	
										10											20												
-----CDR1-----																																	
30																																	
A	W	Y	Q	Q	K	P	G	K	A	P	K	V	L	I	Y	S	A	S	Y	R	Y	T	G	V	P	S	R	F	S	G	S	G	S
-----CDR2-----																																	
50																																	
G	T	D	F	T	F	T	I	S	S	L	Q	P	E	D	I	A	T	Y	Y	C	Q	Q	H	Y	I	T	P	L	T	F	G	G	
												80																					
-----CDR3-----																																	
90																																	
G	T	K	V	E	I	K																											
100																																	
106A																																	

Light Chain KV4:

D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	V	S	I	A	V			
									10										20																
																									-----CDR1-----										
																																			30
A	W	Y	Q	Q	K	P	G	K	A	P	K	V	L	I	Y	S	A	S	Y	R	Y	T	G	V	P	S	R	F	S	G	S	G	S		
																									-----CDR2-----										
																																			50
																																			60
G	T	D	F	T	F	T	I	S	S	L	Q	P	E	D	I	A	V	Y	Y	C	Q	Q	H	Y	I	T	P	L	T	F	G	G			
																									-----CDR3-----										
																																			90
																																			100
G	T	K	V	E	I	K																													
																																			106A

FIGURE 23B

Light Chain KV3:

```

D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q D V S I A V
10
20
30
CDR1-----
A W Y Q Q K P G K A P K V L I Y S A S Y R Y T G V P D R F S G S G S
40
50
60
CDR2-----
G T D F T F T I S S L Q P E D I A V Y Y C Q Q H Y I T P L T F G G
70
80
90
100
CDR3-----
G T K V E I K
106A

```

Light Chain KV2:

```

D I V M T Q S P S L S A S V G D R V T I T C K A S Q D V S I A V
    10                                20                      30-----CDR1-----

A W Y Q Q K P G Q A P K V L I Y S A S Y R Y T G V P D R F T G S G S
    40                                -----CDR2-----                          60

G T D F T F T I S S L Q P E D I A V Y Y C Q Q H Y I T P L T F G G
    70                                80                      90          100-----CDR3-----

G T K V E I K
    106A

```

WO 2008/144891

PCT/CA2008/000979

27/29

FIGURE 23C

Light Chain KV1:

D	I	V	M	T	Q	S	P	S	F	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	V	S	I	A	V					
									10										20																		
																				-----CDR1-----																	
A	W	Y	Q	Q	K	P	G	K	A	P	K	V	L	I	Y	S	A	S	Y	R	Y	T	G	V	P	D	R	F	T	G	S	G	S				
																50																					
																				-----CDR2-----																	
G	T	D	F	T	F	T	I	S	S	L	Q	A	E	D	I	A	V	Y	Y	C	Q	Q	H	Y	I	T	P	L	T	F	G	G					
																				-----CDR3-----																	
G	T	K	V	E	I	K																															
																														100							

WO 2008/144891

PCT/CA2008/000979

28/29

FIGURE 24

Matrix: ratio observed binding/expected

	VH0	VH1	VH2	VH3	VH4	VH5	VH6
VK0	0.963	1.068	0.965	0.916	1.574	1.579	4.836
VK1	0.965	1.029	0.997	1.025	1.689	1.873	4.366
VK2	1.158	1.189	1.067	1.077	1.732	1.885	4.489
VK3	1.015	0.989	1.142	1.057	1.825	1.788	3.853
VK4	1.047	1.118	0.946	1.032	1.701	1.687	4.122
VK5	1.291	1.248	1.334	1.844	1.968	1.969	4.269

WO 2008/144891

PCT/CA2008/000979

29/29

FIGURE 25

Antibody	KD (M)	Ka (1/Ms)	Kd (1/s)
AR47A6.4.2*	3.03E-09	1.29E+05	3.81E-04
(hu)AR47A6.4.2 Variant HV2/KV3	6.90E-10	2.86E+05	1.97E-04
(hu)AR47A6.4.2 Variant HV2/KV4	6.97E-10	3.01E+05	2.10E-04
(hu)AR47A6.4.2 Variant HV3/KV3	6.13E-10	2.89E+05	1.77E-04
(hu)AR47A6.4.2 Variant HV3/KV4	6.31E-10	2.83E+05	1.78E-04

* Average of 3 independent experiments

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/000979

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07K 16/30** (2006.01), **A61K 39/395** (2006.01), **A61K 47/48** (2006.01), **A61K 51/10** (2006.01), **A61P 35/00** (2006.01), **A61P 37/04** (2006.01), **C07K 16/28** (2006.01), **C07K 16/46** (2006.01), **C12P 21/08** (2006.01), **G01N 33/574** (2006.01), **G01N 33/577** (2006.01)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **C07K 16/30** (2006.01), **A61K 39/395** (2006.01), **A61K 47/48** (2006.01), **A61K 51/10** (2006.01), **A61P 35/00** (2006.01), **A61P 37/04**

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

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

Databases: Canadian Patent Database, Delphion, USPTO, QWEB, CAPlus, Genome Quest, Pubmed and Scopus. **Keywords:** TROP-2, EGP-1, GA733-1, antibody, hybridoma, AR47A6.4.2, 141205-05, humanized, CDMAB, chimeric, inhibit*, cancer, Arius, Young

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2003/074566 A2 (IMMUNOMEDICS INC) 12 September 2003 Whole document	38, 39 and 48 - 60
X, P, &	US 2007/0202113 A1 (ARIUS RESEARCH INC.) 30 August 2007 Whole document - cited in the description as US Application No. 11/709,676 -	1 - 60
X, P, &	US 2007/0202043 A1 (ARIUS RESEARCH INC.) 30 August 2007 Whole document	1 - 60

☐ Further documents are listed in the continuation of Box C.	☒ See patent family annex.
* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 22 July 2008 (22-07-2008)	Date of mailing of the international search report 14 August 2008 (14-08-2008)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Jacinth Abraham 819- 934-7598

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/000979**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

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

1. ☒ Claim Nos. : 1 - 21, 23 - 28, 30 - 37 and 40 - 47
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1 - 21, 23 - 28, 30 - 37 and 40 - 47 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 1 - 21, 23 - 28, 30 - 37 and 40 - 47.
2. ☐ Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :
3. ☐ Claim Nos. :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family membersInternational application No.
PCT/CA2008/000979

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO 2003074566 A2	12-09-2003	AU 2003209447A1	16-09-2003
		CA 2478047A1	12-09-2003
		CN 1649903A	03-08-2005
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		WO 2007095748A1	30-08-2007
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		US 2008131428A1	05-06-2008
		WO 2007095748A1	30-08-2007
		WO 2007095749A1	30-08-2007

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- (22) **International Filing Date:**
21 July 2010 (21.07.2010)
- (25) **Filing Language:** English
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- (30) **Priority Data:**
61/227,599 22 July 2009 (22.07.2009) US
- (71) **Applicant (for all designated States except US):** Enzon Pharmaceuticals, Inc. [US/US]; 685 Route 202/206, Bridgewater, New Jersey 08807 (US).
- (72) **Inventor; and**
- (75) **Inventor/Applicant (for US only):** SAPRA, Puja [IN/US]; 254 Wayne Avenue, River Edge, New Jersey 07661 (US).
- (74) **Agent:** Mercanti, Michael N.; Lucas & Mercanti, LLP, 475 Park Avenue South, 15th Floor, New York, New York 10016 (US).
- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) **Title:** METHODS OF TREATING HER2 POSITIVE CANCER WITH HER2 RECEPTOR ANTAGONIST IN COMBINATION WITH MULTI-ARM POLYMERIC CONJUGATES OF 7-ETHYL-10-HYDROXYCAMPTOTHECIN

(57) **Abstract:** The present invention relates to methods of treating a HER2 positive cancer in mammals. The present invention includes administering a HER2 antagonist in combination with a polymeric prodrug of 7-ethyl-10-hydroxycamptothecin to the mammals in need thereof.

WO 2011/011474 A1

WO 2011/011474

PCT/US2010/042686

**METHODS OF TREATING HER2 POSITIVE CANCER WITH HER2 RECEPTOR
ANTAGONIST IN COMBINATION WITH MULTI-ARM POLYMERIC
CONJUGATES OF 7-ETHYL-10-HYDROXYCAMPTOTHECIN**

5

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of priority from U.S. Provisional Patent Application Serial No. 61/227,599, filed July 22, 2009, the contents of which are incorporated herein by reference.

10

FIELD OF THE INVENTION

The present invention relates to methods of treating a HER2 positive cancer. In particular, the invention relates to methods of treating a HER2 positive cancer in a mammal by administering a HER2 antagonist in combination with polyethylene glycol conjugates of 7-ethyl-10-hydroxycamptothecin.

15

BACKGROUND OF THE INVENTION

Breast cancer is the most common type of cancer among women in the United States. Recent studies show that approximately 20-25% of breast cancers are HER2 (Human Epidermal Growth Factor Receptor 2) positive. The HER2 protein, also called the HER2 receptor or HER2/neu or ErbB2, is found on the surface of some normal cells in the body. HER2 plays a role in regulating cell growth and survival. HER2 protein, encoding genes and antibodies to HER2 protein are described in detail in U.S. Patent No. 6,165,464, incorporated by reference herein in its entirety.

20

25

30

Studies show that breast cancer may be more aggressive when the breast cancer tumors over-express the HER2 protein. HER2 positive tumors grow and spread more quickly than tumors that are not HER2 positive. In HER2 positive breast cancer, the cancer cells have an abnormally high number of HER2 gene copies per cell. See *Slamon DJ. et al.*, Science 244:707-712, 1989; and *Pegram M. et al.*, Semin. Oncol. 27: 13-19. 2000. It has been reported that HER positive breast cancer recurs 2.5 times more than non-HER2 positive cancer. It has also been suggested that HER2 overexpression is associated with resistance to chemotherapeutic agents.

Trastuzumab is a humanized monoclonal antibody which binds selectively to the domain IV of HER2 (or HER2/neu) receptor. Trastuzumab inhibits tumor cell growth by

WO 2011/011474

PCT/US2010/042686

binding to the HER2 protein. Clinical studies showed that the use of trastuzumab reduced the risk of a relapse among those with the aggressive HER2 positive cancer by more than half.

There have been various trials to treat cancer with trastuzumab in combination with chemotherapeutic agents in an attempt to achieve synergistic effects or reduce side effects of therapeutic agents. To name a few, the combination therapy with trastuzumab includes docetaxel/gefitinib/trastuzumab, capecitabine/paclitaxel/trastuzumab, carboplatin/docetaxel/trastuzumab, carboplatin/gemcitabine/paclitaxel/trastuzumab, carboplatin/paclitaxel/trastuzumab, cisplatin/docetaxel/trastuzumab, cyclophosphamide/doxorubicin/trastuzumab, cyclophosphamide/fluorouracil/methotrexate/trastuzumab, etc. See also, for example, U.S. Patent Nos. 6,313,138; 6,462,017; 6,537,988; and 6,846,816. It has been shown that trastuzumab in combination with chemotherapy extended survival in women both in early stage and late stage metastatic cancer. For example, median survival increased to 26.2 months for patients receiving trastuzumab and chemotherapy, compared with 20.0 months patients receiving chemotherapy alone.

Unfortunately, patients need to receive trastuzumab therapy over a long period of time such as a year. Such long term treatment with trastuzumab has adverse effects. There have been reports that the treatment with trastuzumab alone or in combination with chemotherapy has resulted in heart failure. It is also reported that it is dangerous for patients to receive trastuzumab in combination with anthracycline-based chemotherapy. A significant number of patients receiving trastuzumab in combination with chemotherapeutic agents such as doxorubicin, cyclophosphamide, and either paclitaxel or docetaxel developed heart failure. As such, trastuzumab-associated therapy requires patients to have their heart function test prior to and during trastuzumab-associated therapy and it is recommended that patients with heart problems not receive or stop trastuzumab-associated therapy. The prolonged use of trastuzumab may also worsen chemotherapy-induced neutropenia.

Thus, there continues to be a need for methods for treating a HER2 positive cancer. The present invention addresses this need.

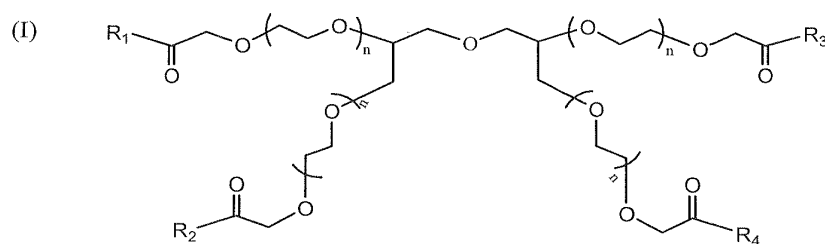
SUMMARY OF THE INVENTION

In one aspect of the present invention, there is provided a method of treating a HER2 positive cancer in a mammal. The method includes administering a HER2 receptor

WO 2011/011474

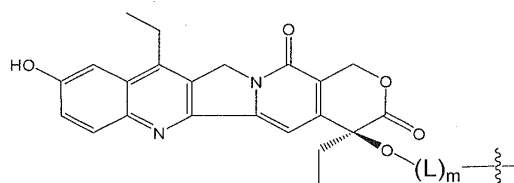
PCT/US2010/042686

antagonist to the mammal in combination with an effective amount of a compound of Formula (I):



wherein

5 R_1 , R_2 , R_3 and R_4 are independently OH or



wherein

L is a bifunctional linker, and each L is the same or different, when (m) is equal to or greater than 2;

10 (m) is 0 or a positive integer; and

(n) is a positive integer;

provided that R_1 , R_2 , R_3 and R_4 are not all OH;

or a pharmaceutically acceptable salt thereof to the mammal.

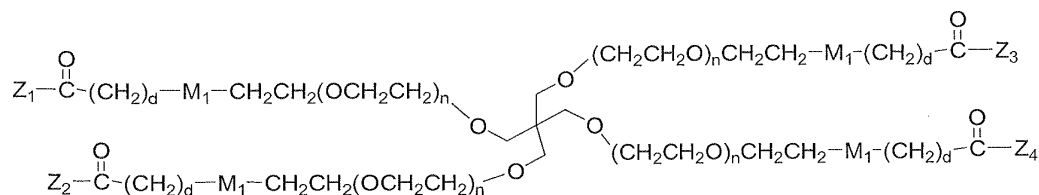
15 In an alternative aspect, there is provided a method of treating a HER2 positive cancer in a mammal. The method includes administering to said mammal a HER2 receptor antagonist in combination with an effective amount of a camptothecin, a camptothecin analog, a polymeric conjugate of a camptothecin or analog thereof, or a pharmaceutically acceptable salt thereof.

20 In one embodiment, the method is conducted by administering a HER2 receptor antagonist plus a polymeric conjugate of a camptothecin or analog thereof to a mammal having a HER2 positive cancer. The polymeric conjugate includes a compound of Formula (II) or (III):

(II)

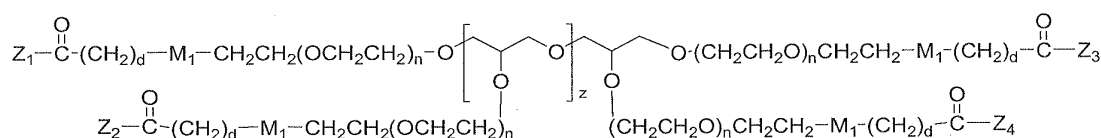
WO 2011/011474

PCT/US2010/042686



or

(III)



5

wherein

Z₁, Z₂, Z₃ and Z₄ are independently OH or (L)_m-D;

L is a bifunctional linker;

D is a camptothecin or a camptothecin analog;

10

M₁ is O, S, or NH;

(d) is zero or a positive integer of from about 1 to about 10;

(z) is zero or a positive integer of from 1 to about 29;

(m) is 0 or a positive integer, wherein each L is the same or different when (m) is

equal to or greater than 2; and

15

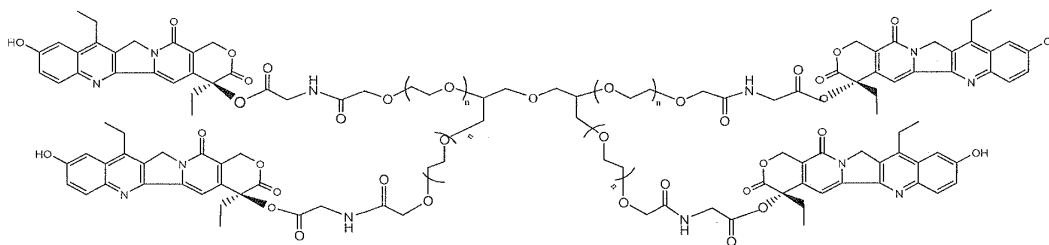
(n) is a positive integer of from about 10 to about 2,300 so that the polymeric portion of the compound has the total number average molecular weight of from about 2,000 to about 100,000 daltons, provided that Z₁, Z₂, Z₃ and Z₄ are not all OH.

In one embodiment, the HER2 antagonist employed in the methods described herein includes trastuzumab marketed under the trademark Herceptin®, as described in detail by U.S. Patent Nos. 5,821,337 and 6,165,464, incorporated by reference herein.

In one preferred embodiment, the polymeric prodrugs of 7-ethyl-10-hydroxycamptothecin employed in the methods described herein include four-arm PEG-7-ethyl-10-hydroxycamptothecin conjugates having the structure of

WO 2011/011474

PCT/US2010/042686



wherein (n) is from about 28 to about 341, preferably from about 114 to about 239, and more preferably about 227.

In yet another embodiment, the method described herein includes:

5 (a) determining the presence of HER2 positive cancer in a mammal having a cancer;
and

(b) administering an effective amount of a HER2 receptor antagonist in combination with an effective amount of a compound of Formula (I) (or Formula (II) or (III)) to a mammal having a HER2 positive cancer.

10 In another aspect, the present invention provides a method of increasing HER2 receptor antagonist effects in a mammal having a HER2 positive cancer.

In yet another aspect, the present invention provides a method of inhibiting the growth or proliferation of HER2 positive cells, as well as a method of delivering a camptothecin such as 7-ethyl-10-hydroxycamptothecin to a HER2 positive cell in a mammal.

15 One advantage of the present invention is that the present invention provides a means to utilize HER2 antagonist-based therapy effectively for the treatment of patients who did not respond to HER2 antagonist-containing therapy, or patients who initially responded but later developed resistance to a HER2 antagonist. Patients can benefit from unexpected lack and/or reduction in resistance to a HER2 antagonist such as trastuzumab and pertuzumab.

20 Another advantage is that the present invention provides a means to treat patients with poor prognosis. HER2 is considered to be correlated with drug resistance and overall poor prognosis. A HER2 antagonist, when administered with the compounds of Formula (I) (alternatively compounds of Formula (II) or (III)) described herein according to the present invention is significantly effective in inhibiting tumor growth and/or proliferation, compared
25 to treatments in which a HER2 antagonist is not administered in combination with the compounds described herein.

Yet another advantage is that the present invention increases the therapeutic efficacy of a HER2 antagonist, and allows certain patients in need to receive HER2-associated therapy

WO 2011/011474

PCT/US2010/042686

for a lesser period or amount, when compared to HER2 therapy alone. Any side-effects associated with or which result from HER2-associated therapy can be alleviated by the enhanced efficacy of HER2 antagonist therapy.

Further advantages will be apparent from the following description and drawings.

5 For purposes of the present invention, “HER2 positive cancer” and “HER2 over-expressing cancer” are used interchangeably. In HER2-positive cancer cells, there is an excess amount of the HER2 protein on the cell surface and/or amplification of the encoding *HER2/neu* gene. Levels of HER2 expression can be measured by techniques known in the art, as well as those methods described later. HER2 positive cancer has greater expression of the
10 HER2 protein or gene, as compared to non-HER2 positive cancer or normal cells or tissues. For example, HER2 is determined by immunohistochemical (“IHC”) assays that measure the amount of HER2 protein expressed on the surface of cancer cells. IHC assays are scored on a scale of 0 to 3+ based on the staining intensity and completeness of cell membrane staining. For example, a cancer that scores 3+ on an IHC assay is considered to be HER2 positive
15 cancer. A cancer that scores 2+ on an IHC assay may be further tested by a fluorescence in-situ hybridization (“FISH”) assay, where a positive FISH assay confirms that the cancer is HER2 positive. A FISH assay measures the number of HER2/neu gene copies present in cancer cells. FISH test results are provided by the ratio of the number of HER2 signals to the number of chromosome 17 signals among 20 interphase nuclei in tumor cells. Normal
20 specimens show a ratio of <2.0, while specimens with amplification of HER2/neu have a ratio of greater than or equal to 2.0 and are defined as HER2-positive (FISH +).

The terms “HER2 receptor antagonist” and “HER2 antagonist” refer to compounds which inhibit expression or function of the HER2 protein or gene. For purposes of the present invention, a HER2 antagonist refers to, e.g., receptor tyrosine kinase inhibitors,
25 especially HER2 receptor protein inhibitors. Simply by way of example, HER2 antagonists include anti-HER2 antibodies. The definition of HER2 antagonists is also intended to include antisense HER2 oligonucleotides.

For purposes of the present invention, the term “adjuvant treatment” refers to treatment given in addition to the primary (initial) treatment. Adjuvant treatment is an
30 additional treatment designed to help reach the ultimate goal.

For purposes of the present invention, the term “early” or “early-stage” breast cancer means that the cancer has not spread beyond the breast or lymph nodes under the arm (known

WO 2011/011474

PCT/US2010/042686

as axillary lymph nodes). Stage 0, I, and II, as well as some stage III cancers, are usually considered early-stage.

For purposes of the present invention, refractory or resistant cancers are defined as cancers that have not responded to previous anticancer therapy or treatment which does not include the compounds of Formula (I) (alternatively, compounds of Formula (II) or (III)) described herein. In one aspect, the cancers are resistant or refractory to a HER2 receptor antagonist such as HER2 antibodies (e.g. trastuzumab and pertuzumab) when used alone or in combination with chemotherapy which does not include the compound of Formula (I) (alternatively, compounds of Formula (II) or (III)). In one embodiment, the cancers are refractory or resistant to Herceptin® treatment alone, or to Herceptin® plus chemotherapy which does not include the compounds of Formula (I) described herein. The cancers can be refractory or resistant at the beginning of treatment, or they may become refractory or resistant during/after treatment. Thus, refractory cancers include tumors that do not respond at the onset of treatment or respond initially for a short period but fail to respond to treatment. Refractory cancers also include tumors that respond to treatment with anticancer therapy but fail to respond to subsequent rounds of therapies. For the purposes of this invention, refractory cancers can also encompass tumors that appear to be inhibited by treatment with anticancer therapy but recur up to five years, sometimes up to ten years or longer, after treatment is discontinued. The anticancer therapy can employ chemotherapeutic agents alone, radiation alone or combinations thereof. For ease of description and not limitation, it will be understood that the refractory cancers are interchangeable with resistant cancers.

For purposes of the present invention, successful treatment of a refractory or resistant cancer shall be understood to mean that refractory or resistant symptoms or conditions are inhibited, minimized or attenuated during and/or after the combination treatment described herein, when compared to that observed in the absence of the combination treatment described herein. The minimized, attenuated or inhibited refractory conditions can be confirmed by clinical markers contemplated by the artisan in the field. In one example, successful treatment of refractory or resistant cancer shall be deemed to occur when at least 5 % or preferably 10%, more preferably 20% or higher (i.e., 30, 40, 50 % or more) inhibition or decrease in tumor growth and/or recurrence including other clinical markers contemplated by the artisan in the field is realized when compared to that observed in the absence of the treatment described herein. Clinical markers which show changes in the severity and magnitude of the refractory cancers can be determined by clinicians.

WO 2011/011474

PCT/US2010/042686

For purposes of the present invention, the terms “cancer” and “tumor” are used interchangeably, unless otherwise indicated. Cancer encompasses benign, malignant and/or metastatic cancer, unless otherwise indicated. Cancers may be more aggressive or less aggressive. The aggressive phenotype refers to the proliferation rate and the ability to form tumors and metastasize. Aggressive cancers proliferate more quickly, and form tumors and metastasize more easily, as compared to less-aggressive tumors.

For purposes of the present invention, “treatment of tumor/cancer” shall be understood to mean inhibition, reduction, or amelioration of tumor growth, tumor burden and metastasis, remission of tumor, or inhibition of recurrences of tumor and/or neoplastic growths realized in patients after completion of the combination therapy described herein, as compared to patients who have not received the combination treatment described herein. Successful treatment is deemed to occur when a patient achieves positive clinical results. For example, successful treatment of a tumor shall be deemed to occur when at least 10% or preferably 20%, more preferably 30 % or higher (i.e., 40%, 50%) decrease in tumor growth including other clinical markers contemplated by the artisan in the field is realized when compared to that observed in the absence of the combination treatment described herein. Other methods for determining changes in a tumor clinical status resulting from the treatment described herein include: biopsies such as a tumor biopsy, an immunohistochemistry study using antibody, radioisotope, dye, and complete blood count (CBC).

For purposes of the present invention, diseases or disorders associated with HER2 over-expression contemplated according to the present invention include conditions in which the HER2 protein or gene plays a role in the pathology or progression of the condition.

The terms “effective amounts” and “sufficient amounts” for purposes of the present invention shall mean an amount which achieves a desired effect or therapeutic effect as such effect is understood by those of ordinary skill in the art. An effective amount for each mammal or human patient to be treated is readily determined by the artisan in a range that provides a desired clinical response while avoiding undesirable effects that are inconsistent with good practice. Dose ranges are provided hereinbelow.

For purposes of the present invention, the term “residue” shall be understood to mean that portion of a compound, to which it refers, e.g., 7-ethyl-10-hydroxycamptothecin, amino acid, etc. that remains after it has undergone a substitution reaction with another compound.

For purposes of the present invention, the term “polymeric residue” or “PEG residue” shall each be understood to mean that portion of the polymer or PEG which remains after it

WO 2011/011474

PCT/US2010/042686

has undergone a reaction with, e.g., an amino acid, 7-ethyl-10-hydroxycamptothecin-containing compounds.

For purposes of the present invention, the term “alkyl” refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. The term “alkyl” also includes alkyl-thio-alkyl, alkoxyalkyl, cycloalkylalkyl, heterocycloalkyl, and C₁₋₆ alkylcarbonylalkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from about 1 to 7 carbons, yet more preferably about 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted, the substituted group(s) preferably include halo, oxy, azido, nitro, cyano, alkyl, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, trihalomethyl, hydroxyl, mercapto, hydroxy, cyano, alkylsilyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, alkenyl, alkynyl, C₁₋₆ hydrocarbonyl, aryl, and amino groups.

For purposes of the present invention, the term “substituted” refers to adding or replacing one or more atoms contained within a functional group or compound with one of the moieties from the group of halo, oxy, azido, nitro, cyano, alkyl, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, trihalomethyl, hydroxyl, mercapto, hydroxy, cyano, alkylsilyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, alkenyl, alkynyl, C₁₋₆ alkylcarbonylalkyl, aryl, and amino groups.

For purposes of the present invention, the term “alkenyl” refers to groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has about 2 to 12 carbons. More preferably, it is a lower alkenyl of from about 2 to 7 carbons, yet more preferably about 2 to 4 carbons. The alkenyl group can be substituted or unsubstituted. When substituted the substituted group(s) include halo, oxy, azido, nitro, cyano, alkyl, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, trihalomethyl, hydroxyl, mercapto, hydroxy, cyano, alkylsilyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, alkenyl, alkynyl, C₁₋₆ hydrocarbonyl, aryl, and amino groups.

For purposes of the present invention, the term “alkynyl” refers to groups containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has about 2 to 12 carbons. More preferably, it is a lower alkynyl of from about 2 to 7 carbons, yet more preferably about 2 to 4 carbons. The alkynyl group can be substituted or unsubstituted. When substituted the substituted group(s) include halo, oxy, azido, nitro, cyano, alkyl, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl,

WO 2011/011474

PCT/US2010/042686

alkylamino, trihalomethyl, hydroxyl, mercapto, hydroxy, cyano, alkylsilyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, alkenyl, alkynyl, C₁₋₆ hydrocarbonyl, aryl, and amino groups. Examples of "alkynyl" include propynyl (i.e., propargyl), and 3-hexynyl.

For purposes of the present invention, the term "aryl" refers to an aromatic hydrocarbon ring system containing at least one aromatic ring. The aromatic ring can optionally be fused or otherwise attached to other aromatic hydrocarbon rings or non-aromatic hydrocarbon rings. Examples of aryl groups include, for example, phenyl, naphthyl, 1,2,3,4-tetrahydronaphthalene and biphenyl. Preferred examples of aryl groups include phenyl and naphthyl.

For purposes of the present invention, the term "cycloalkyl" refers to a C₃₋₈ cyclic hydrocarbon. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

For purposes of the present invention, the term "cycloalkenyl" refers to a C₃₋₈ cyclic hydrocarbon containing at least one carbon-carbon double bond. Examples of cycloalkenyl include cyclopentenyl, cyclopentadienyl, cyclohexenyl, 1,3-cyclohexadienyl, cycloheptenyl, cycloheptatrienyl, and cyclooctenyl.

For purposes of the present invention, the term "cycloalkylalkyl" refers to an alkyl group substituted with a C₃₋₈ cycloalkyl group. Examples of cycloalkylalkyl groups include cyclopropylmethyl and cyclopentylethyl.

For purposes of the present invention, the term "alkoxy" refers to an alkyl group of indicated number of carbon atoms attached to the parent molecular moiety through an oxygen bridge. Examples of alkoxy groups include, for example, methoxy, ethoxy, propoxy and isopropoxy.

For purposes of the present invention, an "alkylaryl" group refers to an aryl group substituted with an alkyl group.

For purposes of the present invention, an "aralkyl" group refers to an alkyl group substituted with an aryl group.

For purposes of the present invention, the term "alkoxyalkyl" group refers to an alkyl group substituted with an alkoxy group.

For purposes of the present invention, the term "amino" refers to a nitrogen containing group as is known in the art derived from ammonia by the replacement of one or more hydrogen radicals by organic radicals. For example, the terms "acylamino" and

WO 2011/011474

PCT/US2010/042686

“alkylamino” refer to specific N-substituted organic radicals with acyl and alkyl substituent groups respectively.

For purposes of the present invention, the term “halogen” or “halo” refers to fluorine, chlorine, bromine, and iodine.

5 For purposes of the present invention, the term “heteroatom” refers to nitrogen, oxygen, and sulfur.

For purposes of the present invention, the term “heterocycloalkyl” refers to a non-aromatic ring system containing at least one heteroatom selected from nitrogen, oxygen, and sulfur. The heterocycloalkyl ring can be optionally fused to or otherwise attached to other
10 heterocycloalkyl rings and/or non-aromatic hydrocarbon rings. Preferred heterocycloalkyl groups have from 3 to 7 members. Examples of heterocycloalkyl groups include, for example, piperazine, morpholine, piperidine, tetrahydrofuran, pyrrolidine, and pyrazole. Preferred heterocycloalkyl groups include piperidinyl, piperazinyl, morpholinyl, and pyrrolidinyl.

15 For purposes of the present invention, the term “heteroaryl” refers to an aromatic ring system containing at least one heteroatom selected from nitrogen, oxygen, and sulfur. The heteroaryl ring can be fused or otherwise attached to one or more heteroaryl rings, aromatic or non-aromatic hydrocarbon rings or heterocycloalkyl rings. Examples of heteroaryl groups include, for example, pyridine, furan, thiophene, 5,6,7,8-tetrahydroisoquinoline and
20 pyrimidine. Preferred examples of heteroaryl groups include thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, benzimidazolyl, furanyl, benzofuranyl, thiazolyl, benzothiazolyl, isoxazolyl, oxadiazolyl, isothiazolyl, benzisothiazolyl, triazolyl, tetrazolyl, pyrrolyl, indolyl, pyrazolyl, and benzopyrazolyl.

In some embodiments, substituted alkyls include carboxyalkyls, aminoalkyls,
25 dialkylaminos, hydroxyalkyls and mercaptoalkyls; substituted alkenyls include carboxyalkenyls, aminoalkenyls, dialkenylaminos, hydroxyalkenyls and mercaptoalkenyls; substituted alkynyls include carboxyalkynyls, aminoalkynyls, dialkynylaminos, hydroxyalkynyls and mercaptoalkynyls; substituted cycloalkyls include moieties such as 4-chlorocyclohexyl.

30 For purposes of the present invention, “positive integer” shall be understood to include an integer equal to or greater than 1 (e.g., an integer from about 1 to about 10, from about 1 to about 6) and as will be understood by those of ordinary skill to be within the realm of reasonableness by the artisan of ordinary skill.

WO 2011/011474

PCT/US2010/042686

For purposes of the present invention, the term “linked” shall be understood to include covalent (preferably) or noncovalent attachment of one group to another, i.e., as a result of a chemical reaction.

For purposes of the present invention, the terms, “nucleic acid” or “nucleotide” apply to deoxyribonucleic acid (“DNA”), ribonucleic acid, (“RNA”) whether single-stranded or double-stranded, unless otherwise specified, and any chemical modifications thereof.

Preferably, a mammal to be treated according to the invention is a human.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 illustrates the stability of 4arm-PEG-Gly-(7-ethyl-10-hydroxycamptothecin) as described in Example 4, in human plasma, phosphate buffer solution, and saline.

FIG. 2 illustrates the effect of pH on stability of 4arm-PEG-Gly-(7-ethyl-10-hydroxycamptothecin) as described in Example 4.

FIG. 3A illustrates pharmacokinetic profiles of 4arm-PEG-Gly-(7-ethyl-10-hydroxycamptothecin) as described in Example 5.

FIG. 3B illustrates pharmacokinetic profiles of 4arm-PEG-Gly-(7-ethyl-10-hydroxycamptothecin) as described in Example 5. Enterohepatic circulation of 4arm-PEG-Gly-(7-ethyl-10-hydroxycamptothecin) conjugates is indicated.

FIG. 4 illustrates the inhibition in tumor growth in mice xenografted with human JIMT-1 breast tumor that is refractory to Herceptin® and pertuzumab, as described in Example 6.

FIG. 5 illustrates the inhibition in tumor growth in mice xenografted with human N87 gastric cancer, as described in Example 7.

DETAILED DESCRIPTION OF THE INVENTION

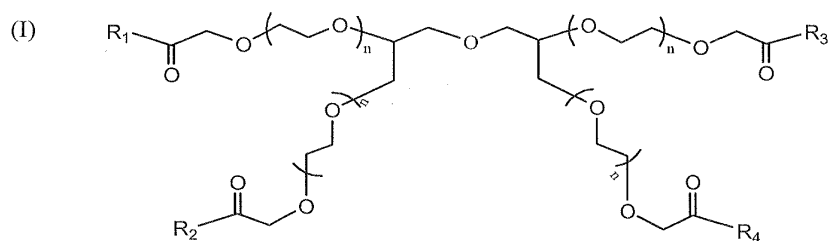
A. OVERVIEW

In one aspect of the invention, there are provided methods of treating a HER2 positive cancer in a mammal. The method includes:

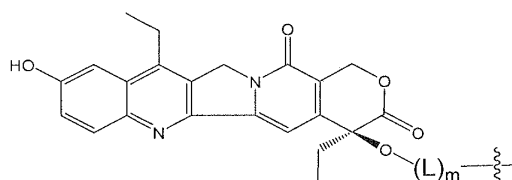
administering a HER2 receptor antagonist in combination with an effective amount of a compound of Formula (I):

WO 2011/011474

PCT/US2010/042686



wherein

R₁, R₂, R₃ and R₄ are independently OH or

5

wherein

L is a bifunctional linker;

(m) is 0 or a positive integer, preferably zero or an integer from about 1 to about 10 (e.g., 1, 2, 3, 4, 5, 6), wherein each L is the same or different when (m) is equal to or greater than 2; and

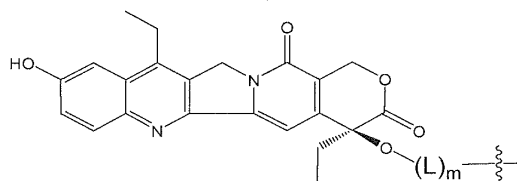
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(n) is a positive integer;

provided that R₁, R₂, R₃ and R₄ are not all OH;

or a pharmaceutically acceptable salt thereof, to said mammal.

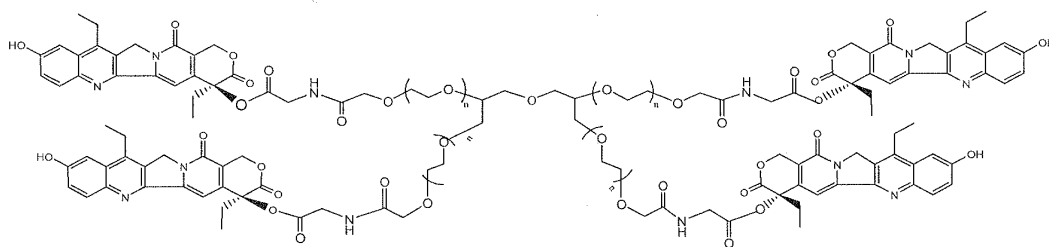
In one preferred embodiment, the method includes administering, to the mammal, a HER2 receptor antagonist in combination with a compound of Formula (I) in which R₁, R₂,

15 R₃ and R₄ are all:

In more preferred aspect, the method includes administering a HER2 receptor antagonist in combination with a compound of Formula (Ia):

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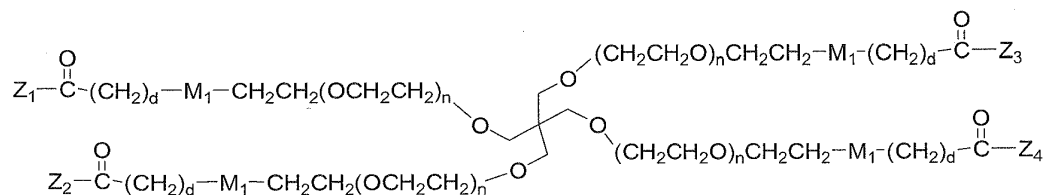


wherein (n) is about 227 so that the polymeric portion of the compound has the total number average molecular weight of about 40,000 daltons.

- In an alternative aspect, there are provided methods of treating a HER2 positive cancer in a mammal. The method includes administering to said mammal a HER2 receptor antagonist in combination with an effective amount of a camptothecin, a camptothecin analog, a polymeric conjugate of a camptothecin or analog thereof, or a pharmaceutically acceptable salt thereof.

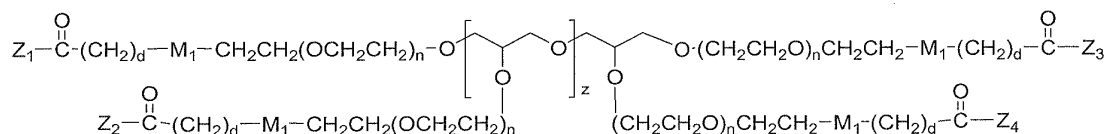
- In one embodiment, the method is conducted by administering a HER2 receptor antagonist plus a polymeric conjugate of a camptothecin or analog thereof to a mammal having a HER2 positive cancer. The polymeric conjugate includes a compound of Formula (II) or (III):

(II)



- or

(III)



wherein

- Z₁, Z₂, Z₃ and Z₄ are independently OH or (L)_m-D;
L is a bifunctional linker;
D is a camptothecin or a camptothecin analog;
M₁ is O, S, or NH, preferably O;

WO 2011/011474

PCT/US2010/042686

(d) is zero or a positive integer of from about 1 to about 10, preferably, 0, 1, 2, or 3, and more preferably, 0 or 1;

(z) is zero or a positive integer of from 1 to about 29, preferably, 1, 5, 13 or 29;

(m) is 0 or a positive integer, preferably zero or an integer from about 1 to about 10 (e.g., 1, 2, 3, 4, 5, 6), wherein each L is the same or different when (m) is equal to or greater than 2; and

(n) is a positive integer of from about 10 to about 2,300 so that the polymeric portion of the compound has the total number average molecular weight of from about 2,000 to about 100,000 daltons,
provided that Z₁, Z₂, Z₃ and Z₄ are not all OH.

In one particular embodiment, SN38 is attached at its 20-OH position to the multi-armed polyethylene glycol of Formula (II) or (III) via the bifunctional linker such as glycine, alanine, methionine, etc. Alternatively, camptothecin, topotecan or CPT-11 is attached at its 20-OH position to the multi-armed polyethylene glycol of Formula (II) or (III) via the bifunctional linker such as glycine, alanine, methionine, sarcosine, etc.

The HER2 antagonist and the compound of Formula (I) (alternatively, compounds of Formula (II) or (III)) are administered in amounts which are sufficient to achieve a desired therapeutic effect.

The HER2 positive cancers which can be treated with the methods described herein include, but are not limited to, solid tumors, breast cancer, gastric cancer, ovarian cancer, stomach cancer, uterine cancer, uterine serous endometrial carcinoma, prostate cancer, bladder cancer, salivary gland carcinoma, renal adenocarcinoma, and mammary gland carcinoma. The forgoing list is not meant to be exclusive and those of ordinary skill will realize that other HER2 cancers not specifically mentioned herein are intended for inclusion.

The HER2 positive cancer can be metastatic or non-metastatic.

In one aspect, the methods described herein can be useful in the treatment of a HER2 positive cancer which is resistant or refractory to a HER2 receptor antagonist such as trastuzumab and pertuzumab when used alone or in combination with chemotherapy which does not include the compound of Formula (I) (alternatively, compounds of Formula (II) or (III)). The combination treatment described herein is also useful for the treatment of a HER2 positive cancer which is sensitive to the anti-HER2 antibodies. Without being bound by any theory, the methods described herein enhance therapeutic efficacy of HER2 antagonist and/or alleviate resistance to the HER2 receptor antagonists by HER2 positive cancer, when

WO 2011/011474

PCT/US2010/042686

compared to the treatment with the anti-HER2 antibodies without the compound of Formula (I) described herein.

In a further aspect, the method described herein includes a step of identifying a patient with a HER2 positive cancer.

5 In an alternate aspect, the present invention provides a method of treating a disease or disorder associated with higher levels of the HER2 protein or gene (e.g., gene expression), compared to that observed in a mammal with normal expression of HER2 (or without excessive expression of HER2). Pathological conditions which involve excessive expression of the HER2 protein or gene benefit from the treatment described herein. The method can be
10 conducted wherein a HER2 receptor antagonist is administered in combination with the compound of Formula (I) (alternatively, compounds of Formula (II) or (III)) or pharmaceutically acceptable salt thereof.

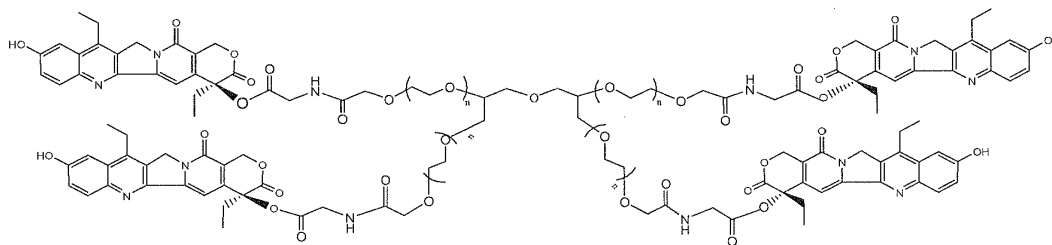
In one embodiment, a HER2 receptor antagonist can be administered with the compound of Formula (I) (alternatively, compounds of Formula (II) or (III)) concurrently or
15 sequentially.

In another embodiment, the HER2 receptor antagonist includes anti-HER2 antibodies, antisense ErbB2 oligonucleotides, and combinations thereof.

In one preferred embodiment, the method includes the steps of:

(a) identifying a mammal having a HER2 positive cancer by e.g., determining the
20 presence, in the mammal, of a cancer that overexpresses HER2; and

(b) administering an effective amount of a HER2 receptor antagonist, preferably trastuzumab, in combination with an effective amount of a compound of Formula (Ia):



25 or a pharmaceutically acceptable salt thereof to the mammal having a HER2 positive cancer, wherein (n) is preferably about 227 so that the total molecular weight of the polymeric portion of the compound of Formula (Ia) is about 40,000 daltons.

WO 2011/011474

PCT/US2010/042686

In an alternate aspect, there are provided methods of treating a tyrosine kinase-dependent disease or disorder in a mammal. The HER2 receptor is a tyrosine kinase and it is implicated in a pathological condition such as cancer. The method includes administering a HER2 receptor antagonist in combination with a compound of Formula (I) (or Formula (II) or (III)) to a mammal having an overexpressing HER2-dependent disease. These methods preferably include the step of identifying a patient having such a disease or disorder.

In yet another aspect, the present invention provides a method of increasing HER2 receptor antagonist effects in a mammal having a HER2 positive cancer. The method includes administering a HER2 receptor antagonist in combination with an effective amount of a compound of Formula (I) (alternatively, compounds of Formula (II) or (III)).

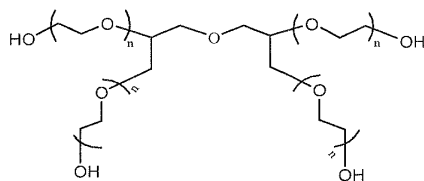
In yet another aspect, the present invention provides a method of inhibiting the growth, proliferation, or metastasis of HER2 positive cells in a mammal by administering a HER2 receptor antagonist in combination with the compound of Formula (I) (alternatively, compounds of Formula (II) or (III)) described herein or pharmaceutically acceptable salt thereof to a mammal or by contacting a HER2 receptor antagonist in combination with the compound of Formula (I) (alternatively, compounds of Formula (II) or (III)) described herein or pharmaceutically acceptable salt thereof with cancer cells or tissues. In one particular embodiment, the method includes:

- (a) determining the presence of a HER2 expression in cells; and
- (b) administering a HER2 receptor antagonist and an effective amount of a compound of Formula (I) (or Formula (II) or (III)) of claim 1 or a pharmaceutically acceptable salt thereof to a mammal in need thereof. In certain aspects, the cells are cancerous cells.

B. POLYMERIC COMPOUNDS

1. MULTI-ARM POLYMERS

The polymeric portion of the compounds described herein includes multi-armed PEG's attached to 20-OH group of 7-ethyl-10-hydroxycamptothecin. In one aspect of the present invention, the polymeric prodrugs of 7-ethyl-10-hydroxycamptothecin include four-arm PEG, prior to conjugation, having the following structure of

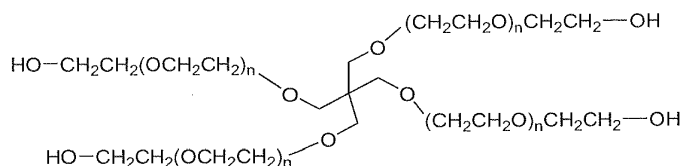


WO 2011/011474

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wherein (n) is a positive integer.

Alternatively, the polymeric compounds employ four-arm PEG, prior to conjugation, having the structure:



5 The multi-armed PEG's are those described in NOF Corp. Drug Delivery System catalog, Ver. 8, April 2006, the disclosure of which is incorporated herein by reference.

In one preferred embodiment of the invention, the degree of polymerization for the polymer (n) is from about 28 to about 341 to provide polymers having the total number average molecular weight of from about 5,000 Da to about 60,000 Da, and preferably from about 114 to about 239 to provide polymers having the total number average molecular weight of from about 20,000 Da to about 42,000 Da. (n) represents the number of repeating units in the polymer chain and is dependent on the molecular weight of the polymer. In one particularly preferred embodiment of the invention, (n) is about 227 to provide the polymeric portion having the total number average molecular weight of about 40,000 Da.

15

2. BIFUNCTIONAL LINKERS

In certain preferred aspects of the present invention, bifunctional linkers include an amino acid. The amino acid which can be selected from any of the known naturally-occurring L-amino acids is, e.g., alanine, valine, leucine, isoleucine, glycine, serine, threonine, methionine, cysteine, phenylalanine, tyrosine, tryptophan, aspartic acid, glutamic acid, lysine, arginine, histidine, proline, and/or a combination thereof, to name but a few. In alternative aspects, L can be a peptide residue. The peptide can range in size, for instance, from about 2 to about 10 amino acid residues (e.g., 2, 3, 4, 5, or 6).

Derivatives and analogs of the naturally occurring amino acids, as well as various art-known non-naturally occurring amino acids (D or L), hydrophobic or non-hydrophobic, are also contemplated to be within the scope of the invention. Simply by way of example, amino acid analogs and derivates include:

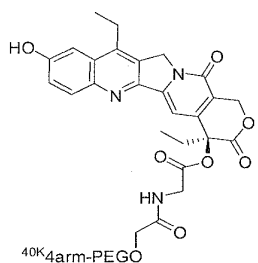
2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, beta-aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, piperidinic acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid,

30

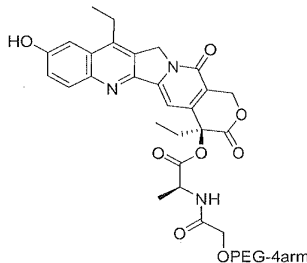
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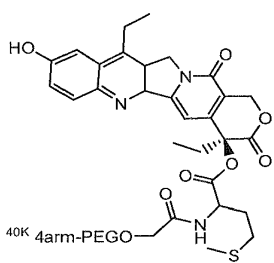
2-aminopimelic acid, 2,4-aminobutyric acid, desmosine, 2,2-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine or sarcosine, N-methylisoleucine, 6-N-methyllysine, N-methylvaline, norvaline, norleucine, ornithine, and
 5 others too numerous to mention, that are listed in 63 Fed. Reg., 29620, 29622, incorporated by reference herein. Some preferred L groups include glycine, alanine, methionine or sarcosine. For example, the compounds can be among:



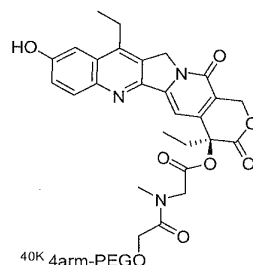
(Compound 9: glycine),



(compound 12: alanine)



(Compound 18: methionine),

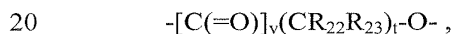
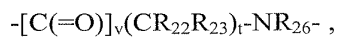
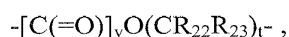


(compound 23: sarcosine).

For ease of the description and not limitation, only one arm of the four-arm PEG is shown. One arm, up to four arms of the four-arm PEG can be conjugated with 7-ethyl-10-hydroxycamptothecin.

15 More preferably, the treatment described herein employs compounds including a glycine as the linker group (L).

In an alternative aspect of the present invention, L after attachment between the polymer and 7-ethyl-10-hydroxycamptothecin can be selected among:



WO 2011/011474

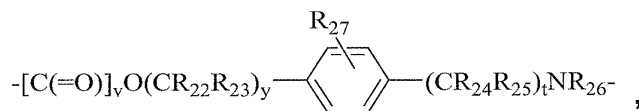
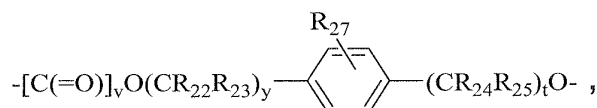
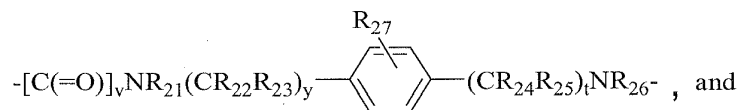
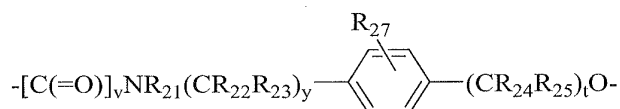
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- $-[C(=O)]_vO(CR_{22}R_{23})_tO^-$,
 $-[C(=O)]_vO(CR_{22}R_{23})_tNR_{26}^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23})_t^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23})_tO^-$,
5 $-[C(=O)]_vNR_{21}(CR_{22}R_{23})_tNR_{26}^-$,
 $-[C(=O)]_v(CR_{22}R_{23}O)_t^-$,
 $-[C(=O)]_vO(CR_{22}R_{23}O)_t^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23}O)_t^-$,
 $-[C(=O)]_v(CR_{22}R_{23}O)_t(CR_{24}R_{25})_y^-$,
10 $-[C(=O)]_vO(CR_{22}R_{23}O)_t(CR_{24}R_{25})_y^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23}O)_t(CR_{24}R_{25})_y^-$,
 $-[C(=O)]_v(CR_{22}R_{23}O)_t(CR_{24}R_{25})_yO^-$,
 $-[C(=O)]_v(CR_{22}R_{23})_t(CR_{24}R_{25}O)_y^-$,
 $-[C(=O)]_vO(CR_{22}R_{23}O)_t(CR_{24}R_{25})_yO^-$,
15 $-[C(=O)]_vO(CR_{22}R_{23})_t(CR_{24}R_{25}O)_y^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23}O)_t(CR_{24}R_{25})_yO^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23})_t(CR_{24}R_{25}O)_y^-$,
 $-[C(=O)]_v(CR_{22}R_{23})_tO-(CR_{28}R_{29})_t^-$,
 $-[C(=O)]_v(CR_{22}R_{23})_tNR_{26}-(CR_{28}R_{29})_t^-$,
20 $-[C(=O)]_v(CR_{22}R_{23})_tS-(CR_{28}R_{29})_t^-$,
 $-[C(=O)]_vO(CR_{22}R_{23})_tO-(CR_{28}R_{29})_t^-$,
 $-[C(=O)]_vO(CR_{22}R_{23})_tNR_{26}-(CR_{28}R_{29})_t^-$,
 $-[C(=O)]_vO(CR_{22}R_{23})_tS-(CR_{28}R_{29})_t^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23})_tO-(CR_{28}R_{29})_t^-$,
25 $-[C(=O)]_vNR_{21}(CR_{22}R_{23})_tNR_{26}-(CR_{28}R_{29})_t^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23})_tS-(CR_{28}R_{29})_t^-$,
 $-[C(=O)]_v(CR_{22}R_{23}CR_{28}R_{29}O)_tNR_{26}^-$,
 $-[C(=O)]_v(CR_{22}R_{23}CR_{28}R_{29}O)_t^-$,
 $-[C(=O)]_vO(CR_{22}R_{23}CR_{28}R_{29}O)_tNR_{26}^-$,
30 $-[C(=O)]_vO(CR_{22}R_{23}CR_{28}R_{29}O)_t^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23}CR_{28}R_{29}O)_tNR_{26}^-$,
 $-[C(=O)]_vNR_{21}(CR_{22}R_{23}CR_{28}R_{29}O)_t^-$,
 $-[C(=O)]_v(CR_{22}R_{23}CR_{28}R_{29}O)_t(CR_{24}R_{25})_y^-$,

WO 2011/011474

PCT/US2010/042686

- $-[C(=O)]_v O(CR_{22}R_{23}CR_{28}R_{29}O)_t (CR_{24}R_{25})_y^-$,
 $-[C(=O)]_v NR_{21}(CR_{22}R_{23}CR_{28}R_{29}O)_t (CR_{24}R_{25})_y^-$,
 $-[C(=O)]_v (CR_{22}R_{23}CR_{28}R_{29}O)_t (CR_{24}R_{25})_y O^-$,
 $-[C(=O)]_v (CR_{22}R_{23})_t (CR_{24}R_{25}CR_{28}R_{29}O)_y^-$,
5 $-[C(=O)]_v (CR_{22}R_{23})_t (CR_{24}R_{25}CR_{28}R_{29}O)_y NR_{26}^-$,
 $-[C(=O)]_v O(CR_{22}R_{23}CR_{28}R_{29}O)_t (CR_{24}R_{25})_y O^-$,
 $-[C(=O)]_v O(CR_{22}R_{23})_t (CR_{24}R_{25}CR_{28}R_{29}O)_y^-$,
 $-[C(=O)]_v O(CR_{22}R_{23})_t (CR_{24}R_{25}CR_{28}R_{29}O)_y NR_{26}^-$,
 $-[C(=O)]_v NR_{21}(CR_{22}R_{23}CR_{28}R_{29}O)_t (CR_{24}R_{25})_y O^-$,
10 $-[C(=O)]_v NR_{21}(CR_{22}R_{23})_t (CR_{24}R_{25}CR_{28}R_{29}O)_y^-$,
 $-[C(=O)]_v NR_{21}(CR_{22}R_{23})_t (CR_{24}R_{25}CR_{28}R_{29}O)_y NR_{26}^-$,



wherein:

- 15 R_{21} - R_{29} are independently selected among hydrogen, amino, substituted amino, azido, carboxy, cyano, halo, hydroxyl, nitro, silyl ether, sulfonyl, mercapto, C_{1-6} alkylmercapto, arylmercapto, substituted arylmercapto, substituted C_{1-6} alkylthio, C_{1-6} alkyls, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-19} branched alkyl, C_{3-8} cycloalkyl, C_{1-6} substituted alkyl, C_{2-6} substituted alkenyl, C_{2-6} substituted alkynyl, C_{3-8} substituted cycloalkyl, aryl, substituted aryl, heteroaryl,
20 substituted heteroaryl, C_{1-6} heteroalkyl, substituted C_{1-6} heteroalkyl, C_{1-6} alkoxy, aryloxy, C_{1-6} heteroalkoxy, heteroaryloxy, C_{2-6} alkanoyl, arylcarbonyl, C_{2-6} alkoxycarbonyl, aryloxycarbonyl, C_{2-6} alkanoyloxy, arylcarbonyloxy, C_{2-6} substituted alkanoyl, substituted arylcarbonyl, C_{2-6} substituted alkanoyloxy, substituted aryloxycarbonyl, C_{2-6} substituted alkanoyloxy, substituted and arylcarbonyloxy;

WO 2011/011474

PCT/US2010/042686

(t), (t') and (y) are independently chosen from zero or a positive integer, preferably from about 1 to about 10 such as 1, 2, 3, 4, 5 and 6; and

(v) is 0 or 1.

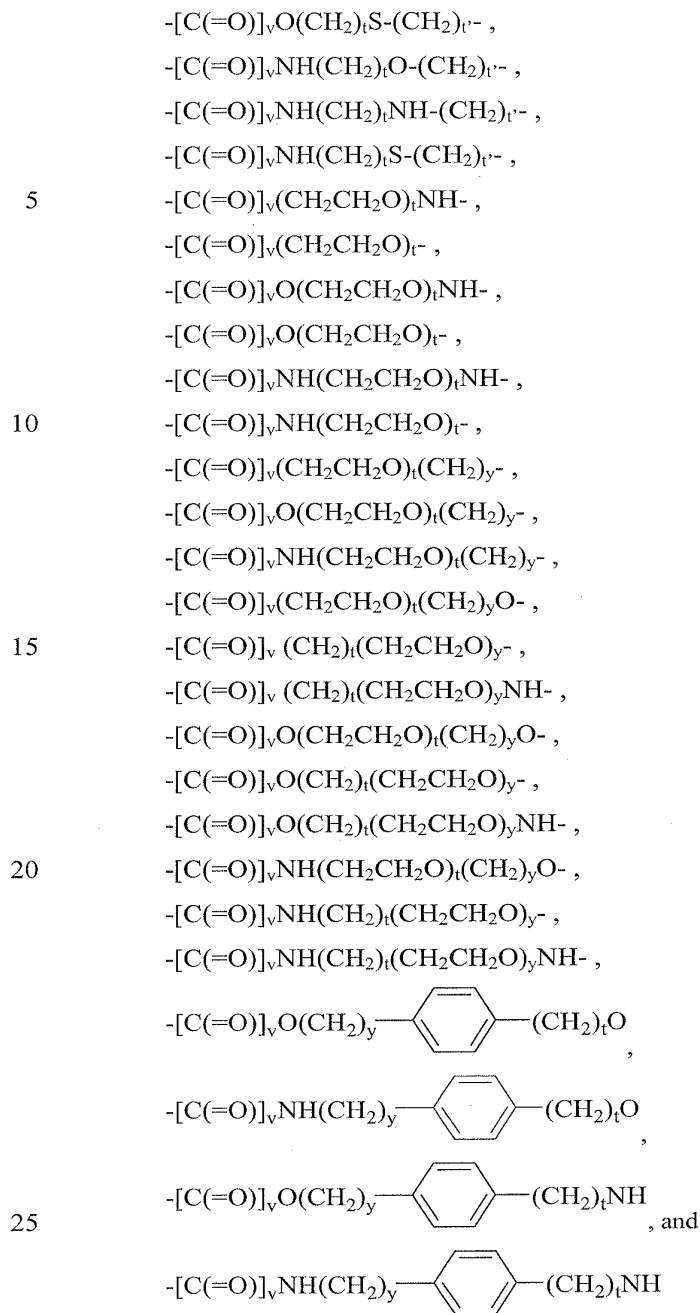
The bifunctional linkers contemplated within the scope of the present invention
5 include those in which combinations of substituents and variables are permissible so that such combinations result in stable compounds.

In some preferred embodiments, L can include:

- [C(=O)]_v(CH₂)_t- ,
- [C(=O)]_v(CH₂)_t-O- ,
- 10 -[C(=O)]_v(CH₂)_t-NH- ,
- [C(=O)]_vO(CH₂)_t- ,
- [C(=O)]_vO(CH₂)_tO- ,
- [C(=O)]_vO(CH₂)_tNH- ,
- [C(=O)]_vNH(CH₂)_t- ,
- 15 -[C(=O)]_vNH(CH₂)_tO- ,
- [C(=O)]_vNH(CH₂)_tNH- ,
- [C(=O)]_v(CH₂O)_t- ,
- [C(=O)]_vO(CH₂O)_t- ,
- [C(=O)]_vNH(CH₂O)_t- ,
- 20 -[C(=O)]_v(CH₂O)_t(CH₂)_y- ,
- [C(=O)]_vO(CH₂O)_t(CH₂)_y- ,
- [C(=O)]_vNH(CH₂O)_t(CH₂)_y- ,
- [C(=O)]_v(CH₂O)_t(CH₂)_yO- ,
- [C(=O)]_v(CH₂)_t(CH₂O)_y- ,
- 25 -[C(=O)]_vO(CH₂O)_t(CH₂)_yO- ,
- [C(=O)]_vO(CH₂)_t(CH₂O)_y- ,
- [C(=O)]_vNH(CH₂O)_t(CH₂)_yO- ,
- [C(=O)]_vNH(CH₂)_t(CH₂O)_y- ,
- [C(=O)]_v(CH₂)_tO-(CH₂)_{t'}- ,
- 30 -[C(=O)]_v(CH₂)_tNH-(CH₂)_{t'}- ,
- [C(=O)]_v(CH₂)_tS-(CH₂)_{t'}- ,
- [C(=O)]_vO(CH₂)_tO-(CH₂)_{t'}- ,
- [C(=O)]_vO(CH₂)_tNH-(CH₂)_{t'}- ,

WO 2011/011474

PCT/US2010/042686



wherein (t), (t') and (y) are independently chosen from zero or a positive integer, preferably from about 1 to about 10 (e.g., 1, 2, 3, 4, 5, and 6); and (v) is 0 or 1.

WO 2011/011474

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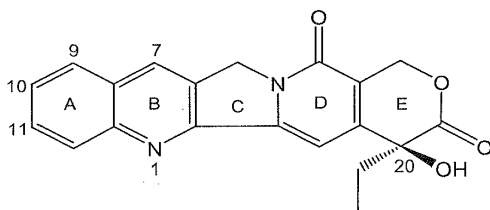
In some aspects of the present invention, the compounds of Formula (I) (or Formula (II) or (III)) include from 1 to about 10 units (e.g., 1, 2, 3, 4, 5, or 6) of the bifunctional linker. In some preferred aspects of the present invention, the compounds include one unit of the bifunctional linker and thus (m) is 1.

5 Additional linkers are found in Table 1 of Greenwald et al. (*Bioorganic & Medicinal Chemistry*, 1998, 6:551-562), the contents of which are incorporated by reference herein.

3. CAMPTOTHECIN AND RELATED CAMPTOTHECIN ANALOGS

10 Camptothecin is a water-insoluble cytotoxic alkaloid produced by *camptoteca accuminata* trees indigenous to China and *nothapodytes foetida* trees indigenous to India. Camptothecin and related compounds and analogs are also known to be potential anticancer or antitumor agents and have been shown to exhibit these activities *in vitro* and *in vivo* in laboratory animals. For example, camptothecin analogs useful in the treatment described herein includes SN38, camptothecin, topotecan, and CPT-11.

15 Camptothecin and certain related analogues share the structure:



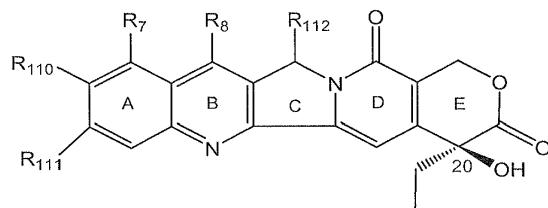
From this core structure, several known analogs have been prepared. For example, the A ring in either or both of the 10- and 11-positions can be substituted with an OH. The A ring can also be substituted with a straight or branched C₁₋₃₀ alkyl or C₁₋₁₇ alkoxy, optionally linked to the ring by a heteroatom i.e.- O or -S. The B ring can be substituted in the 7-position with a
 20 straight or branched C₁₋₃₀ alkyl (preferably C₂ alkyl), C₅₋₈ cycloalkyl, C₁₋₃₀ alkoxy, phenyl alkyl, etc., alkyl carbamate, alkyl carbazides, phenyl hydrazine derivatives, etc. Other substitutions are possible in the C, D and E rings. See, for example, U.S. Patent Nos. 5,004,758; 4,943,579; RE 32,518, the contents of which are incorporated herein by reference.
 25 As the artisan will appreciate, the 10-hydroxycamptothecin, 11-hydroxycamptothecin and the 10,11-dihydroxycamptothecin analogs occur naturally as one of the minor components in *C. Acuminata* and its relatives. Additional substitutions to these compounds, i.e. 7-alkyl-, 7-substituted alkyl-, 7-amino-, 7-aminoalkyl-, 7-aralkyl-, 9-alkyl-, 9-aralkyl- camptothecin etc.

WO 2011/011474

PCT/US2010/042686

derivatives can be made using known synthetic techniques without undue experimentation.
Some camptotheca alkaloids have the structure shown below:

(IV)



5 wherein

R₇ is selected among NO₂, NH₂, N₃, hydrogen, halogen, F, Cl, Br, I, COOH, OH, O-C₁₋₈ alkyl, SH, S-C₁₋₃ alkyl, CN, CH₂NH₂, NH-C₁₋₃ alkyl, CH₂-NH-C₁₋₃ alkyl, N(C₁₋₃ alkyl)₂, CH₂N(C₁₋₃ alkyl)₂, O-, NH- and S-CH₂CH₂N(CH₂CH₂OH)₂, O-, NH- and S-CH₂CH₂CH₂N(CH₂CH₂OH)₂, O-, NH- and S-CH₂CH₂N(CH₂CH₂CH₂OH)₂, O-, NH- and S-CH₂CH₂CH₂N(CH₂CH₂CH₂OH)₂, O-, NH- and S-CH₂CH₂N(C₁₋₃ alkyl)₂, O-, NH- and S-CH₂CH₂CH₂N(C₁₋₃ alkyl)₂, CHO and C₁₋₃ alkyl;

R₈ is selected among hydrogen, C₁₋₈ alkyl and CH₂NR₉R₁₀,

wherein

R₉ is selected from the group consisting of hydrogen, C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkyl-C₁₋₆ alkyl, C₂₋₆ alkenyl, hydroxy-C₁₋₆ alkyl, and C₁₋₆ alkoxy-C₁₋₆ alkyl; and

R₁₀ is selected among hydrogen, C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkyl-C₁₋₆ alkyl, C₂₋₆ alkenyl, hydroxy-C₁₋₆ alkyl, C₁₋₆ alkoxy-C₁₋₆ alkyl, and COR₁₁, wherein R₁₁ is selected from the group consisting of hydrogen, C₁₋₆ alkyl, perhalo-C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkyl-C₁₋₆ alkyl, C₂₋₆ alkenyl, hydroxy-C₁₋₆ alkyl, C₁₋₆ alkoxy, and C₁₋₆ alkoxy-C₁₋₆ alkyl;

R₁₁₀-R₁₁₁ are each independently selected among hydrogen, halo, acyl, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, alkynyl, cycloalkyl, hydroxy, cyano, nitro, azido, amido, hydrazine, amino, substituted amino, hydroxycarbonyl, alkoxy carbonyl, alkylcarbonyloxy, alkylcarbonylamino, carbamoyloxy, arylsulfonyloxy, alkylsulfonyloxy, -C(R₁₁₇)=N-(O)_j-R₁₁₈ wherein R₁₁₇ is H, alkyl, alkenyl, cycloalkyl, or aryl, (j) is 0 or 1, and R₁₁₈ is H, alkyl, alkenyl, cycloalkyl, or heterocycloalkyl, and R₁₁₉C(O)O- wherein R₁₁₉ is halogen, amino, substituted amino, heterocycloalkyl, substituted heterocycloalkyl, or R₁₂₀-O-

WO 2011/011474

PCT/US2010/042686

(CH₂)_k- where (k) is an integer of 1-10 and R₁₂₀ is alkyl, phenyl, substituted phenyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, or substituted heterocycloalkyl; or

R₇ together with R₁₁₀, or R₁₁₀ together with R₁₁₁, form substituted or unsubstituted methylenedioxy, ethylenedioxy, or ethyleneoxy; and

5 R₁₁₂ is H or OR', wherein R' is alkyl, alkenyl, cycloalkyl, haloalkyl, or hydroxyalkyl.

Preferred aryl groups are phenyl and naphthyl. Preferred heterocycloalkyl rings include bipiperidine. Suitable heterocyclic rings when R₉ and R₁₀ are taken together with the nitrogen atom to which they are attached include: aziridine, azetidine, pyrrolidine, piperidine, hexamethylenimine, imidazolidine, pyrazolidine, isoxazolidine, piperazine, N-

10 methylpiperazine, tetrahydroazepine, N-methyl-tetrahydroazepine, thiazolidine, etc.

For ease of description and not limitation, the description refers to 7-ethyl-10-hydroxycamptothecin, or CPT-11 as the camptothecin analog, as the preferred and illustrated compound. It will be understood that the claimed invention includes all such derivatives and analogs so long as the analog has an OH, such as the 20-OH group, for the point of

15 attachment to the polymer. The camptothecin or camptothecin analogs can be racemic mixtures or optically pure isomer. Preferably, a substantially pure and active form of such as the 20(S) camptothecin or camptothecin analog is employed in the multi-arm polymeric prodrugs.

20 4. SYNTHESIS OF POLYMERIC COMPOUNDS

Generally, the polymeric compounds employed in the treatment described herein are prepared by reacting one or more equivalents of an activated multi-arm polymer with, for example, one or more equivalents per active site of amino acid-(20)-7-ethyl-10-hydroxycamptothecin under conditions which are sufficient to effectively cause the amino

25 group to undergo a reaction with the carboxylic acid of the polymer and form a linkage.

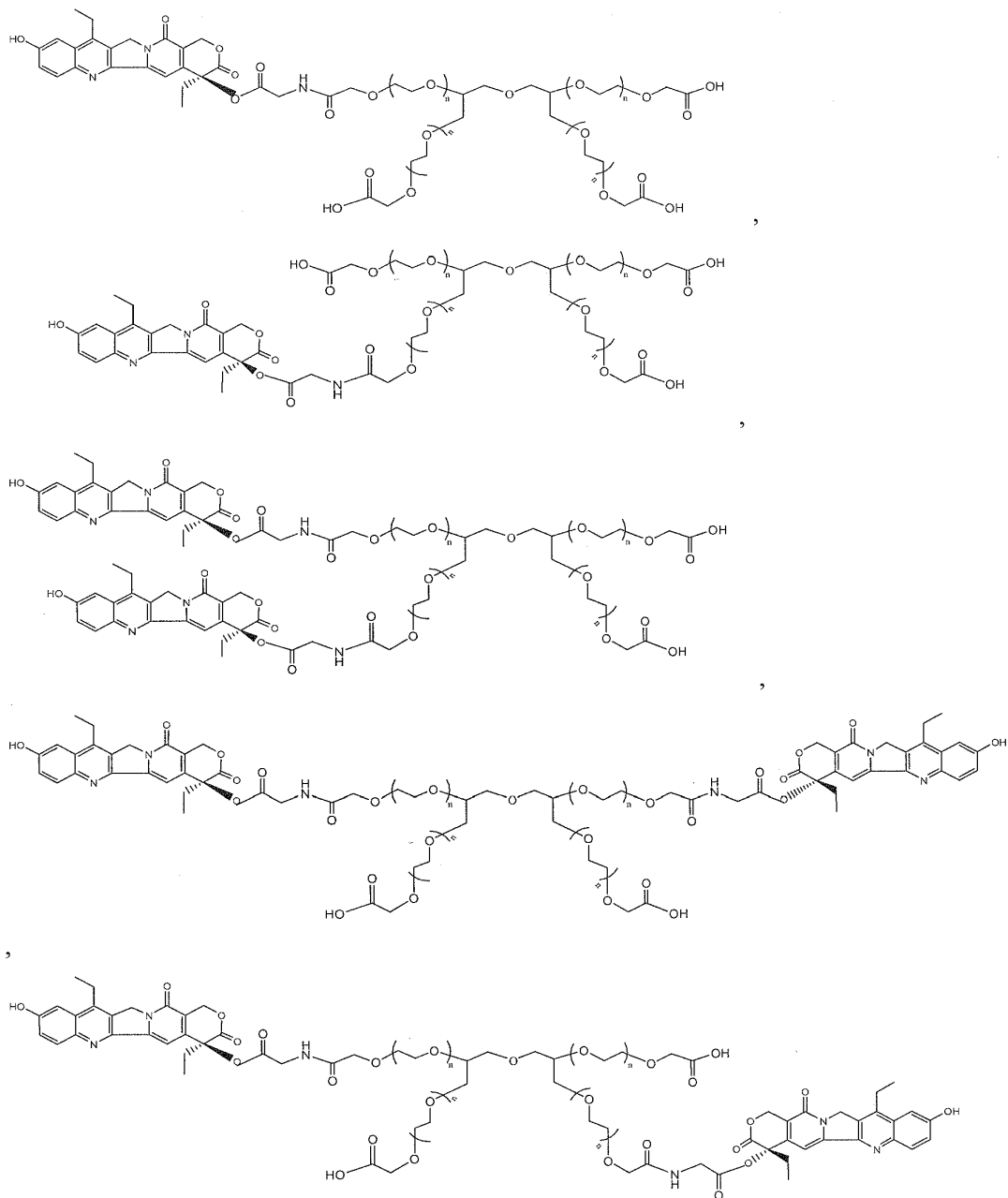
Details of the synthesis are described in U.S. Patent No. 7,462,627, the contents of which are incorporated herein by reference in its entirety.

Examples of preferred bifunctional linker groups include glycine, alanine, methionine, sarcosine, etc. and syntheses are described in the Examples of U.S. Patent No. 7,462,627.

30 According to the present invention, the compounds administered include:

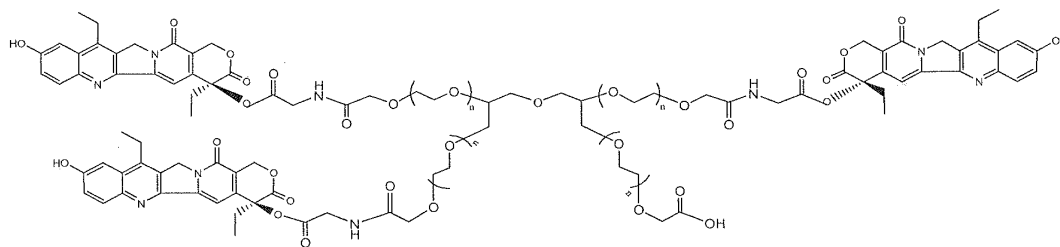
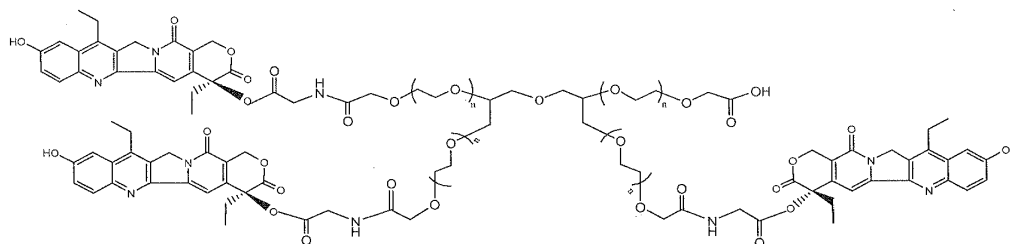
WO 2011/011474

PCT/US2010/042686

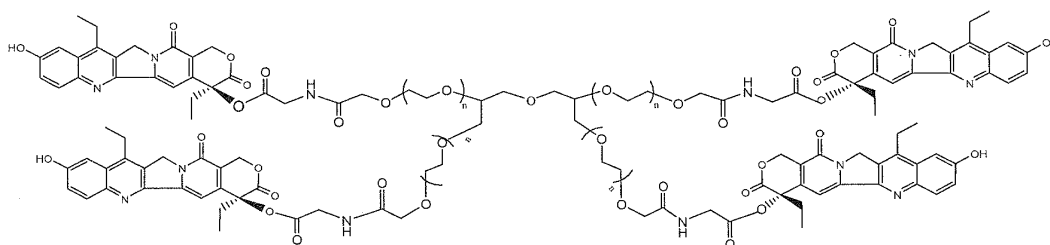


WO 2011/011474

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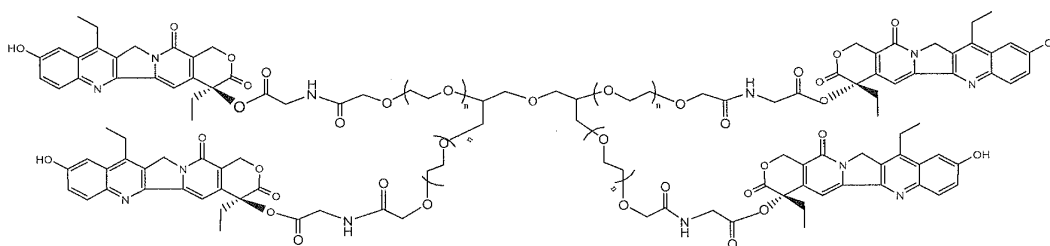


and



5

One particularly preferred embodiment includes administering a compound having the structure: (Ia)

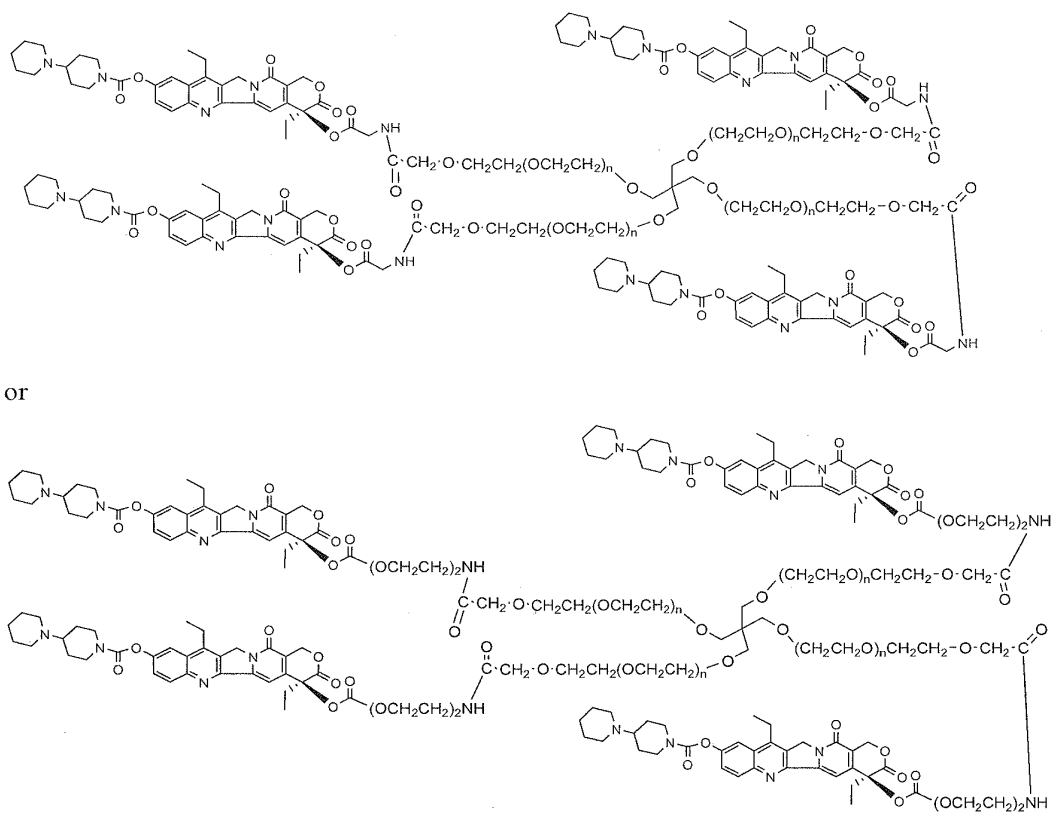


- 10 wherein all four arms of the polymer are conjugated to 7-ethyl-10-hydroxycamptothecin through glycine and the polymer portion has the total number average molecular weight of about 40,000 daltons.

An alternative embodiment useful in the treatment described herein includes

WO 2011/011474

PCT/US2010/042686



wherein (n) is an integer of from about 28 to about 341, so that the total molecular weight of the polymeric portion of the compound of Formula (II) ranges from about 5,000 to about 60,000 daltons, preferably about 20,000 or 40,000 daltons.

In a further embodiment, the treatment described herein employs polymeric compounds described in WO2005/028539, the contents of which are incorporated herein by reference in its entirety.

C. HER2 ANTAGONISTS

Many types of cancers have been associated with increased levels of the HER2 protein and gene. The HER2 protein catalyzes the transfer of the terminal phosphate from ATP to tyrosine residues of protein substrates. The HER2 receptor antagonist and HER2 antagonist generally refer to compounds which inhibit function or expression of the HER2 protein or gene. HER2 receptor antagonists can inhibit HER2 receptor function directly or via downstream or upstream cellular signaling pathway in which the HER2 protein is involved. In particular, HER2 receptor antagonists used in the combination treatment

WO 2011/011474

PCT/US2010/042686

described herein includes anti-HER2 antibodies and antisense HER2 oligonucleotides which directly inhibit HER2 receptor function or expression of HER2 receptor, instead of inhibiting the HER2 receptor function via downstream or upstream signaling pathway.

In one embodiment, the combination treatment described herein is conducted by administering an anti-HER2 receptor antibody in combination with a compound of Formula (I) (or Formula (II) or (III)). The antibody binds to the HER2 receptor protein (p185). Preferably, the antibody useful in the treatment described herein binds to the extracellular domain of the HER2 receptor such as HER2 domain II and/or IV. One particular embodiment employs trastuzumab. Another particular embodiment employs pertuzumab.

Trastuzumab under the tradename Herceptin® is a recombinant humanized monoclonal antibody directed against the human epidermal growth factor receptor 2 (HER2). After binding to HER2 receptor on the tumor cell surface, trastuzumab induces an antibody-dependent cell-mediated cytotoxicity against tumor cells that overexpress HER2 receptor. HER2 is overexpressed by many adenocarcinomas, particularly breast adenocarcinomas. Trastuzumab is registered in CAS Registry No. 180288-69-1. Detailed information about trastuzumab is described in U.S. Patent No. 6,165,464, the contents of which are incorporated herein by reference.

Pertuzumab under the tradename Omnitarg™ is also a recombinant humanized monoclonal antibody (2C4) directed against the extracellular dimerization domain of the HER2 receptor. (CAS Registry No. 380610-27-5). Pertuzumab binds to the dimerization domain of the HER2 receptor and inhibits the ability of the HER2 receptor protein to dimerize with other HER tyrosine kinase receptor proteins. The inhibition of receptor protein dimerization prevents the activation of HER signaling pathways, resulting in tumor cell apoptosis. Pertuzumab, also known as rhuMab 2C4, is described, for example in U.S. Patent Nos. 6,949,245 and 5,821,337, incorporated by reference herein.

In another embodiment, the methods described herein can be conducted wherein the compound of Formula (I) (or Formula (II) or (III)) is administered with antisense HER2 (ErbB2) oligonucleotides or pharmaceutically acceptable salt thereof. The antisense HER2 oligonucleotides can be administered concurrently or sequentially.

In one embodiment, the antisense HER2 oligonucleotide includes nucleic acids complementary to at least 8 consecutive nucleotides of HER2 pre-mRNA or mRNA.

An “oligonucleotide” is generally a relatively short polynucleotide, e.g., ranging in size from about 2 to about 200 nucleotides, or preferably from about 8 to about 50

WO 2011/011474

PCT/US2010/042686

nucleotides, or more preferably from about 8 to about 30 nucleotides. The oligonucleotides according to the invention are generally synthetic nucleic acids, and are single stranded, unless otherwise specified. The terms, "polynucleotide" and "polynucleic acid" may also be used synonymously herein.

5 The oligonucleotides (analogues) are not limited to a single species of oligonucleotide but, instead, are designed to work with a wide variety of such moieties. The nucleic acids molecules contemplated can include a phosphorothioate internucleotide linkage modification, sugar modification, nucleic acid base modification and/or phosphate backbone modification. The oligonucleotides can contain natural phosphorodiester backbone or phosphorothioate
10 backbone or any other modified backbone analogues such as LNA (Locked Nucleic Acid), PNA (nucleic acid with peptide backbone), CpG oligomers, and the like, such as those disclosed at Tides 2002, Oligonucleotide and Peptide Technology Conferences, May 6-8, 2002, Las Vegas, NV and Oligonucleotide & Peptide Technologies, 18th & 19th November 2003, Hamburg, Germany, the contents of which are incorporated herein by reference.

15 Modifications to the oligonucleotides contemplated by the invention include, for example, the addition or substitution of functional moieties that incorporate additional charge, polarizability, hydrogen bonding, electrostatic interaction, and functionality to an oligonucleotide. Such modifications include, but are not limited to, 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications,
20 modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodouracil, backbone modifications, methylations, base-pairing combinations such as the isobases isocytidine and isoguanidine, and analogous combinations. Oligonucleotides contemplated within the scope of the present invention can also include 3' and/or 5' cap structure.

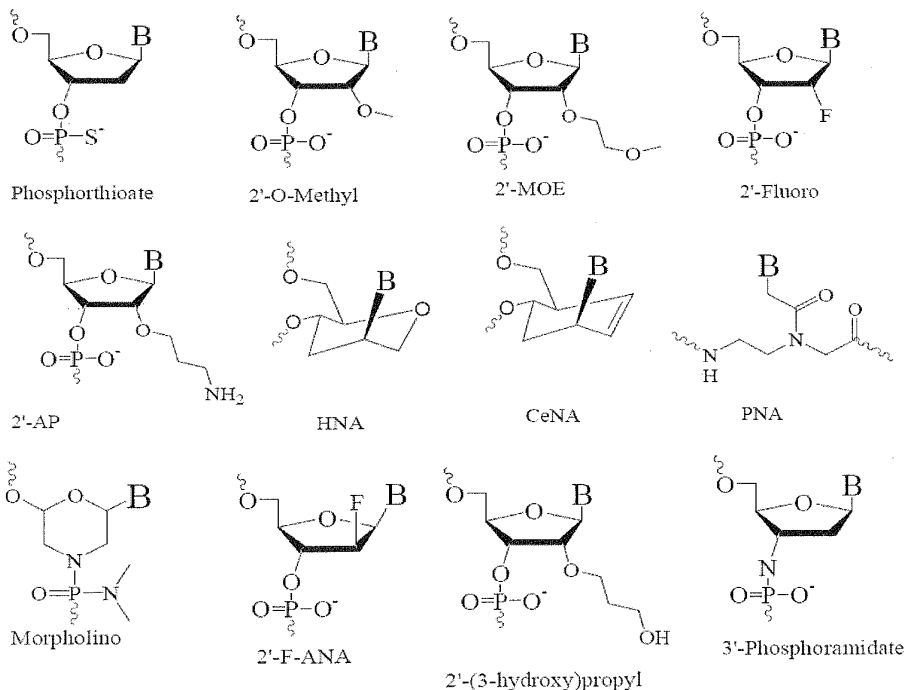
25 For purposes of the present invention, "cap structure" shall be understood to mean chemical modifications, which have been incorporated at either terminus of the oligonucleotide. The cap can be present at the 5'-terminus (5'-cap) or at the 3'-terminus (3'-cap) or can be present on both termini. A non-limiting example of the 5'-cap includes inverted abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl)
30 nucleotide, 4'-thio nucleotide, carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; threopentofuransyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-

WO 2011/011474

PCT/US2010/042686

- inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety. Details are described in WO 97/26270, incorporated by
- 5 reference herein. The 3'-cap can include for example 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate, 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate;
- 10 threo-pentofuransyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties.
- 15 See also Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; the contents of which are incorporated by reference herein.

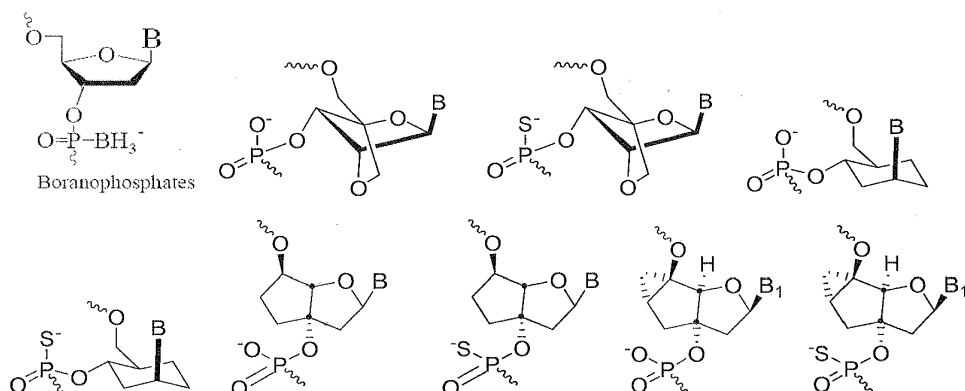
A non-limiting list of nucleoside analogs have the structure:



20

WO 2011/011474

PCT/US2010/042686



See more examples of nucleoside analogues described in Freier & Altmann; *Nucl. Acid Res.*, 1997, 25, 4429-4443 and Uhlmann; *Curr. Opinion in Drug Development*, 2000, 3(2), 293-

5 213, the contents of each of which are incorporated herein by reference.

The term "antisense," as used herein, refers to nucleotide sequences which are complementary to a specific DNA or RNA sequence that encodes a gene product or that encodes a control sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. In the normal operation of cellular

10 metabolism, the sense strand of a DNA molecule is the strand that encodes polypeptides and/or other gene products. The antisense strand serves as a template for synthesis of a messenger RNA ("mRNA") transcript (a sense strand) which, in turn, directs synthesis of any encoded gene product. Antisense nucleic acid molecules may be produced by any art-known methods, including synthesis by ligating the gene(s) of interest in a reverse orientation to a

15 viral promoter which permits the synthesis of a complementary strand. Once introduced into a cell, this transcribed strand combines with natural sequences produced by the cell to form duplexes. These duplexes then block either the further transcription or translation. The designations "negative" or (-) are also art-known to refer to the antisense strand, and "positive" or (+) are also art-known to refer to the sense strand.

20 For purposes of the present invention, "complementary" shall be understood to mean that a nucleic acid sequence forms hydrogen bond(s) with another nucleic acid sequence. A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule which can form hydrogen bonds, i.e., Watson-Crick base pairing, with a second nucleic acid sequence, i.e., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and

25 100% complementary. "Perfectly complementary" means that all the contiguous residues of

WO 2011/011474

PCT/US2010/042686

a nucleic acid sequence form hydrogen bonds with the same number of contiguous residues in a second nucleic acid sequence.

The oligonucleotides or oligonucleotide derivatives useful in the method described herein can include from about 10 to about 1000 nucleic acids, and preferably relatively short polynucleotides, *e.g.*, ranging in size from about 8 to about 30 nucleotides in length (*e.g.*, about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30).

In one aspect of useful nucleic acids used in the method described herein, oligonucleotides and oligodeoxynucleotides with natural phosphodiester backbone or phosphorothioate backbone or any other modified backbone analogues include:

- 10 LNA (Locked Nucleic Acid);
- PNA (nucleic acid with peptide backbone);
- short interfering RNA (siRNA);
- microRNA (miRNA);
- nucleic acid with peptide backbone (PNA);
- 15 phosphorodiamidate morpholino oligonucleotides (PMO);
- tricyclo-DNA;
- decoy ODN (double stranded oligonucleotide);
- catalytic RNA sequence (RNAi);
- ribozymes;
- 20 aptamers;
- spiegelmers (L-conformational oligonucleotides);
- CpG oligomers, and the like, such as those disclosed at:

Tides 2002, Oligonucleotide and Peptide Technology Conferences, May 6-8, 2002, Las Vegas, NV and Oligonucleotide & Peptide Technologies, 18th & 19th November 2003, Hamburg, Germany, the contents of which are incorporated herein by reference.

In another aspect of the nucleic acids used in the method described herein, oligonucleotides can optionally include any suitable art-known nucleotide analogs and derivatives, including those listed by Table 1, below:

30 **TABLE 1. Representative Nucleotide Analogs And Derivatives**

4-acetylcytidine	5-methoxyaminomethyl-2-thiouridine
5-(carboxyhydroxymethyl)uridine	beta, D-mannosylqueosine
2'-O-methylcytidine	5-methoxycarbonylmethyl-2-thiouridine
5-methoxycarbonylmethyluridine	5-carboxymethylaminomethyl-2-thiouridine
5-methoxyuridine	5-carboxymethylaminomethyluridine

WO 2011/011474

PCT/US2010/042686

Dihydrouridine	2-methylthio-N6-isopentenyladenosine
2'-O-methylpseudouridine	N-[(9-beta-D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl]threonine
D-galactosylqueuosine	N-[(9-beta-D-ribofuranosylpurine-6-yl)N-methylcarbamoyl]threonine
2'-O-methylguanosine	uridine-5-oxyacetic acid-methylester
2'-halo-adenosine	2'-halo-cytidine
2'-halo-guanosine	2'-halo-thymine
2'-halo-uridine	2'-halo-methylcytidine
2'-amino-adenosine	2'-amino-cytidine
2'-amino-guanosine	2'-amino-thymine
2'-amino-uridine	2'-amino-methylcytidine
Inosine	uridine-5-oxyacetic acid
N6-isopentenyladenosine	Wybutoxosine
1-methyladenosine	Pseudouridine
1-methylpseudouridine	Queuosine
1-methylguanosine	2-thiocytidine
1-methylinosine	5-methyl-2-thiouridine
2,2-dimethylguanosine	2-thiouridine
2-methyladenosine	4-thiouridine
2-methylguanosine	5-methyluridine
3-methylcytidine	N-[(9-beta-D-ribofuranosylpurine-6-yl)-carbamoyl]threonine
5-methylcytidine	2'-O-methyl-5-methyluridine
N6-methyladenosine	2'-O-methyluridine
7-methylguanosine	Wybutosine
5-methylaminomethyluridine	3-(3-amino-3-carboxy-propyl)uridine
Locked-adenosine	Locked-cytidine
Locked-guanosine	Locked-thymine
Locked-uridine	Locked-methylcytidine

In one particular embodiment, the antisense HER2 (ErbB2) oligonucleotide includes nucleotides that are complementary to at least 8 consecutive nucleotides of the sequence set forth in SEQ ID NO: 1 (GenBank Accession No. X03363). *See also*, Yamamoto, T. et al.

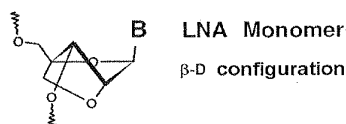
- 5 Nature 319:230-234, 1986; *Papewalis, J. et al. Nucleic Acids Res.* 1:5452, 1991, the contents of each of which are incorporated herein by reference in its entirety.

Preferably, the oligonucleotides according to the invention described herein include one or more phosphorothioate internucleotide linkages (backbone) and one or more locked nucleic acids (LNA). Preferably, LNA monomers include 2'-O, 4'-C methylene

- 10 bicyclonucleotide as shown below:

WO 2011/011474

PCT/US2010/042686



D. SELECTION OF PATIENTS WITH HER2 POSITIVE CANCER

The treatment described herein benefits patients having a HER2 positive cancer. The treatment described herein significantly extends survival. Selection of patients having a HER2 positive cancer to receive the treatment described herein is predetermined by measuring levels of HER2 expression. In addition to HER2 expression levels, the patient's clinical history should be considered in selecting patients for the treatment described herein.

HER2 protein or gene levels can be measured by techniques known in the art, including, but not limited to, immunohistochemistry (IHC), silver *in situ* hybridization (SISH), chromogenic *in situ* hybridization (CISH), fluorescence *in situ* hybridization (FISH), virtual karyotyping, PCR-based methods, and other methods known in the art. Each type of assays has strength and limitations. Thus, selection of patients with a HER2 positive cancer can be more reliable when confirmed by a combination of the assays, rather than relying on a single assay to rule out potential benefit of the treatment described herein. Currently, the recommended assays are a combination of IHC and FISH.

Generally speaking, HER2 expression in cells or tissues can be assessed by measuring levels of HER2 receptor protein expression or HER2 gene amplification. FDA has approved several HER2 tests which aid to determine HER2 expression and there are several tests commercially available. These assays utilize IHC and/or FISH assays. For example, the commercially available assays include Dako HercepTest™ (Carpinteria, CA, USA) and Ventana Pathway® HER-2/neu (AZ, USA), which measure the level of HER2 protein (IHC assays); and PathVysion® and HER2 FISH pharmDx™, which measure the level of gene amplification (FISH assays). Detailed information and guidelines for assessing HER2 expression in a test specimen are provided in the package insert of each of the test kits, the contents of the package insert of each of the aforementioned test kits are incorporated herein by reference. The assessment and validation of HER2 expression measurement should follow guidelines found in the package insert of the test kits.

Both HercepTest™ and Pathway™ test kits utilize IHC and measure levels of HER2 protein in a test tissue/cell. These are highly standardized, semi-quantitative assays.

WO 2011/011474

PCT/US2010/042686

Interpretation of IHC test results use scoring system on a scale of 0 to 3+: 0 (negative), 1+ (negative), 2+ (borderline/weak positive), or 3+ (positive), based on the reviewer's interpretation of staining intensity and completeness of cell membrane staining. Score 0 indicates that there are <20,000 receptors per cell, and that there is no visible membrane staining or membrane staining is observed in less than 10 % of the tumor cells. Score 1+ indicates there are ~100,000 receptors per cell, that a faint membrane staining is observed in more than 10% of the tumor cells, but the membranes are partially stained. Score 2+ indicates that there are ~500,000 receptors per cell, that a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells. Score 3+ indicates that there are ~2,000,000 receptors per cell, and that a strong complete membrane staining is obtained in more than 10% of the tumor cells.

With FISH testing, tumors are interpreted as HER2 negative (FISH-) or positive (FISH+) by counting the HER2/neu gene copy number. The presence of HER2 protein overexpression and gene amplification are highly correlated. FISH analysis reveals that some patients with IHC 2+ or IHC 3+ do not have gene amplification (FISH-), suggesting that these patients may be false positives. According to PathVysion® technology, approximately 40% of IHC-positive patients (2+/ 3+) did not have gene amplification, FISH negative. Thus, it is recommended that a patient with IHC 2+ be referred to a FISH test.

The combination treatment described herein is preferably given to IHC 2+ or 3+ and/or FISH+ patients. Further, the combination treatment can be given to patients with a HER2 status comparable to IHC 2+ or 3+ and/or FISH+, when other techniques such as PCR methods are used for selection of HER2 positive patients.

E. COMPOSITIONS/FORMULATIONS

Pharmaceutical compositions containing the polymer conjugates described herein and the HER2 antagonists may be manufactured by processes well known in the art, e.g., using a variety of well-known mixing, dissolving, granulating, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. The compositions may be formulated in conjunction with one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Parenteral routes are preferred in many aspects of the invention.

WO 2011/011474

PCT/US2010/042686

For injection, including, without limitation, intravenous, intramuscular and subcutaneous injection, the compounds of Formula (I) (alternatively, Formula (II) or (III)) described herein may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as physiological saline buffer or polar solvents including, without
5 limitation, a pyrrolidone or dimethylsulfoxide.

The compounds described herein may also be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers. Useful compositions include, without limitation, suspensions, solutions or emulsions in oily or aqueous vehicles,
10 and may contain adjuncts such as suspending, stabilizing and/or dispersing agents. Pharmaceutical compositions for parenteral administration include aqueous solutions of a water soluble form, such as, without limitation, a salt (preferred) of the active compound. Additionally, suspensions of the active compounds may be prepared in a lipophilic vehicle. Suitable lipophilic vehicles include fatty oils such as sesame oil, synthetic fatty acid esters
15 such as ethyl oleate and triglycerides, or materials such as liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers and/or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active
20 ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use. In one embodiment, trastuzumab can be reconstituted with bacteriostatic water for injection.

For oral administration, the compounds can be formulated by combining the active compounds with pharmaceutically acceptable carriers well-known in the art. Such carriers
25 enable the compounds of the invention to be formulated as tablets, pills, lozenges, dragees, capsules, liquids, gels, syrups, pastes, slurries, solutions, suspensions, concentrated solutions and suspensions for diluting in the drinking water of a patient, premixes for dilution in the feed of a patient, and the like, for oral ingestion by a patient. Pharmaceutical preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and
30 processing the mixture of granules, after adding other suitable auxiliaries if desired, to obtain tablets or dragee cores. Useful excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol, cellulose preparations such as, for example, maize starch, wheat starch, rice starch and potato starch and other materials such as gelatin, gum

WO 2011/011474

PCT/US2010/042686

tragacanth, methyl cellulose, hydroxypropyl- methylcellulose, sodium carboxy-methylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid. A salt such as sodium alginate may also be used.

- 5 For administration by inhalation, the compounds of the present invention can conveniently be delivered in the form of an aerosol spray using a pressurized pack or a nebulizer and a suitable propellant.

- The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other
10 glycerides.

- In addition to the formulations described previously, the compounds may also be formulated as depot preparations. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. A compound of this invention may be formulated for this route of administration with
15 suitable polymeric or hydrophobic materials (for instance, in an emulsion with a pharmacologically acceptable oil), with ion exchange resins, or as a sparingly soluble derivative such as, without limitation, a sparingly soluble salt.

 Other delivery systems such as liposomes and emulsions can also be used.

- Additionally, the compounds may be delivered using a sustained-release system, such
20 as semi-permeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the particular compound, additional stabilization
25 strategies may be employed.

F. DOSAGES

- For any compound used in the methods of the present invention, the therapeutically effective amount can be estimated initially from *in vitro* assays. Then, the dosage can be
30 formulated for use in animal models so as to achieve a circulating concentration range that includes the effective dosage. Such information can then be used to more accurately determine dosages useful in patients.

WO 2011/011474

PCT/US2010/042686

The amount of the composition, e.g., used as a prodrug, that is administered will depend upon the parent molecule included therein (in this case, 7-ethyl-10-hydroxycamptothecin). Generally, the amount of prodrug used in the methods described herein is that amount which effectively achieves the desired therapeutic result in mammals. Naturally, the dosages of the various prodrug compounds can vary somewhat depending upon the parent compound, rate of *in vivo* hydrolysis, molecular weight of the polymer, etc. In addition, the dosage, of course, can vary depending upon the dosage form and route of administration.

In general, however, the polymeric ester derivatives of 7-ethyl-10-hydroxycamptothecin described herein can be administered in amounts ranging from about 0.3 to about 90 mg/m² body surface, and preferably from about 0.5 to about 50 mg/m² body surface/dose, yet preferably from about 1 to about 18 mg/m² body surface/dose, and even more preferably from about 1.25 mg/m² body surface/dose to about 16.5 mg/m² body surface/dose for systemic delivery. Some particular doses include one of the following: 1.25, 2.5, 5, 9, 10, 12, 13, 14, 15, 16 and 16.5 mg/m²/dose. One preferred dosage includes 5 mg/m² body surface/dose.

The compounds of Formula (I) (or Formula (II) or (III)) described herein can be administered in amounts ranging from about 0.3 to about 90 mg/m² body surface/week such as, for example, from about 1 to about 18 mg/m² body surface/week. In particular embodiments, the dose regimens can be, for example, from about 5 to about 7 mg/m² body surface weekly for 3 weeks in 4-week cycles, from about 1.25 to about 45 mg/m² one injection every 3 weeks, and/or from about 1 to about 16 mg/m² three injections weekly in a four week cycle.

The treatment protocol can be based, for example, on a single dose administered once every three weeks or divided into multiple doses which are given as part of a multi-week treatment protocol. Thus, the treatment regimens can include, e.g., one dose every three weeks for each treatment cycle and, alternatively one dose weekly for three weeks followed by one week off for each cycle. It is also contemplated that the treatment will be given for one or more cycles until the desired clinical result is obtained.

The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the prodrug selected based on clinical experience and the treatment indication. Moreover, the exact formulation, route of administration and dosage can be selected by the individual physician in view of the patient's condition. The precise dose will depend on the stage and severity of the condition, and the individual characteristics of the

WO 2011/011474

PCT/US2010/042686

patient being treated, as will be appreciated by one of ordinary skill in the art.

Additionally, toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals using methods well-known in the art.

5 In some preferred embodiments, the treatment protocol includes administering the amount ranging from about 1.25 to about 16.5 mg/m² body surface/dose weekly for three weeks, followed by one week without treatment and repeating for about 3 cycles or more until the desired results are observed. The amount administered per each cycle can range from about 2.5 to about 16.5 mg/m² body surface/dose.

10 In one particular embodiment, the polymeric ester derivatives of 7-ethyl-10-hydroxycamptothecin can be administered in one dose, such as 5, 9 or 10 mg/m² weekly for three weeks, followed by one week without treatment. The dosage of the treatment cycle can be designed as an escalating dose regimen when two or more treatment cycles are applied. The polymeric drug is preferably administered via IV infusion.

15 In another particular embodiment, the compound of Formula (I) (or Formula (II) or (III)) is administered in a dose from about 12 to about 16 mg/m² body surface/dose. The dose can be given weekly. The treatment protocol includes administering the compound of Formula (I) (or Formula (II) or (III)) in amounts ranging from about 12 to about 16 mg/m² body surface/dose weekly for three weeks, followed by one week without treatment.

20 In yet another particular embodiment, the dose regiment can be about 10 mg/m² body surface/dose every three weeks.

Alternative embodiments include: for the treatment of pediatric patients, a regimen based on a protocol of about 1.85 mg/m² body surface/dose daily for 5 days every three weeks, a protocol of from about 1.85 to about 7.5 mg/m² body surface/dose daily for 3 days
25 every 25 days, or a protocol of about 22.5 mg/m² body surface/dose once every three weeks, and for the treatment of adult patients, a protocol based on about 13 mg/m² body surface/dose every three weeks or about 4.5 mg/m² body surface/dose weekly for four weeks every six weeks. The compounds described herein can be administered in combination with a second therapeutic agent. In one embodiment, the combination therapy includes a protocol of about
30 0.75 mg/m² body surface/dose daily for 5 days each cycle in combination with a second agent.

Alternatively, the compounds can be administered based on body weight. The dosage range for systemic delivery of a compound of Formula (I) (or Formula (II) or (III)) in a mammal will be from about 1 to about 100 mg/kg/week and is preferably from about 2 to

WO 2011/011474

PCT/US2010/042686

about 60 mg/kg/week. Thus, the amounts can range from about 0.1 mg/kg body weight/dose to about 30 mg/kg body weight/dose, preferably, from about 0.3 mg/kg to about 10 mg/kg. Specific doses such as 5 or 10 mg/kg at q2d x 5 regimen (multiple dose) or 20 or 30 mg/kg on a single dose regimen can be administered.

5 In all aspects of the invention where polymeric conjugates are administered, the dosage amount mentioned is based on the amount of an active agent (preferably, 7-ethyl-10-hydroxycamptothecin) rather than the amount of polymeric conjugate administered. It is contemplated that the treatment will be given for one or more cycles until the desired clinical result is obtained. The exact amount, frequency and period of administration of the
10 compound of the present invention will vary, of course, depending upon the sex, age and medical condition of the patient as well as the severity of the disease as determined by the attending clinician. The weight given above represents the weight of 7-ethyl-10-hydroxycamptothecin present in the PEG-conjugated 7-ethyl-10-hydroxy-camptothecin employed for treatment. The actual weight of the PEG-conjugated 7-ethyl-10-
15 hydroxycamptothecin will vary depending on the loading of the PEG (e.g., optionally from one to four moles of 7-ethyl-10-hydroxycamptothecin per mole of PEG.).

The HER2 antagonists can be administered in combination with the compound of Formula (I) (or Formula (II) or (III)) concurrently or sequentially. The combination therapy protocol includes administering an anti-HER2 antibody ranging from about 0.5/kg to about
20 15 mg/kg body weight, i.e., from about 2 mg/kg to about 8 mg/kg/dose such as 2, 4, 5, 6, 8 mg/kg/dose.

In one embodiment, trastuzumab is administered based on a protocol: initial dose at 4 mg/kg i.v. followed by 2 mg/kg/dose i.v. weekly during and after the combination therapy, or initial dose at 8 mg/kg i.v. followed by 6 mg/kg i.v. every three weeks, until a desired clinical
25 result is achieved. Detailed dosing information of trastuzumab is described in the package insert of Herceptin®, the contents of which are incorporated herein by reference.

In another embodiment, pertuzumab is administered in an amount ranging from about 0.5 to about 15 mg/kg/dose i.v. every three weeks during and after the combination treatment described herein. Pertuzumab is given based on a protocol: 5 mg/kg/dose every three weeks.
30 See *Agus, D.B., et al.*, Journal of Clinical Oncology, 23:2534-2543, 2005, the contents of which are incorporated herein by reference.

The combination therapy protocol includes administering an antisense oligonucleotide in an amount of from about 2 to about 100 mg/kg/dose (e.g., 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30,

WO 2011/011474

PCT/US2010/042686

35, 40, 50, 60, 70, 100 mg/kg/dose). For example, the combination therapy regimen dose includes treatment with an antisense HER2 oligonucleotide in an amount of from about 2 to about 50 mg/kg/dose. Preferably, the antisense oligonucleotide administered in the combination therapy is in an amount of from about 4 to about 25 mg/kg/dose.

5 In one aspect of the combination therapy, the protocol includes administering an antisense HER2 oligonucleotide in an amount of about 4 to about 18 mg/kg/dose weekly, or about 4 to about 9.5 mg/kg/dose weekly during the combination therapy.

In one particular embodiment, the combination therapy protocol includes an antisense HER2 oligonucleotide in an amount of about 4 to about 18 mg/kg/dose weekly for 3 weeks in
10 a six week cycle (i.e. about 8 mg/kg/dose). Another particular embodiment includes about 4 to about 9.5 mg/kg/dose weekly (i.e., about 4.1 mg/kg/dose).

Where the HER2 antagonists encompassed by the present invention are administered in combination with the compounds of Formula (I) (or Formula (II) or (III)) described herein, the individual components of the combinations may be administered either sequentially or
15 simultaneously in separate or combined pharmaceutical formulations by any convenient route. When the HER2 antagonist and the compound of Formula (I) (or Formula (II) or (III)) is administered sequentially, either the compound of Formula (I) (or Formula (II) or (III)) or the HER antagonist may be administered first. For example, the HER2 antagonist and the compound of Formula (I) (or Formula (II) or (III)) may be administered in a sequential
20 manner in a regimen that will provide beneficial effects of the combination. When the HER2 antagonist and the compound of Formula (I) (or Formula (II) or (III)) is administered in a simultaneous manner, the combination may be administered either in the same or different pharmaceutical compositions.

Further aspects of the present invention include combining the HER2 antagonist and
25 the compounds described herein with other chemotherapy or radiotherapy for additive benefit.

EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention.

30

EXAMPLE 1. TOXICITY DATA

A maximum tolerated dose ("MTD") of 4arm-PEG-Gly-(7-ethyl-10-hydroxycamptothecin) (compound **9**) was studied using nude mice. Mice were monitored for

WO 2011/011474

PCT/US2010/042686

14 days for mortality and signs of illness and sacrificed when body weight loss was >20% of the pretreatment body weight.

Table 2, below, shows the maximum tolerated dose of each compound for both single dose and multiple dose administration. Each dose for multiple dose administration was given 5 mice every other day for 10 days and the mice were observed for another 4 days, thus for total 14 days.

TABLE 2. MTD Data in Nude Mice

Compound	Dose Level (mg/kg)	Survival/Total	Comments
Compound 9 Single dose	25	5/5	
	30	5/5	
	35	4/5	Mouse euthanized due to >20% body weight loss
Compound 9 Multiple dose*	10	5/5	
	15	3/5	Mice euthanized due to >20% body weight loss
	20	0/5	Mice euthanized due to >20% body weight loss

The MTD found for 4arm-PEG-Gly-(7-ethyl-10-hydroxycamptothecin) (compound 9) 10 was 30 mg/kg when given as single dose, and 10 mg/kg when given as multiple dose (q2d x 5). 15

EXAMPLE 2. Properties of PEG Conjugates

Table 3, below, shows solubility of four different PEG-(7-ethyl-10- 15 hydroxycamptothecin) conjugates in aqueous saline solution. All four PEG-(7-ethyl-10-hydroxycamptothecin) conjugates showed good solubility of up to 4 mg/mL equivalent of 7-ethyl-10-hydroxycamptothecin. In human plasma, 7-ethyl-10-hydroxycamptothecin was steadily released from the PEG conjugates with a doubling time of 22 to 52 minutes and the release appeared to be pH and concentration dependent as described in the following 20

EXAMPLE 3.

TABLE 3. Properties of PEG-7-ethyl-10-hydroxycamptothecin Conjugates

WO 2011/011474

PCT/US2010/042686

Compound	Solubility in Saline (mg/mL) ^a	t _{1/2} (min) in Human Plasma ^b	Doubling Time in Plasma (min) ^c		
			Human	Mouse	Rat
Compound 9 (Gly)	180	12.3	31.4	49.5	570
Compound 12 (Ala)	121	12.5	51.9	45.8	753
Compound 23 (Sar)	ND	19.0	28.8	43.4	481
Compound 18 (Met)	142	26.8	22.2	41.9	1920

^a 7-ethyl-10-hydroxycamptothecin is not soluble in saline.^b PEG conjugate half life.^c 7-ethyl-10-hydroxycamptothecin formation rate from conjugates.

- 5 PEG-7-ethyl-10-hydroxycamptothecin conjugates show good stability in saline and other aqueous medium for up to 24 hours at room temperature.

EXAMPLE 4. Effects Of Concentration and pH on Stability

10 Based on our previous work, acylation at the 20-OH position protects the lactone ring in the active closed form. The aqueous stability and hydrolysis properties in rat and human plasma were monitored using UV based HPLC methods. 4armPEG-Gly-(7-ethyl-10-hydroxycamptothecin) conjugates were incubated with each sample for 5 minutes at room temperature.

15 Stability of PEG-7-ethyl-10-hydroxycamptothecin conjugates in buffer was pH dependent. Figure 1 shows 4armPEG-Gly-(7-ethyl-10-hydroxycamptothecin) stability in various samples. Figure 2 shows that the rate of 7-ethyl-10-hydroxycamptothecin release from PEG-Gly-(7-ethyl-10-hydroxycamptothecin) increases with increased pH.

EXAMPLE 5. Pharmacokinetic Properties

20 Tumor free Balb/C mice were injected with a single injection of 20 mg/kg 4armPEG-Gly-(7-ethyl-10-hydroxycamptothecin) conjugates. At various time points mice were sacrificed and plasma was analyzed for intact conjugates and released 7-ethyl-10-hydroxycamptothecin by HPLC. Pharmacokinetic analysis was done using non-compartmental analysis (WinNonlin). Details are set forth in Table 4, below.

25

Table 4. Pharmacokinetic Data

Parameter	Compound 9	7-ethyl-10-hydroxycamptothecin Released
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WO 2011/011474

PCT/US2010/042686

		from Compound 9
AUC (h*µg/mL)	124,000	98.3
Terminal t _{1/2} (Hr)	19.3	14.2
C _{max} (µg/mL)	20,500	13.2
CL (mL/hr/kg)	5.3	202
Vss (mL/kg)	131	3094

As shown in Figures 3A and 3B, PEGylation of 7-ethyl-10-hydroxycamptothecin allows long circulation half life and high exposure to native drug 7-ethyl-10-hydroxycamptothecin.

Enterohepatic circulation of 4armPEG-Gly-(7-ethyl-10-hydroxycamptothecin) conjugates

5 was observed. The pharmacokinetic profile of PEG-Gly-(7-ethyl-10-hydroxycamptothecin) in mice was biphasic showing a rapid plasma distribution phase during the initial 2 hours followed by a 18-22 hours terminal elimination half-life for the conjugate and a concomitant 18-26 hours terminal elimination half-life for 7-ethyl-10-hydroxycamptothecin (FIG. 3B).

Additionally, pharmacokinetic profiles of 4arm PEG-Gly-(7-ethyl-10-
10 hydroxycamptothecin) were investigated in rats. In rats, dose levels of 3, 10 and 30 mg/kg (7-ethyl-10-hydroxycamptothecin equivalent) were used. The pharmacokinetic profiles in rats were consistent with those of mice.

In rats, 4arm PEG-Gly-(7-ethyl-10-hydroxycamptothecin) showed a biphasic clearance from the circulation with an elimination half life of 12-18 hours in rats. 7-ethyl-10-
15 hydroxycamptothecin released from 4armPEG-Gly-7-ethyl-10-hydroxycamptothecin conjugates had an apparent elimination half life of 21-22 hours. The maximum plasma concentration (C_{max}) and area under the curve (AUC) increased in a dose dependent manner in rats. The apparent half life of released 7-ethyl-10-hydroxycamptothecin from 4armPEG-Gly conjugates in mice or rats is significantly longer than the reported apparent half life of
20 released 7-ethyl-10-hydroxycamptothecin from CPT-11 and the exposure of released 7-ethyl-10-hydroxycamptothecin from 4arm PEG-Gly-(7-ethyl-10-hydroxycamptothecin) is significantly higher than the reported exposure of released 7-ethyl-10-hydroxycamptothecin from CPT-11. The clearance of the parent compound was 0.35 mL/hr/kg in rats. The estimated volume of distribution at steady state (Vss) of the parent compound was 5.49
25 mL/kg. The clearance of the released 7-ethyl-10-hydroxycamptothecin was 131 mL/hr/kg in rats. The estimated Vss of released 7-ethyl-10-hydroxycamptothecin was 2384 mL/kg in rats. Enterohepatic circulation of released 7-ethyl-10-hydroxycamptothecin was observed both in mice and rats.

WO 2011/011474

PCT/US2010/042686

EXAMPLE 6. Therapeutic Efficacy in Human Breast Tumor Xenografted Mice Refractory to Herceptin®

Therapeutic efficacy of HER2 receptor antagonist-containing therapies against a refractory human JIMT-1 breast tumor grown in nude mice was measured. Tumors were established by implanting small tumor fragments into a single subcutaneous site on the left auxiliary flank region of nude mice. The tumor implantation site was observed twice weekly and measured once palpable. The tumor volume for each mouse was determined by measuring two dimensions with calipers and calculated. When tumors reached an average volume of 100 mm³, the mice were divided into their experimental groups consisting of: untreated controls, Herceptin® only, compound **9** only, and a combination of compound **9** and Herceptin®. Herceptin® was given 5 mg/kg body weight/dose at q7d intraperitoneally. Compound **9** was given 4 mg/kg/dose at q2d x 5 intravenously. For the combination treatment, Herceptin® and compound **9** were given 5 mg/kg/dose at q7d and 4 mg/kg/dose at q2d x 5, respectively. Compound **9** was administered 1 minute following Herceptin® treatment on day 0. On no other day did treatment of compound **9** and Herceptin® coincide. In these experiments, the amount of compound **9** administered was based on the amount of 7-ethyl-10-hydroxycamptothecin, not the amount of polymeric conjugate administered. Mouse weight and tumor sizes were measured at the beginning of the study and at day 28.

The treatment with Herceptin® and compound **9** alone led to about 28% and 51% TGI, respectively as of day 28. The treatment with the combination of Herceptin® and compound **9** resulted in about 81% TGI. The results are set forth in Table 5.

Table 5. Therapeutic Efficacy in JIMT-1 Breast Tumor Xenografted Mouse Model

Treatment	TGI (%)
Control	-
Herceptin® i.p.	28.34
compound 9 i.v.	50.74
Herceptin® i.p. & compound 9 i.v.	80.80

Tumor volume measured at various time points are shown in FIG. 4. A combination of compound **9** and Herceptin® inhibited tumor growth significantly as compared to that of compound **9** or Herceptin® alone. The results show that Herceptin®, when administered with compound **9**, is significantly more effective than either Herceptin® or compound **9** alone in the treatment of breast cancer.

The therapy using compounds described herein in combination with the HER2 receptor antagonist unexpectedly ameliorates and/or avoids resistance associated with HER2

WO 2011/011474

PCT/US2010/042686

antagonist-containing therapy. The human JIMT-1 breast tumor is refractory to a HER2 antibody such as trastuzumab and pertuzumab. The therapy described herein provides ways to treat cancers refractory to HER2 antagonists more effectively by avoiding and reducing potential drug resistance. Patients and clinicians can benefit from unexpected lack and/or reduction of resistance to HER2 antagonist-containing therapy, when a HER2 antagonist is administered together with the compounds described herein.

EXAMPLE 7. Therapeutic Efficacy in Human Gastric Carcinoma Xenografted Mice

Therapeutic efficacy of HER2 receptor antagonist-containing therapies against a human gastric carcinoma N87 grown in nude mice was determined. Human N87 gastric carcinoma was established in nude mice by subcutaneous injection. Groups of mice were randomly divided and treated with Herceptin® alone, compound 9 alone, and a combination of both. Herceptin® was given 20 mg/kg body weight/dose at q7d intraperitoneally. Compound 9 was given 5 mg/kg/dose at q2d x 5 intravenously. For the combination treatment, compound 9 and Herceptin® were given 5 mg/kg/dose at q2d x 5 and 20 mg/kg/dose at q7d, respectively. The amounts of compound 9 administered were based on the amount of 7-ethyl-10-hydroxycamptothecin.

The results are set forth in FIG. 5. Tumors continued to grow in the mice treated with Herceptin® alone. On day 40, tumor volume increased by 613 % compared to day 0. The tumor volume in the mice treated with Herceptin® alone was comparable to the control untreated mice. Herceptin® alone did not inhibit gastric tumor growth. The treatment with compound 9 alone inhibited tumor growth effectively. In the mice treated with the combination of Herceptin® and compound 9, tumor volume decreased by 23% by day 40 compared to day 0. The tumors receiving combined treatment regressed (below baseline values) from day 5 until day 48 of the study. 71% of animals treated with Herceptin® alone were sacrificed by day 52 due to excessive tumor burden ($>1700 \text{ mm}^3$) or tumor ulceration. In the group treated with Herceptin® plus compound 9, 86% of mice survived until day 95 (the last day of the study). Among the 5 surviving mice (71%) treated with four-arm ^{40}K -PEG-Gly-(7-ethyl-10-hydroxycamptothecin), all had tumors $<1500 \text{ mm}^3$ by day 80. The results show that therapeutic efficacy of the HER2 receptor antagonist and survival rate, when administered in combination with the compounds described herein, were enhanced significantly. The treatment described herein provides ways to utilize HER2 antagonist-based therapy more effectively.

WO 2011/011474

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Various references are cited herein. The contents of all of which are hereby incorporated by reference herein in their entireties.

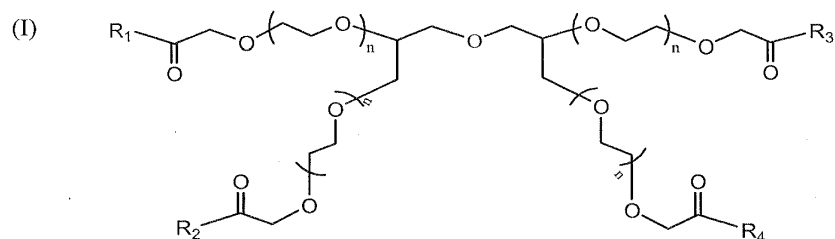
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We claim:

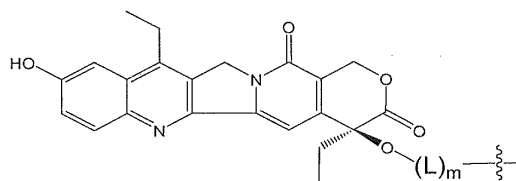
1. A method of treating a HER2 positive cancer in a mammal, comprising administering a HER2 receptor antagonist in combination with an effective amount of a compound of

5 Formula (I):



wherein

R₁, R₂, R₃ and R₄ are independently OH or



10

wherein

L is a bifunctional linker;

(m) is 0 or a positive integer, wherein each L is the same or different when (m)

is equal to or greater than 2; and

(n) is a positive integer;

15

provided that R₁, R₂, R₃ and R₄ are not all OH;

or a pharmaceutically acceptable salt thereof, to said mammal.

2. The method of claim 1, wherein the HER2 receptor antagonist is selected from the group consisting of anti-HER2 antibodies, antisense ErbB2 oligonucleotides, and

20

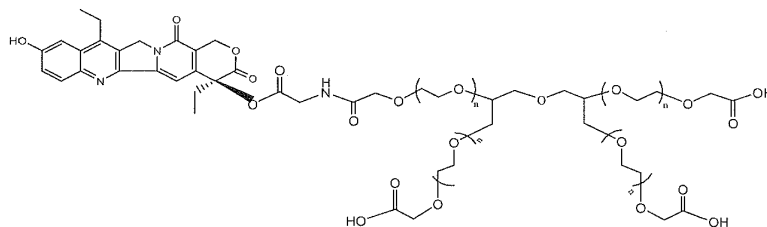
combinations thereof.

3. The method of claim 2, wherein the anti-HER2 antibodies bind to the extracellular domain of HER2 Domain IV or HER2 Domain II.

WO 2011/011474

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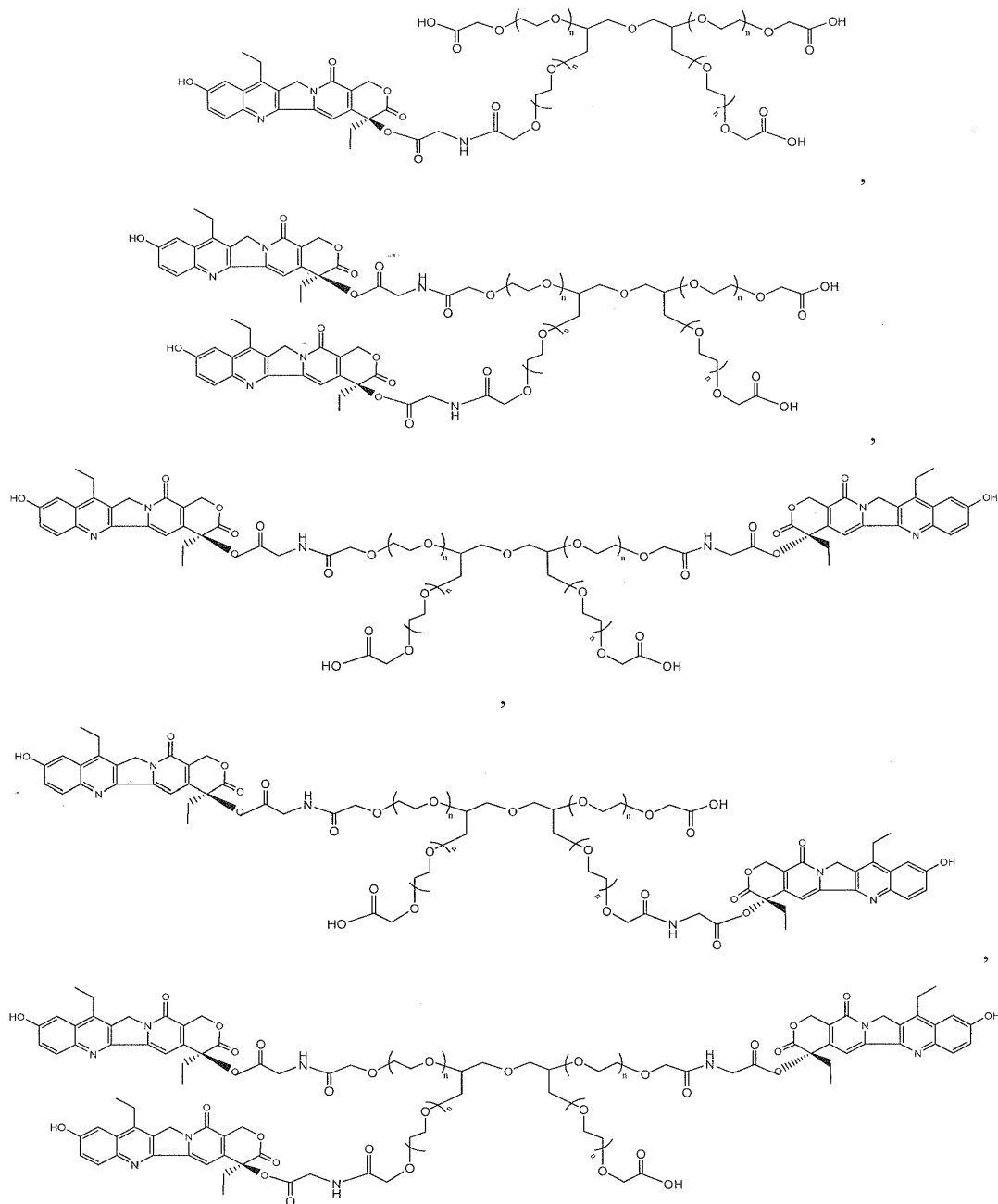
4. The method of claim 2, wherein the anti-HER2 antibody comprises trastuzumab or pertuzumab.
5. The method of claim 1, wherein the HER2 positive cancer is metastatic or non-metastatic.
6. The method of claim 1, wherein the HER2 positive cancer is resistant or refractory to the HER2 receptor antagonist.
- 10 7. The method of claim 1, wherein the HER2 positive cancer is selected from the group consisting of solid tumors, breast cancer, gastric cancer, ovarian cancer, stomach cancer, uterine cancer, uterine serous endometrial carcinoma, prostate cancer, bladder cancer, salivary gland carcinoma, renal adenocarcinoma, and mammary gland carcinoma.
- 15 8. The method of claim 1, wherein (n) is an integer of from about 28 to about 341, so that the total molecular weight of the polymeric portion of the compound of Formula (I) ranges from about 5,000 to about 60,000 daltons.
9. The method of claim 8, wherein (n) is an integer of from about 114 to about 239, so
20 that the total molecular weight of the polymeric portion of the compound of Formula (I) ranges from about 20,000 to about 42,000 daltons.
10. The method of claim 1, wherein the compound of Formula (I) is selected from the group consisting of



25

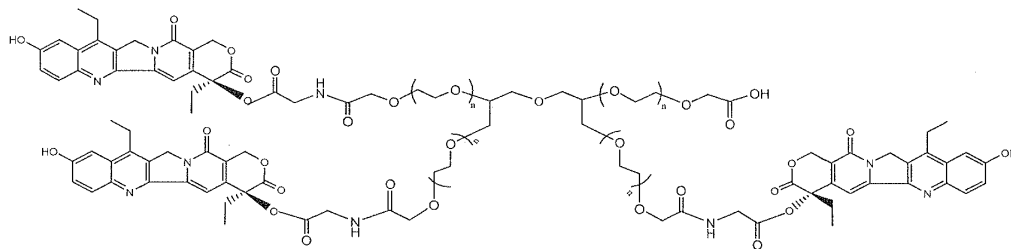
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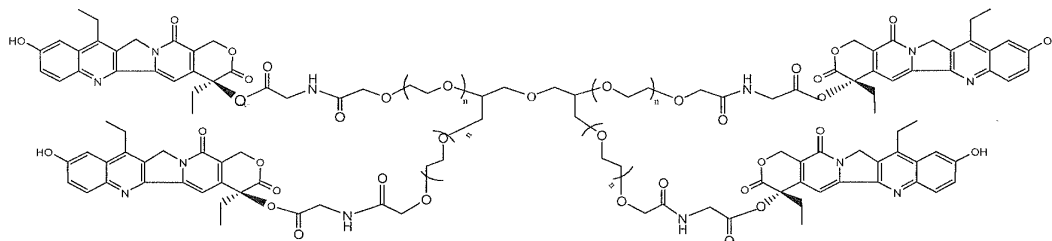


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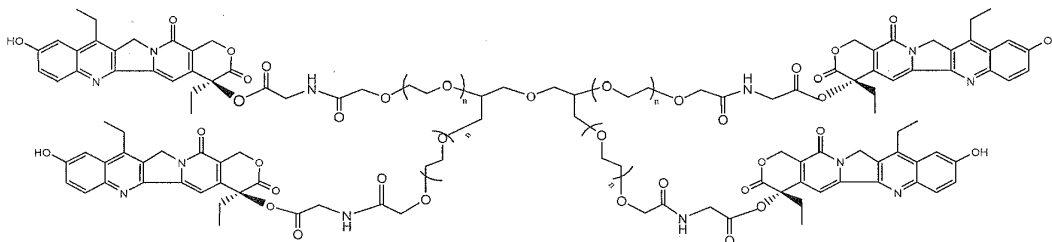


and



5

11. The method of claim 1, wherein the compound of Formula (I) is
(Ia)



10

12. The method of claim 1, wherein the compound of Formula (I) is administered to the mammal, in amounts of from about 0.5 mg/m² body surface/dose to about 50 mg/m² body surface/dose, and wherein the amount is the weight of 7-ethyl-10-hydroxycamptothecin included in the compound of Formula (I).

15

13. The method of claim 1, wherein the compound of Formula (I) is administered to the mammal, in amounts of from about 1 mg/m² body surface/dose to about 18 mg/m² body surface/dose, and the amount is the weight of 7-ethyl-10-hydroxycamptothecin included in

WO 2011/011474

PCT/US2010/042686

the compound of Formula (I).

14. The method of claim 1, wherein the compound of Formula (I) is administered to the mammal, according to a protocol of from about 1.25 mg/m² body surface/dose to about 16.5 mg/m² body surface/dose given weekly for three weeks, followed by 1 week without treatment, and the amount is the weight of 7-ethyl-10-hydroxycamptothecin included in the compound of Formula (I).

15. The method of claim 14, wherein the amount administered to the mammal weekly, is about 5 mg/m² body surface/dose, and the amount is the weight of 7-ethyl-10-hydroxycamptothecin included in the compound of Formula (I).

16. The method of claim 4, wherein the anti-HER2 receptor antibody is administered to the mammal, in an amount of from about 2 mg/kg to about 8 mg/kg.

17. The method of claim 2, wherein the antisense ErbB2 oligonucleotide or a pharmaceutically acceptable salt thereof is administered to the mammal, in combination with the compound of Formula (I) or an pharmaceutically acceptable salt thereof.

18. The method of claim 17, wherein the antisense ErbB2 oligonucleotide is complementary to at least 8 consecutive nucleotides of ErbB2 pre-mRNA or mRNA.

19. The method of claim 17, wherein the antisense ErbB2 oligonucleotide comprises from about 8 to 50 nucleotides in length.

20. The method of claim 17, wherein the antisense ErbB2 oligonucleotide comprises nucleotides that are complementary to at least 8 consecutive nucleotides set forth in SEQ ID NO: 1.

21. The method of claim 17, wherein the antisense ErbB2 oligonucleotide comprises one or more phosphorothioate internucleotide linkages.

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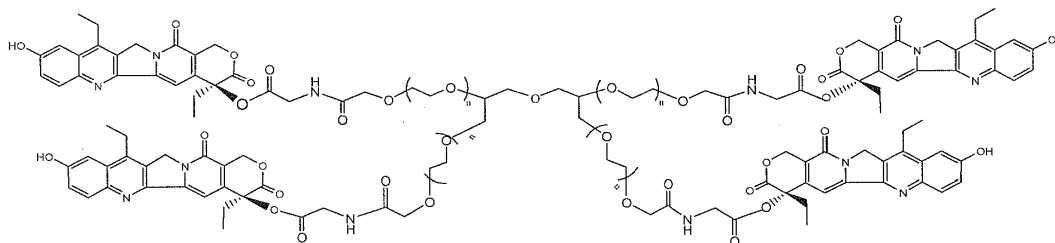
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22. The method of claim 17, wherein the antisense ErbB2 oligonucleotide includes one or more locked nucleic acids (LNA).

23. The method of claim 17, wherein the antisense ErbB2 oligonucleotide is administered
5 in an amount of from about 2 to about 50 mg/kg/dose.

24. The method of claim 1, further comprising determining the presence of a HER2
HER2 positive cancer in the mammal.

10 25. A method of treating a HER2 positive cancer in a mammal, comprising:
(a) identifying a mammal having a HER2 positive cancer by determining the presence,
in the mammal, of a cancer that overexpresses HER2; and
(b) administering, to the mammal, an effective amount of a HER2 receptor antagonist
comprising trastuzumab in combination with an effective amount of a compound of Formula
15 (Ia):



or a pharmaceutically acceptable salt thereof to the mammal having a HER2 positive cancer,
wherein (n) is about 227 so that the total molecular weight of the polymeric portion of
20 the compound of Formula (Ia) is about 40,000 daltons.

26. A method of increasing HER2 receptor antagonist effects in a mammal having a
HER2 positive cancer, comprising administering, to the mammal, a HER2 receptor antagonist
in combination with an effective amount of a compound of Formula (I) of claim 1 or a
25 pharmaceutically acceptable salt thereof.

27. A method of inhibiting the growth or proliferation of HER2 positive cells in a
mammal, comprising

WO 2011/011474

PCT/US2010/042686

(a) determining the presence of a HER2 expression in cells in a mammal; and
 (b) administering a HER2 receptor antagonist, to the mammal, in combination with a compound of Formula (I) of claim 1 or a pharmaceutically acceptable salt thereof to a mammal having HER2 positive cells.

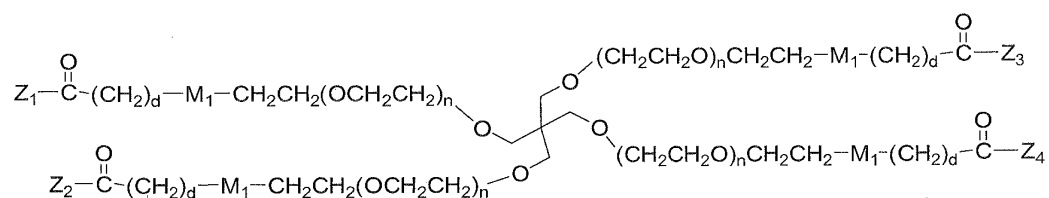
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28. A method of treating a HER2 positive cancer in a mammal, comprising administering to said mammal a HER2 receptor antagonist in combination with an effective amount of a camptothecin, a camptothecin analog, a polymeric conjugate of a camptothecin or analog thereof, or a pharmaceutically acceptable salt thereof.

10

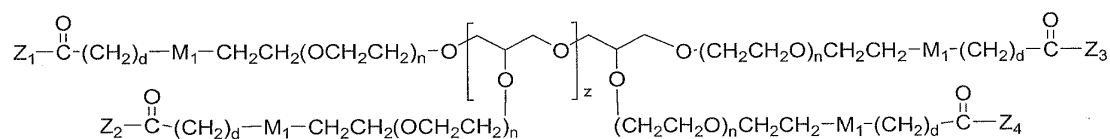
29. The method of claim 28, wherein the polymeric conjugate is a compound of Formula (II) or Formula (III):

(II)



15 or

(III)



wherein

20 Z_1 , Z_2 , Z_3 and Z_4 are independently OH or $(\text{L})_m\text{-D}$;

L is a bifunctional linker;

D is a camptothecin or a camptothecin analog;

M_1 is O, S, or NH;

(d) is zero or a positive integer of from about 1 to about 10;

25 (z) is zero or a positive integer of from 1 to about 29;

(m) is 0 or a positive integer; and

WO 2011/011474

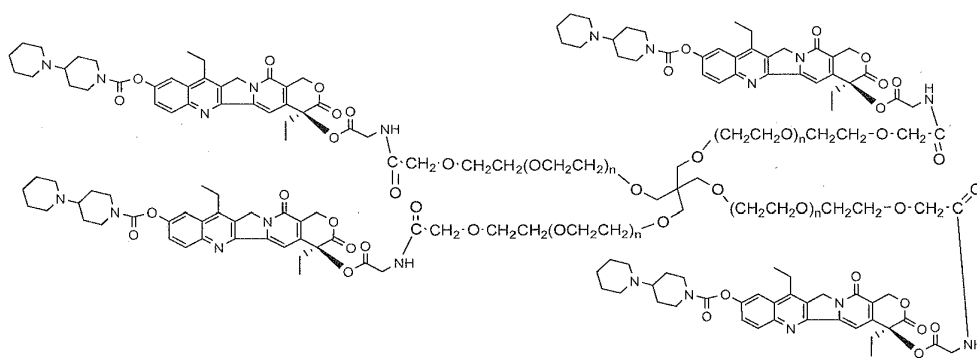
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(n) is a positive integer of from about 10 to about 2,300 so that the polymeric portion of the compound has the total number average molecular weight of from about 2,000 to about 100,000 daltons,
provided that Z₁, Z₂, Z₃ and Z₄ are not all OH.

5

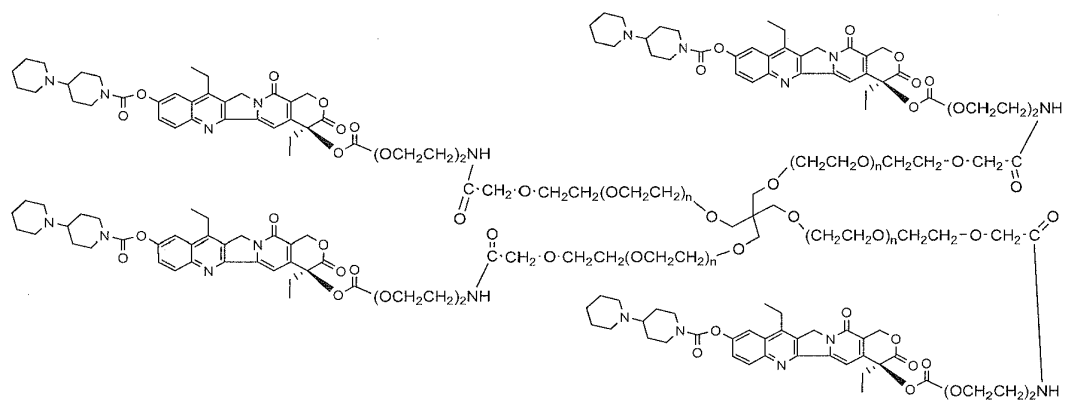
30. The method of claim 29, wherein D is selected from the group consisting of camptothecin, SN38, topotecan, and CPT-11.

31. The method of claim 29, wherein the polymeric conjugate is



10

or



wherein (n) is an integer of from about 28 to about 341, so that the total molecular weight of the polymeric portion of the compound of Formula (II) ranges from about 5,000 to about 60,000 daltons.

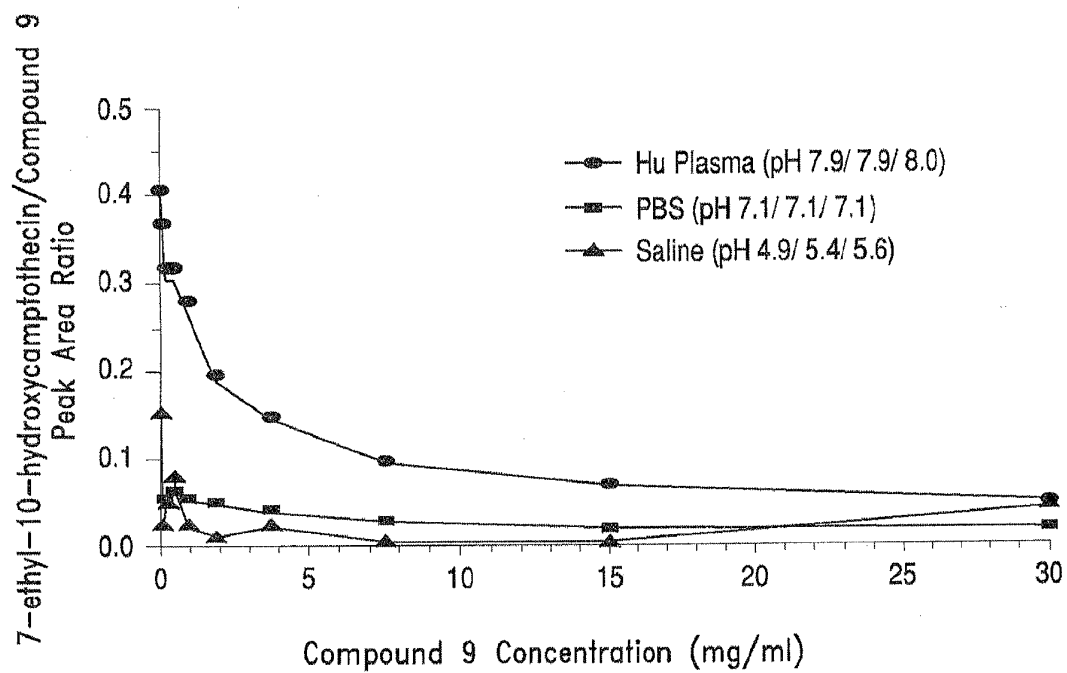
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WO 2011/011474

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FIG. 1

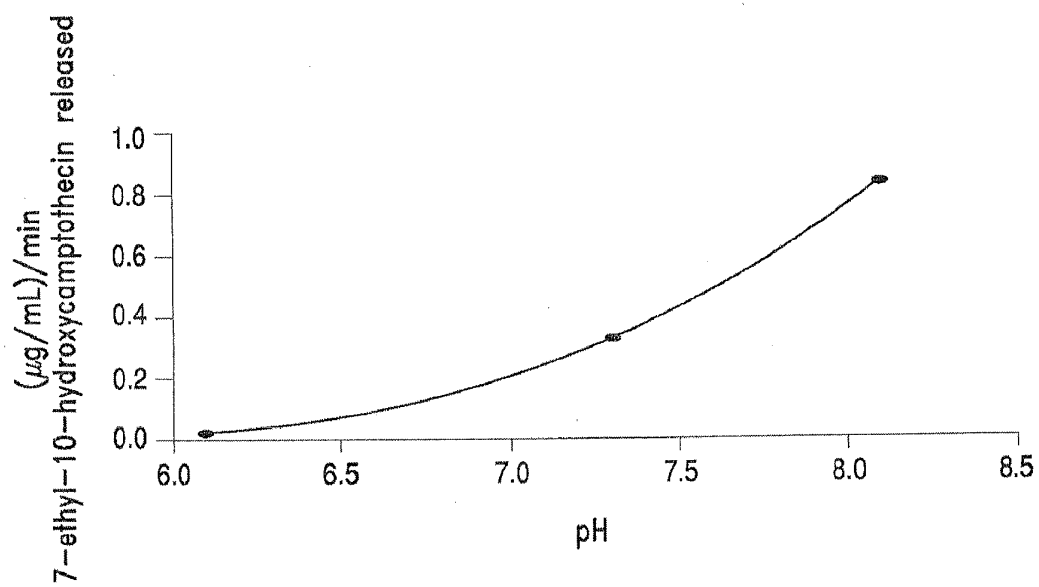


WO 2011/011474

PCT/US2010/042686

2/5

FIG. 2



WO 2011/011474

PCT/US2010/042686

3/5

FIG. 3A

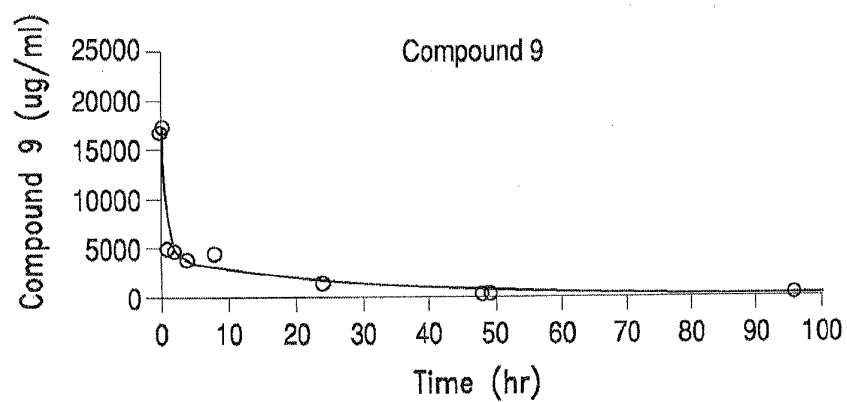
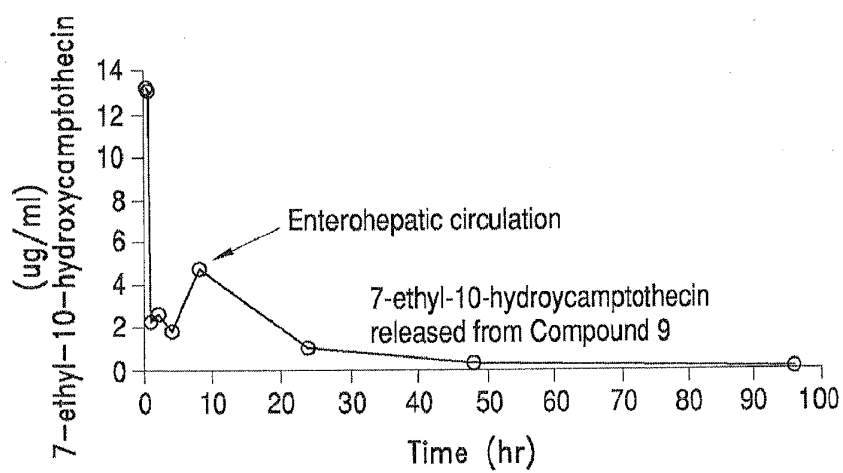


FIG. 3B

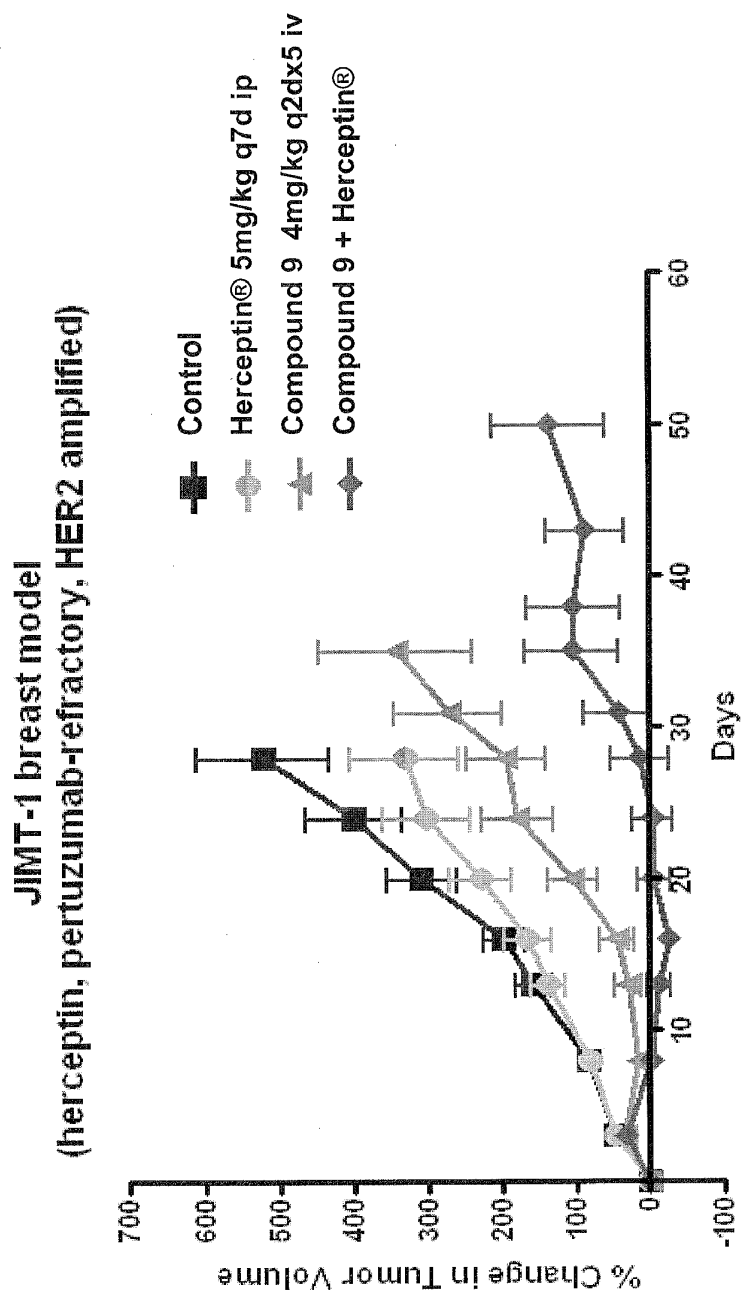


WO 2011/011474

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FIG. 4



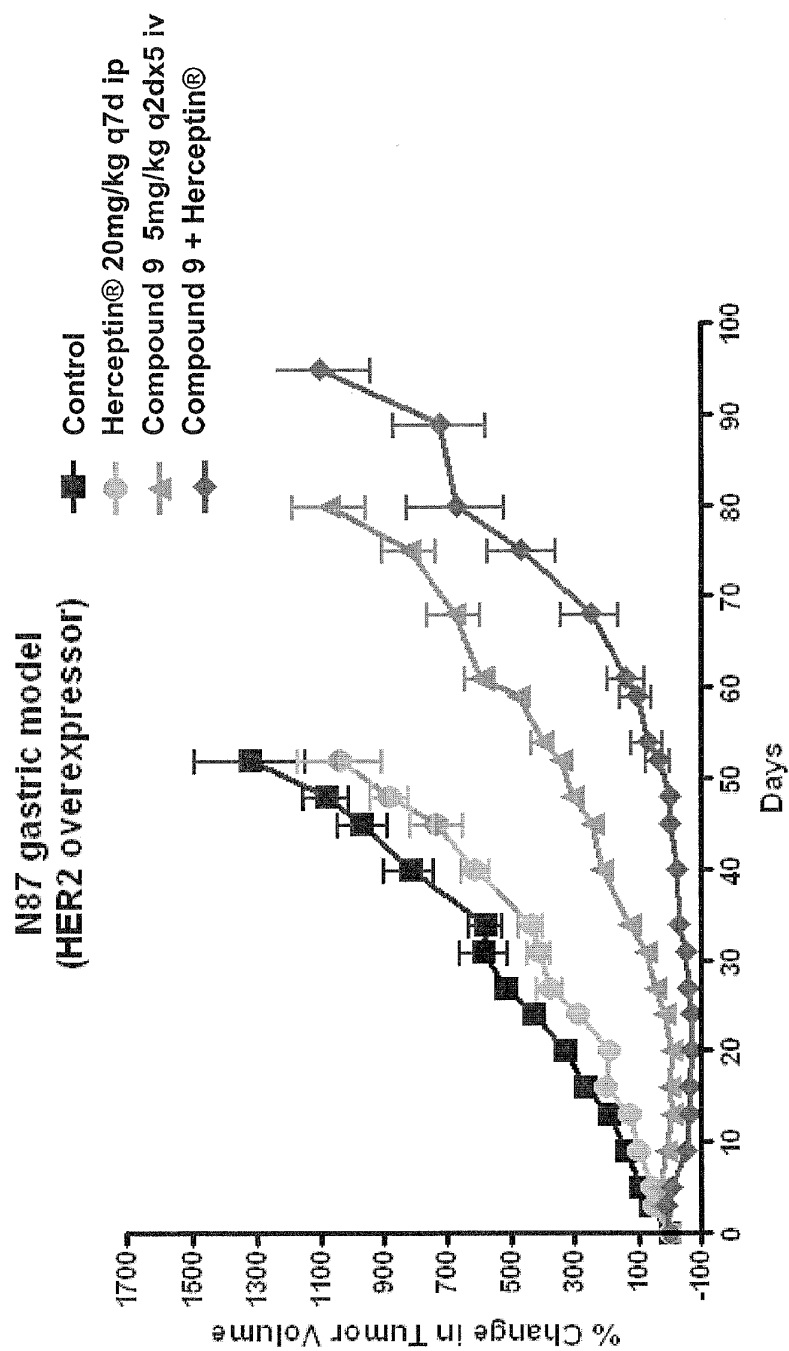
SUBSTITUTE SHEET (RULE 26)

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FIG. 5



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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/42686A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395; A61K 31/44 (2010.01)

USPC - 424/155.1; 514/283

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 424/155.1; 514/283Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 424/142.1; 514/17; 514/171; 514/183; 514/251; 514/254.07; 514/263.32; 514/320; 514/34; 514/44R; 514/49; 514/493; 514/54; 514/575; 514/650; 514/651; 514/8 (keywords below)Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGPB, EPAB, JPAB), Google Patents/Scholar: Her2, camptothecin conjugate, PEG, trastuzumab, petuzumab, antisense, LNA
GenCore 6.3: SEQ ID NO:1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2008/0102069 A1 (Friess et al.) 01 May 2008 (01.05.2008) para [0009], [0015], [0018], [0039], [0078], [0081]-[0082]	28 ----- 1-27, 29-31
Y	US 7,462,627 B2 (Zhao et al.) 09 December 2008 (09.12.2008) col 1, ln 44-53, col 4, ln 3-4, col 9, ln 1-67, col 15, ln 1-38, col 17, ln 57 to col 18, ln 4	1-27
Y	US 2008/0194612 A1 (Zhao et al.) 14 August 2008 (14.08.2008) para [0011], [0030], [0136], [0139], [0155]-[0156]	29-31
Y	US 6,395,266 B1 (Martinez et al.) 28 May 2002 (28.05.2002) col 11, ln 10-30; col 23, ln 1-29 and 44-60	12-15
Y	US 5,968,748 A (Bennett et al.) 19 October 1999 (19.10.1999) col 12, ln 1-16, col 17, ln 10-30, ln 36-55, SEQ ID NO 1	17-23
Y	US 2009/0105139 A1 (Kole et al.) 23 April 2009 (23.04.2009) para [0015], [0018]	22

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
02 November 2010 (02.11.2010)

Date of mailing of the international search report

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P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201Authorized officer:
Lee W. YoungPCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

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(19) 世界知的所有権機関
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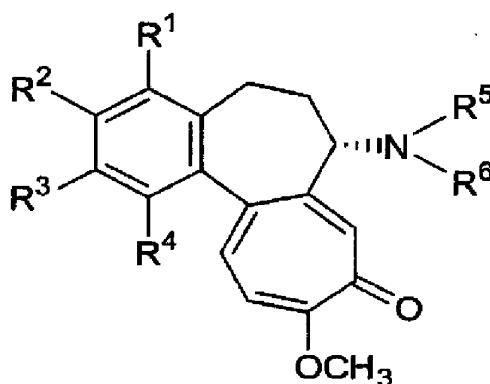
- (51) 国際特許分類:
C07C 225/20 (2006.01) *C07C 233/32* (2006.01)
A61K 31/135 (2006.01) *C07C 233/41* (2006.01)
A61K 31/165 (2006.01) *C07C 271/24* (2006.01)
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- (21) 国際出願番号: PCT/JP2010/005136
- (22) 国際出願日: 2010 年 8 月 20 日(20.08.2010)
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- (30) 優先権データ:
 特願 2009-191464 2009 年 8 月 20 日(20.08.2009) JP
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- (71) 出願人 (米国を除く全ての指定国について): 国立大学法人千葉大学(NATIONAL UNIVERSITY CORPORATION CHIBA UNIVERSITY) [JP/JP]; 〒2638522 千葉県千葉市稲毛区弥生町 1 番 3 3 号 Chiba (JP). 株式会社ヤクルト本社(KABUSHIKI KAISHA YAKULT HONSHA) [JP/JP]; 〒1058660 東京都港区東新橋 1 丁目 1 番 1 9 号 Tokyo (JP).
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[続葉有]

(54) Title: COLCHICINE DERIVATIVES

(54) 発明の名称: コルヒチン誘導体



(1)

(57) **Abstract:** Provided are 4-modified colchicine compounds and medicines using the same. Specifically provided are colchicine derivatives represented by general formula (1), salts thereof, and solvates of the same. In general formula (1), R^1 is a halogen atom, a hydroxyl group, a nitro group, an amino group, or a mono-, di- or tri-fluoromethyl group; R^2 , R^3 and R^4 are each a methoxy group or a hydroxyl group, or alternatively R^2 and R^3 , or R^3 and R^4 together represent a methylenedioxy group; R^5 and R^6 may be the same or different and are each a hydrogen atom, a C_{1-6} alkyl group, an arylalkyl group, a C_{2-6} alkenyl group, $-\text{COR}^7$, $-\text{COOR}^8$, $-\text{SO}_2\text{R}^9$, $-\text{CONR}^{10}\text{R}^{11}$, or $-\text{CSNR}^{12}\text{R}^{13}$, or alternatively R^5 and R^6 together with the nitrogen atom to which R^5 and R^6 are bonded may form a three- to seven-membered cyclic amino group; R^7 is a C_{1-6} alkyl group or the like; R^8 is a C_{1-6} alkyl group or the like; R^9 is a C_{1-6} alkyl group or the like; R^{10} and R^{11} may be the same or different and are each a hydrogen atom, a C_{1-6} alkyl group, or the like; and R^{12} and R^{13} may be the same or different and are each a hydrogen atom, an alkyl group, or the like.

(57) 要約:

[続葉有]

WO 2011/021397 A1



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CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS,
JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR,
LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW,
MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH,
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SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
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コルヒチンの 4 位修飾化合物及びこれを用いた医薬を提供する。次の一般式 (1) (式中、 R^1 はハロゲン原子、ヒドロキシ基、ニトロ基、アミノ基又はモノー、ジー若しくはトリフルオロメチル基を示し; R^2 、 R^3 及び R^4 はそれぞれメトキシ基、又はヒドロキシ基を示すか、 R^2 と R^3 又は R^3 と R^4 が一緒になってメチレンジオキシ基を示し; R^5 及び R^6 は同一又は異なって、水素原子、炭素数 1~6 のアルキル基、アリールアルキル基、炭素数 2~6 のアルケニル基、 $-COR^7$ 、 $-COOR^8$ 、 $-SO_2R^9$ 、 $-CONR^{10}R^{11}$ 又は $-CSNR^{12}R^{13}$ を示すか、 R^5 と R^6 が結合する窒素原子と一緒にあって 3~7 員の環状アミノ基を示し; R^7 は炭素数 1~6 のアルキル基等を示し; R^8 は炭素数 1~6 のアルキル基等を示し; R^9 は炭素数 1~6 のアルキル基等を示し; R^{10} 及び R^{11} は同一又は異なって、水素原子、炭素数 1~6 のアルキル基等を示し; R^{12} 及び R^{13} は同一又は異なって、水素原子、アルキル基等を示す。) で表されるコルヒチン誘導体、その塩又はそれらの溶媒和物。

WO 2011/021397

1

PCT/JP2010/005136

明 細 書

発明の名称： コルヒチン誘導体

技術分野

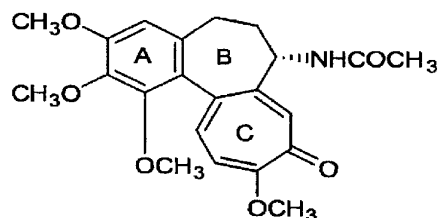
[0001] 本発明は、抗癌剤等の医薬として有用なコルヒチン誘導体及びこれを含有する医薬に関する。

背景技術

[0002] コルヒチンは、トロポロン環を有するイソキノリンアルカロイドの一種であり、ユリ科イヌサフランの種子や球根に多く含まれており、古くから痛風の治療薬として用いられてきた。コルヒチンは、チューブリンに結合することにより細胞分裂を抑制する作用を有することが見出され、当該チューブリンは細胞骨格として細胞の構造を維持するだけでなく、シグナル伝達における輸送路としても作用し、細胞分裂において紡錘体を形成する機能を有する。従って、コルヒチンは、ビンブラスチンやタキソールと同様に抗癌剤として有用であるとして注目された。しかし、コルヒチンは正常細胞の分裂も抑制してしまうため、実用化には至っていない。

[0003] コルヒチンをリード化合物として種々の誘導体が合成され、その生物活性に関する報告がなされている。その中で、コルヒチンは、A環とC環を介してチューブリンと相互作用することが明らかとなり、A環やC環の置換基の修飾やC環をベンゼン環に変えた化合物などが合成されている（非特許文献1～8）。

[0004] [化1]



コルヒチン

WO 2011/021397

2

PCT/JP2010/005136

[0005] 近年では、B環部分がチューブリンへの不可逆的な結合に関与しているのではないかと考えられるようになり、B環側鎖を変換したコルヒチン誘導体も注目を集めている（非特許文献9、10）。

先行技術文献

非特許文献

- [0006] 非特許文献1: Biochem. Pharm., 1976, 25, 1383-1387
非特許文献2: J. Med. Chem., 1985, 28, 1204-1208
非特許文献3: J. Med. Chem., 1981, 24, 257-261
非特許文献4: Arch. Pharm. Chem. Life Sci., 2005, 338, 582-589
非特許文献5: Biochemistry, 1982, 21, 534-543
非特許文献6: Biochem. J., 1997, 327, 658-688
非特許文献7: Heterocycles, 2005, 65, 541-551
非特許文献8: Bioorg. Med. Chem., 2005, 15, 235-238
非特許文献9: J. Org. Chem., 2004, 69, 8987-8996
非特許文献10: Biochemistry, 2005, 44, 3249-3258

発明の概要

発明が解決しようとする課題

[0007] しかしながら、従来のコルヒチンのA環部分の修飾は、そのほとんどが、1～3位のメトキシ基の変換であり、4位への修飾はほとんど行われていなかった。

従って、本発明の課題は、コルヒチンの4位修飾化合物及びこれを用いた

WO 2011/021397

3

PCT/JP2010/005136

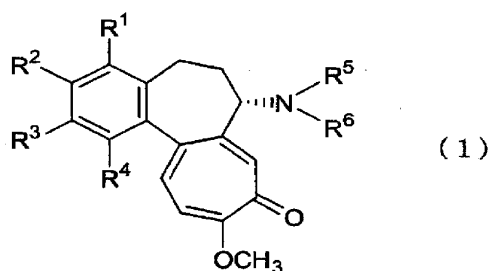
医薬を提供することにある。

課題を解決するための手段

[0008] そこで、本発明者は、コルヒチンのA環の4位にハロゲン原子等の置換基を導入し、かつB環の側鎖を変換したところ、優れた抗癌活性を有し、かつ毒性が低い化合物が得られ、当該化合物は抗癌剤等の医薬として有用であることを見出し、本発明を完成した。

[0009] すなわち、本発明は、次の一般式（1）

[0010] [化2]



[0011] （式中、R¹はハロゲン原子、ヒドロキシ基、ニトロ基、アミノ基又はモノー、ジー若しくはトリフルオロメチル基を示し；
R²、R³及びR⁴はそれぞれメトキシ基、又はヒドロキシ基を示すか、R²とR³又はR³とR⁴が一緒になってメチレンジオキシ基、又はエチレンジオキシ基を示し；
R⁵及びR⁶は同一又は異なって、水素原子、炭素数1～6のアルキル基、アリールアルキル基、炭素数2～6のアルケニル基、 $-\text{COR}^7$ 、 $-\text{COOR}^8$ 、 $-\text{SO}_2\text{R}^9$ 、 $-\text{CONR}^{10}\text{R}^{11}$ 又は $-\text{CSNR}^{12}\text{R}^{13}$ を示すか、R⁵とR⁶が結合する窒素原子と一緒にあって3～7員の環状アミノ基を示し；
R⁷は水素原子、炭素数6～14のアリール基、ヘテロアリール基、環状アミノ基、炭素数3～7の環状アルキル基又は炭素数1～6のアルキル基を示し、当該アルキル基には環状アルキル基、ハロゲン原子、アミノ基、アルキルアミノ基、アリールアルキルアミノ基、ジアルキルアミノ基、環状アミノ基、アルコキシカルボニルアミノ基、アリールアミノ基、ヘテロアリールアミ

ノ基、カルボキシル基、アルコキシカルボニル基、ヒドロキシ基、アシルオキシ基、ジアルキルアミノアシルオキシ基、アルキルオキシ基、アリール基、アリールオキシ基、アリールアルキルオキシ基、ヘテロアリール基、ヘテロアリールオキシ基、チオール基、アシルチオ基、ジアルキルアミノアシルチオ基、アルキルチオ基、アルキルスルホニル基、アリールチオ基、ヘテロアリールチオ基、カルバモイルオキシ基及び環状アミノカルボニルオキシ基から選ばれる1～3個が置換していてもよく、当該アリール基にはハロゲン原子、アルコキシ基、ニトロ基、シアノ基、アミノ基、アルキルアミノ基、ジアルキルアミノ基、アルキル基、アリール基、アルキルアミノアルキル基及びヒドロキシアルキルオキシ基から選ばれる1～3個が置換していてもよく、当該環状アミノ基にはアルキル基、アルコキシカルボニル基及びアシル基から選ばれる1～2個が置換していてもよい；

R⁸は炭素数1～6のアルキル基、炭素数6～14のアリール基、ヘテロアリール基、炭素数3～7の環状アルキル基又は環状アミノ基を示し、当該アルキル基にはアミノ基、アルキルアミノ基、ジアルキルアミノ基、環状アミノ基、カルボキシル基、アルコキシカルボニル基、アリール基及びヘテロアリール基から選ばれる1～3個が置換していてもよく、当該環状アミノ基にはアルキル基及びアルコキシカルボニル基から選ばれる1～2個が置換していてもよく、当該環状アルキル基にはカルボキシル基及びアルコキシカルボニル基から選ばれる1～3個が置換していてもよい；

R⁹は炭素数1～6のアルキル基、炭素数3～7の環状アルキル基、炭素数6～14のアリール基又はヘテロアリール基を示し、当該アルキル基には1～3個のハロゲン原子が置換していてもよく、当該アリール基には1～3個のアルキル基が置換していてもよい；

R¹⁰とR¹¹は同一又は異なって、水素原子、炭素数1～6のアルキル基、炭素数6～14のアリール基、ヘテロアリール基、炭素数3～7の環状アルキル基又は炭素数1～7のアシル基を示すか、R¹⁰とR¹¹が結合する窒素原子と一緒になって3～7員環の環状アミノ基を示し、当該アルキル基にはアリール

基及びヘテロアリール基から選ばれる 1～3 個が置換していてもよく、炭素数 1～7 のアシル基には 1～3 個のハロゲン原子が置換していてもよく、当該環状アミノ基にはさらに酸素原子、窒素原子又は硫黄原子が含まれていてもよい；

R^{12} と R^{13} は同一又は異なって、水素原子、炭素数 1～6 のアルキル基、炭素数 6～14 のアリール基、ヘテロアリール基又は炭素数 3～7 の環状アルキル基を示すか、 R^{12} と R^{13} が結合する窒素原子と一緒になって 3～7 員環の環状アミノ基を示し、当該アルキル基にはアリール基、ヘテロアリール基、環状アミノ基、アミノ基、アルキルアミノ基、ジアルキルアミノ基、ヒドロキシ基及びアルキルオキシ基から選ばれる 1～3 個が置換していてもよく、当該環状アルキル基には 1～3 個のヒドロキシ基が置換していてもよく、当該アリール基にはジアルキルアミノ基、アルキルオキシ基、アルキル基、シアノ基、ハロゲン原子、ヒドロキシアルキル基、ヒドロキシアルキルオキシ基、スルファモイル基及びヒドロキシ基から選ばれる 1～3 個が置換していてもよく、当該ヘテロアリール基にはアルキル基、シアノ基、アリール基及びヒドロキシアルキル基から選ばれる 1～3 個が置換していてもよく、当該環状アミノ基にはさらに酸素原子、窒素原子又は硫黄原子が含まれていてもよく、さらにアルキル基、ヒドロキシ基、ハロゲン原子、アミノ基、アシル基及びジアルキルアミノ基から選ばれる 1～3 個が置換していてもよい；

ただし、 R^1 が塩素原子、臭素原子又はヨウ素原子であり、 R^2 、 R^3 及び R^4 がメトキシ基であり、 R^5 が水素原子であり、かつ R^6 がアセチル基である場合を除く。 R^1 がヒドロキシ基のとき、 R^2 、 R^3 及び R^4 はメトキシ基である。) で表されるコルヒチン誘導体、その塩又はそれらの溶媒和物を提供するものである。

[0012] また、本発明は、一般式 (1) で表されるコルヒチン誘導体、その塩又はそれらの溶媒和物を含有する医薬を提供するものである。

また、本発明は、一般式 (1) で表されるコルヒチン誘導体、その塩又はそれらの溶媒和物、及び薬学的に許容される担体を含有する医薬組成物を提

WO 2011/021397

7

PCT/JP2010/005136

—SO₂R⁹、—CONR¹⁰R¹¹又は—CSNR¹²R¹³を示すか、R⁵とR⁶が結合する窒素原子と一緒にあって3～7員の環状アミノ基を示す。ここで、炭素数1～6のアルキル基としては、メチル基、エチル基、n-プロピル基、イソプロピル基、n-ブチル基、イソブチル基、Sec-ブチル基、tert-ブチル基、n-ペンチル基、n-ヘキシル基等の直鎖又は分岐鎖のアルキル基が挙げられるが、炭素数1～4のアルキル基がより好ましく、特にメチル基、エチル基、n-プロピル基、イソブチル基が好ましい。アリールアルキル基としては、ベンジル基、フェネチル基等が挙げられるがベンジル基が好ましい。炭素数2～6のアルケニル基としてはビニル基、アリル基、2-ブテニル基、イソブテニル基等が挙げられるが、炭素数3～5の直鎖又は分岐鎖のアルケニル基がより好ましく、特にアリル基、イソブテニル基が好ましい。

[0018] 上記—COR⁷におけるR⁷は、水素原子、炭素数6～14のアリール基、ヘテロアリール基、環状アミノ基、炭素数3～7の環状アルキル基又は炭素数1～6のアルキル基を示し、当該アルキル基には環状アルキル基、ハロゲン原子、アミノ基、アルキルアミノ基、アリールアルキルアミノ基、ジアルキルアミノ基、環状アミノ基、アルコキシカルボニルアミノ基、アリールアミノ基、ヘテロアリールアミノ基、カルボキシ基、アルコキシカルボニル基、ヒドロキシ基、アシルオキシ基、ジアルキルアミノアシルオキシ基、アルキルオキシ基、アリール基、アリールオキシ基、アリールアルキルオキシ基、ヘテロアリール基、ヘテロアリールオキシ基、チオール基、アシルチオ基、ジアルキルアミノアシルチオ基、アルキルチオ基、アルキルスルホニル基、アリールチオ基、ヘテロアリールチオ基、カルバモイルオキシ基及び環状アミノカルボニルオキシ基から選ばれる1～3個が置換していてもよく、当該アリール基にはハロゲン原子、アルコキシ基、ニトロ基、シアノ基、アミノ基、アルキルアミノ基、ジアルキルアミノ基、アルキル基、アリール基、アルキルアミノアルキル基及びヒドロキシアルキルオキシ基から選ばれる1～3個が置換していてもよく、当該環状アミノ基にはアルキル基、アルコキ

シカルボニル基及びアシル基から選ばれる 1～2 個が置換していてもよい。ここで、炭素数 1～6 のアルキル基としては、前記 R⁵ 及び R⁶ と同様の基が挙げられ、炭素数 1～4 のアルキル基が好ましく、特にメチル基、エチル基、n-プロピル基、イソプロピル基、イソブチル基が好ましい。また、炭素数 6～14 のアリール基としては、フェニル基、ナフチル基、トリル基、キシリル基等が挙げられるが、フェニル基が特に好ましい。ヘテロアリール基としては、フリル基、チエニル基、ピロリル基、チアゾリル基、チアジアゾリル基、ピラゾリル基、ピリジニル基、ピラジニル基、ピリミジニル基、ピリダジニル基、オキサゾリル基、イミダゾリル基、ベンゾチアゾリル基、ベンゾオキサゾリル基、インドリル基、ピラジニル基、インダゾリル基、ベンゾチアジアゾリル基、ベンゾイミダゾリル基、ベンゾチアゾリル基、チアジアゾリル基、イソオキサゾリル基、トリアゾリル基等が挙げられるが、フリル基、チエニル基、ピロリル基、ピリジニル基、ベンゾチアゾリル基、ベンゾオキサゾリル基、インドリル基が特に好ましい。環状アミノ基としては、ピロリジノ基、ピペリジノ基、N-メチルピペリジノ基、N-(*t*-ブトキシカルボニル)ピペリジノ基、N-アセチルピペリジノ、N-ベンゾイルピペリジノ、N-(ベンジルオキシカルボニル)ピペリジノ基等が挙げられるが、N-メチルピペリジノ基、N-(*t*-ブトキシカルボニル)ピペリジノ基、N-ベンゾイルピペリジノ、N-(ベンジルオキシカルボニル)ピペリジノ基が特に好ましい。炭素数 3～7 の環状アルキル基としては、シクロプロピル基、シクロブチル基、シクロペンチル基、シクロヘキシル基等が挙げられ、炭素数 3～6 の環状アルキル基が好ましく、シクロプロピル、シクロペンチル基、シクロヘキシル基が特に好ましい。

[0019] R⁷で示されるアルキル基上の置換基のうち、環状アルキル基としては、炭素数 3～7 の環状アルキル基、例えばシクロプロピル基、シクロヘキシル基が挙げられる。ハロゲン原子としては、フッ素原子、塩素原子、臭素原子、ヨウ素原子が挙げられる。アルキルアミノ基としては、C₁₋₆アルキルアミノ基、例えばメチルアミノ基、エチルアミノ基が挙げられる。アリールアルキル

アミノ基としては、例えばベンジルアミノ基が挙げられる。ジアルキルアミノ基としては、ジ(C₁₋₆アルキル)アミノ基、例えばジメチルアミノ基、ジエチルアミノ基が挙げられる。環状アミノ基としては、C₃₋₇環状アミノ基、例えばアジリジノ基、ピロリジノ基、ピペリジノ基等が挙げられる。アルコキシカルボニルアミノ基としては、t-ブトキシカルボニルアミノ基等が挙げられる。アリールアミノ基としては、アニリノ基等が挙げられる。ヘテロアリールアミノ基としては、フリルアミノ基、チエニルアミノ基、ピリジニルアミノ基等が挙げられる。アルコキシカルボニル基としては、C₁₋₆₇アルコキシカルボニル基、例えばメトキシカルボニル基、エトキシカルボニル基、tert-ブチルオキシカルボニル基、ベンジルオキシカルボニル基等が挙げられる。アシルオキシ基としては、C₁₋₆アシルオキシ基、例えばアセチルオキシ基、プロピオニルオキシ基等が挙げられる。ジアルキルアミノアシルオキシ基としては、ジ(C₁₋₆アルキル)アミノアシルオキシ基、例えばジメチルアミノアセチルオキシ基、ジエチルアミノアセチルオキシ基、ジメチルアミノプロピオニルオキシ基、ジエチルアミノブチロイルオキシ基等が挙げられる。アルキルオキシ基としては、C₁₋₆アルキルオキシ基、例えばメトキシ基、エトキシ基等が挙げられる。アリール基としては、例えばフェニル基、トリル基等が挙げられる。アリールオキシ基としては、例えばフェニルオキシ基、トリルオキシ基等が挙げられる。アリールアルキルオキシ基としては、例えばベンジルオキシ基、トリフェニルメチルオキシ基等が挙げられる。ヘテロアリール基としては、ピリジル基、フリル基、オキサゾリル基等が挙げられる。ヘテロアリールオキシ基としては、例えばピリジルオキシ基、フリルオキシ基、オキサゾリルオキシ基等が挙げられる。アシルチオ基としては、C₁₋₆アシルチオ基、例えばアセチルチオ基、プロピオニルチオ基が挙げられる。ジアルキルアミノアシルチオ基としては、ジ(C₁₋₆アルキル)アミノアシルチオ基、例えばジメチルアミノアセチルチオ基、ジメチルアミノプロピオニルチオ基、ジエチルアミノアセチルチオ基、ジエチルアミノブチリルチオ基等が挙げられる。アルキルチオ基としては、C₁₋₆アルキルチオ基、例えばメチルチオ基、エ

チルチオ基等が挙げられる。アルキルスルホニル基としては、 C_{1-6} アルキルスルホニル基、例えばメチルスルホニル基、エチルスルホニル基等が挙げられる。アリールチオ基としては、例えばフェニルチオ基、トリルチオ基等が挙げられる。ヘテロアリールチオ基としては、例えばピリジルチオ基、フリルチオ基、オキサゾリルチオ等が挙げられる。カルバモイルオキシ基としては、例えば4-ピペリジノピペリジン-1-カルボニルオキシ基、1-メチルピペリジン-4-カルボニルオキシ基等が挙げられる。環状アミノカルバモイルオキシ基としては、例えば1-(ベンジルオキシカルボニル)ピペリジン-4-カルボニルオキシ基等が挙げられる。 R^7 で示されるアリール基上の置換基のうち、ハロゲン原子としては前記と同様の基が挙げられる。アルコキシ基としては前記と同様の基が挙げられる。アルキルアミノ基としては前記と同様の基が挙げられる。ジアルキルアミノ基としては前記と同様の基が挙げられる。アルキル基としては前記と同様の基が挙げられる。アリール基としては前記と同様の基が挙げられる。アルキルアミノアルキル基としては、 C_{1-6} アルキルアミノ C_{1-6} アルキル基、例えばジメチルアミノメチル基、モルホリノ-4-メチル基等が挙げられる。ヒドロキシアルキルオキシ基としては、例えば2-ヒドロキシエトキシ基、3-ヒドロキシプロポキシ基、2-ヒドロキシプロポキシ基等が挙げられる。 R^7 で示される環状アミノ基上の置換基のうち、アルキル基としては前記と同様の基が挙げられる。アルコキシカルボニル基としては前記と同様の基が挙げられる。アシル基としてはアセチル基、プロピオニル基、ベンゾイル基等が挙げられる。

[0020] 上記-COOR⁸におけるR⁸は、炭素数1～6のアルキル基、炭素数6～14のアリール基、ヘテロアリール基、炭素数3～7の環状アルキル基又は環状アミノ基を示し、当該アルキル基にはアミノ基、アルキルアミノ基、ジアルキルアミノ基、環状アミノ基、カルボキシ基、アルコキシカルボニル基、アリール基及びヘテロアリール基から選ばれる1～3個が置換していてもよく、当該環状アミノ基にはアルキル基及びアルコキシカルボニル基から選ばれる1～2個が置換していてもよく、当該環状アルキル基にはカルボキシ

ル基及びアルコキシカルボニル基から選ばれる 1～3 個が置換していてもよい。ここで、炭素数 1～6 のアルキル基としては、前記と同じ基が挙げられ、炭素数 1～4 のアルキル基が好ましく、特にメチル基、エチル基、イソプロピル基、イソブチル基、*t*-ブチル基が好ましい。炭素数 6～14 のアリール基としては、前記 R⁷と同様の基が挙げられ、フェニル基が特に好ましい。ヘテロアリール基としては、前記 R⁷と同様の基が挙げられ、ピリジル基、インドリル基が特に好ましい。炭素数 3～7 の環状アルキル基としては前記 R⁷と同様の基が挙げられ、シクロペンチル基、シクロヘキシル基が特に好ましい。環状アミノ基としては、前記 R⁷と同様の基が挙げられ、N-メチルピペリジノ基、N-(*t*-ブトキシカルボニル)ピペリジノ基、N-(ベンジルオキシカルボニル)ピペリジノ基が特に好ましい。R⁸で示されるアルキル基上の置換基としては、アミノ基、アルキルアミノ基、ジアルキルアミノ基、環状アミノ基、カルボキシル基、アルコキシカルボニル基、アリール基又はヘテロアリール基が挙げられ、アルキルアミノ基としては、メチルアミノ基、エチルアミノ基が特に好ましい。ジアルキルアミノ基としては、ジメチルアミノ基、ジエチルアミノ基が特に好ましい。環状アミノ基としては、ピロリジノ基、ピペリジノ基が特に好ましい。アルコキシカルボニル基としては、メトキシカルボニル基、エトキシカルボニル基、ベンジルオキシカルボニル基が特に好ましい。アリール基としては、フェニル基、トリル基が特に好ましい。ヘテロアリール基としては、ピリジル基が特に好ましい。

[0021] 上記 $-SO_2R^9$ における R⁹は、炭素数 1～6 のアルキル基、炭素数 3～7 の環状アルキル基、炭素数 6～14 のアリール基又はヘテロアリール基を示す。当該アルキル基には 1～3 個のハロゲン原子が置換していてもよく、当該アリール基には 1～3 個の炭素数 1～6 のアルキル基が置換していてもよい。炭素数 1～6 のアルキル基としては、前記と同じ基が挙げられ、炭素数 1～4 のアルキル基、特にメチル基、エチル基、イソプロピル基、イソブチル基が好ましい。炭素数 3～7 の環状アルキル基としては、前記と同じ基が挙げられ、シクロプロピル基、シクロヘキシル基が好ましい。炭素数 6～14

のアリール基としては、前記と同じ基が挙げられ、特にフェニル基、トリル基が好ましい。ヘテロアリール基としては、前記と同じ基が挙げられ、チエニル基が好ましい。R⁹で示されるアルキル基上の置換基としては、ハロゲン原子が挙げられ、ハロゲン原子としては、前記R⁷と同様の基が挙げられる。R⁹で示されるアリール基上の置換基としては、炭素数1～6のアルキル基が挙げられ、炭素数1～6のアルキル基としては、前記R⁷と同様の基が挙げられる。

[0022] 上記の-C(=O)NR¹⁰R¹¹におけるR¹⁰とR¹¹は、同一又は異なって、水素原子、炭素数1～6のアルキル基、炭素数6～14のアリール基、ヘテロアリール基、炭素数3～7の環状アルキル基又は炭素数1～7のアシル基を示すか、R¹⁰とR¹¹が結合する窒素原子と一緒にあって3～7員環の環状アミノ基を示し、当該アルキル基にはアリール基及び1～3個のヘテロアリール基から選ばれる1～3個が置換していてもよく、炭素数1～7のアシル基には1～3個のハロゲン原子が置換していてもよく、当該環状アミノ基にはさらに酸素原子、窒素原子又は硫黄原子が含まれてもよい。ここで、炭素数1～6のアルキル基としては、前記と同じ基が挙げられ、特にメチル基、エチル基、ヘキシル基が好ましい。炭素数6～14のアリール基としては、前記R⁷と同様の基が挙げられ、フェニル基が特に好ましい。ヘテロアリール基としては、前記R⁷と同様の基が挙げられ、ピリジル基、インドリル基が特に好ましい。R¹⁰及びR¹¹で示されるアルキル基上の置換基としてはアリール基、又はヘテロアリール基が挙げられ、アリール基としては前記R⁷と同様の基が挙げられ、フェニル基が特に好ましい。ヘテロアリール基としては、前記R⁷と同様の基が挙げられ、フリル基、チエニル基が特に好ましい。炭素数3～7の環状アルキル基としては、前記R⁷と同じ基が挙げられ、シクロヘキシル基が特に好ましい。炭素数1～7のアシル基としては、例えばアセチル基が挙げられる。当該アシル基上に置換するハロゲン原子としては、上記R⁷と同様の基が挙げられる。R¹⁰及びR¹¹が、窒素原子と一緒にあって形成する3～7員環の環状アミノ基としてはアジリジノ基、ピロリジノ基、ピペリジノ基が挙げ

られ、ピペリジノ基が特に好ましい。R¹⁰及びR¹¹が窒素原子と一緒にあって形成する3～7員環の環状アミノ基でさらに酸素原子、窒素原子又は硫黄原子を含む環状アミノ基としては、モルホリノ基、ピペラジノ基、N-メチルピペラジノ基、チオモルホリノ基、チオモルホリノー1, 1-ジオキシド基等が挙げられ、モルホリノ基、チオモルホリノ基が特に好ましい。

[0023] 上記の-C-S-N-R¹²-R¹³におけるR¹²とR¹³は、同一又は異なって、水素原子、炭素数1～6のアルキル基、炭素数6～14のアリール基、ヘテロアリール基又は炭素数3～7の環状アルキル基を示すか、R¹²とR¹³が結合する窒素原子と一緒にあって3～7員環の環状アミノ基を示し、当該アルキル基にはアリール基、ヘテロアリール基、環状アミノ基、アミノ基、アルキルアミノ基、ジアルキルアミノ基、ヒドロキシ基及びアルキルオキシ基から選ばれる1～3個が置換していてもよく、当該環状アルキル基には1～3個のヒドロキシ基が置換していてもよく、当該アリール基にはジアルキルアミノ基、アルキルオキシ基、アルキル基、シアノ基、ハロゲン原子、ヒドロキシアルキル基、ヒドロキシアルキルオキシ基、スルファモイル基及びヒドロキシ基から選ばれる1～3個が置換してもよく、当該ヘテロアリール基にはアルキル基、シアノ基、アリール基及びヒドロキシアルキル基から選ばれる1～3個が置換していてもよく、当該環状アミノ基にはさらに酸素原子、窒素原子又は硫黄原子が含まれてもよく、さらにはアルキル基、ヒドロキシ基、ハロゲン原子、アミノ基及びジアルキルアミノ基から選ばれる1～3個が置換していてもよい。ここで、炭素数1～6のアルキル基としては、前記と同じ基が挙げられ、炭素数1～4のアルキル基、特にメチル基、エチル基、イソブチル基が好ましい。炭素数6～14のアリール基としては、前記R⁷と同様の基が挙げられ、フェニル基が特に好ましい。ヘテロアリール基としては、前記R⁷と同様の基が挙げられ、ピリジル基、インドリル基、ピリダジニル基、ピラジニル基、ベンゾチアジアゾリル基、チアゾリル基、イソオキサゾリル基、ピラゾリル基、トリアゾリル基が特に好ましい。R¹²及びR¹³で示されるアルキル基上の置換基としてはアリール基、ヘテロアリール基、環状アミノ基

、アミノ基、ジアルキルアミノ基、ヒドロキシ基及びアルキルオキシ基が挙げられ、アリール基としては前記 R^7 と同様の基が挙げられ、フェニル基が特に好ましい。ヘテロアリール基としては、前記 R^7 と同様の基が挙げられ、フリル基、チエニル基が特に好ましい。環状アミノ基としては、前記 R^7 と同様の基が挙げられ、モルホリノ基が好ましい。アルキルアミノ基としては、前記 R^7 と同様の基が挙げられる。ジアルキルアミノ基としては、前記 R^7 と同様の基が挙げられる。アルキルオキシ基としては、前記 R^7 と同様の基が挙げられ、特にメトキシ基が好ましい。炭素数3～7の環状アルキル基としては、前記 R^7 と同じ基が挙げられ、シクロヘキシル基が特に好ましい。 R^{12} 及び R^{13} が、窒素原子と一緒に形成する3～7員環の環状アミノ基としてはアジリジノ基、ピロリジノ基、ピペリジノ基、アゼパノ基等が挙げられ、アジリジノ基、ピロリジノ基、ピペリジノ基が特に好ましい。 R^{12} 及び R^{13} が窒素原子と一緒に形成する3～7員環の環状アミノ基でさらに酸素原子、窒素原子又は硫黄原子を含む環状アミノ基としては、モルホリノ基、ピペラジノ基、N-メチルピペラジノ基、チオモルホリノ基、チオモルホリノー1, 1-ジオキシド基等が挙げられ、モルホリノ基、チオモルホリノ基が特に好ましい。当該環状アミノ基上に置換するアルキル基としては、前記 R^7 と同じ基が挙げられ、メチル基が特に好ましい。当該環状アミノ基上に置換するハロゲン原子としては、前記 R^7 と同じ基が挙げられ、フッ素が特に好ましい。当該環状アミノ基上に置換するアシル基としては、前記 R^7 と同じ基が挙げられる。当該環状アミノ基上に置換するジアルキルアミノ基としては、前記 R^7 と同じ基が挙げられる。当該ヘテロアリール基上に置換するアルキル基としてはメチル基、エチル基等が挙げられ、アリール基としてはフェニル基等が挙げられ、ヒドロキシアルキル基としては2-ヒドロキシエチル基、3-ヒドロキシプロピル基、2-ヒドロキシプロピル基等が挙げられる。

[0024] R^5 及び R^6 が、窒素原子と一緒に形成する3～7員の環状アミノ基としては、アジリジノ基、ピロリジノ基、ピペリジノ基等が挙げられる。

[0025] R^5 及び R^6 は、水素原子及び炭素数1～6のアルキル基、アリールアルキル

WO 2011/021397

15

PCT/JP2010/005136

基、炭素数 2 ～ 6 のアルケニル基を示す場合は、同一でも異なってもよく、 R^5 又は R^6 の一方が $-\text{COR}^7$ 、 $-\text{COOR}^8$ 、 $-\text{SO}_2\text{R}^9$ 、 $-\text{CONR}^{10}\text{R}^{11}$ 又は $-\text{CSNR}^{12}\text{R}^{13}$ を示す場合には、他方は水素原子であるのが好ましい。

[0026] R^5 及び R^6 の好ましい組み合わせについて説明する。以下の (1) ～ (3) の組み合わせが特に好ましい。

(1) R^5 及び R^6 が水素原子の場合。

(2) R^5 が水素原子であり、 R^6 が $-\text{COR}^7$ 、 $-\text{COOR}^8$ 、 $-\text{SO}_2\text{R}^9$ 、 $-\text{CONR}^{10}\text{R}^{11}$ 又は $-\text{CSNR}^{12}\text{R}^{13}$ である場合。

(3) R^5 が $-\text{COR}^7$ で、 R^6 が $-\text{COOR}^8$ の場合。

[0027] 上記 (2) のうち、 R^6 は $-\text{COR}^7$ 、 $-\text{COOR}^8$ 、 $-\text{SO}_2\text{R}^9$ 又は $-\text{CSNR}^{12}\text{R}^{13}$ がさらに好ましい。

R^6 が $-\text{COR}^7$ の場合、 R^7 は水素原子；炭素数 2 ～ 6 のアルキル基；炭素数 3 ～ 7 の環状アルキル基； C_{3-7} 環状アルキル基、ハロゲン原子、アミノ基、 C_{1-6} アルキルアミノ基、 C_{6-14} アリールアルキルアミノ基、ジ (C_{1-6} アルキル) アミノ基、環状アミノ基、 C_{1-6} アルコキシカルボニルアミノ基、 C_{6-14} アリールアミノ基、ヘテロアリールアミノ基、カルボキシ基、 C_{1-6} アルコキシカルボニル基、ヒドロキシ基、アシルオキシ基、ジ (C_{1-6} アルキル) アミノアシルオキシ基、 C_{1-6} アルキルオキシ基、 C_{6-14} アリール基、 C_{6-14} アリールオキシ基、 C_{6-14} アリールアルキルオキシ基、ヘテロアリール基、ヘテロアリールオキシ基、チオール基、アシルチオ基、ジ (C_{1-6} アルキル) アミノアシルチオ基、 C_{1-6} アルキルチオ基、 C_{1-6} アルキルスルホニル基、 C_{6-14} アリールチオ基及びヘテロアリールチオ基から選ばれる 1 ～ 3 個が置換した炭素数 1 ～ 6 のアルキル基；あるいはハロゲン原子、 C_{1-6} アルコキシ基、ニトロ基、シアノ基、アミノ基、 C_{1-6} アルキルアミノ基、ジ (C_{1-6} アルキル) アミノ基、 C_{1-6} アルキル基、 C_{6-14} アリール基及び C_{1-6} アルキルアミノアルキル基から選ばれる 1 ～ 3 個が置換していてもよい C_{6-14} アリール基又はヘテロアリール基が好ましい。

[0028] R^6 が $-\text{COOR}^8$ の場合、 R^8 は炭素数 1 ～ 6 のアルキル基、炭素数 6 ～ 14 のアリール基、ヘテロアリール基、炭素数 3 ～ 7 の環状アルキル基又は環状

アミノ基が好ましく、当該アルキル基にはアミノ基、 C_{1-6} アルキルアミノ基、ジ(C_{1-6} アルキル)アミノ基、環状アミノ基、カルボキシ基、 C_{1-6} アルコキシカルボニル基、 C_{6-14} アリール基及びヘテロアリール基から選ばれる1～3個が置換していてもよく、当該環状アミノ基には C_{1-6} アルキル基又は C_{1-6} アルコキシカルボニル基が置換していてもよく、当該環状アルキル基にはカルボキシ基、アルコキシカルボニル基が置換していてもよい。

[0029] R^6 が $-SO_2R^9$ の場合、 R^9 は C_{6-14} アリール基又はヘテロアリール基が好ましい。 R^6 が $-CSNR^{12}R^{13}$ の場合、 R^{12} と R^{13} は同一又は異なって、水素原子、炭素数1～4のアルキル基、炭素数5～6の環状アルキル基、アリール基、ヘテロアリール基又は R^{12} と R^{13} が窒素原子と一緒に形成する4～6員環の環状アミノ基が好ましく、当該アルキル基にはアルキルオキシ基、ヒドロキシ基が置換していてもよく、当該アリール基にはジアルキルアミノ基、アルキルオキシ基、アルキル基、シアノ基、ハロゲン原子、ヒドロキシアルキル基及びヒドロキシアルキルオキシ基から選ばれる1～3個が置換していてもよく、当該ヘテロアリール基にはアルキル基、シアノ基、アリール基、ヒドロキシアルキル基から選ばれる1～3個が置換していてもよく、当該環状アミノ基には酸素原子、硫黄原子が含まれていてもよく、さらにはアルキル基、ハロゲン原子から選ばれる1～3個が置換していてもよい。ただし、 R^5 が水素原子であり、 R^6 がアセチル基の場合、 R^1 は塩素原子、臭素原子又はヨウ素原子ではない。

[0030] 本発明において、下記から選ばれるコルヒチン誘導体、その塩又はそれらの溶媒和物が好ましい。

- 4-ニトロコルヒチン、
- 4-ヒドロキシコルヒチン、
- 4-フルオロコルヒチン、
- 4-アミノコルヒチン、
- 4-クロロアセチルコルヒチン、
- 4-フルオロアセチルコルヒチン、

WO 2011/021397

17

PCT/JP2010/005136

- 4-クロロ-N-プロピオニルデアセチルコルヒチン、
4-クロロ-N-ホルミルデアセチルコルヒチン、
4-クロロ-N-(クロロアセチル)デアセチルコルヒチン、
4-クロロ-N-(tert-ブトキシカルボニル)コルヒチン、
4-クロロ-N-(tert-ブトキシカルボニル)デアセチルコルヒチン、
[0031] 4-クロロ-N, N-ジメチルデアセチルコルヒチン、
4-クロロ-N, N-ジエチルデアセチルコルヒチン、
4-クロロ-7-(ピペラジン-1-イル)-7-デ(アセチルアミノ)
コルヒチン、
N-(tert-ブチルオキシカルボニル)-4-クロロ-N-メチルデアセチルコルヒチン、
N-(tert-ブチルオキシカルボニル)-4-クロロ-N-エチルデアセチルコルヒチン、
N-アリル-4-クロロ-N-(tert-ブチルオキシカルボニル)デアセチルコルヒチン、
N-(tert-ブチルオキシカルボニル)-4-クロロ-N-(2-メチルアリル)デアセチルコルヒチン、
N-ベンジル-N-(tert-ブチルオキシカルボニル)-4-クロロデアセチルコルヒチン、
4-クロロ-N-メチルデアセチルコルヒチン、
4-クロロ-N-エチルデアセチルコルヒチン、
N-アリル-4-クロロデアセチルコルヒチン、
4-クロロ-N-(2-メチルアリル)デアセチルコルヒチン、
N-ベンジル-4-クロロデアセチルコルヒチン、
4-クロロ-N-プロピルデアセチルコルヒチン、
N-イソブチル-4-クロロデアセチルコルヒチン、
N-(アセトキシアセチル)-4-クロロデアセチルコルヒチン、
4-クロロ-N-(ヒドロキシアセチル)デアセチルコルヒチン、

WO 2011/021397

18

PCT/JP2010/005136

4-クロロ-N-(1-メチルピペラジン-4-イルオキシカルボニル)
デアセチルコルヒチン、

4-クロロ-N-[1-(ベンジルオキシカルボニル)ピペリジン-4-
イルオキシカルボニル]デアセチルコルヒチン、

[0032] 4-クロロ-N-(ピペリジン-4-イルオキシカルボニル)デアセチル
コルヒチン、

N-[trans-1-(ベンジルオキシカルボニル)シクロヘキサン-
4-イルオキシカルボニル]-4-クロロデアセチルコルヒチン、

4-クロロ-N-(チオモルホリン-4-カルボニル)デアセチルコルヒ
チン、

4-クロロ-N-(1,1-ジオキソチオモルホリン-4-カルボニル)
デアセチルコルヒチン、

4-クロロ-N-(1-メチルピペラジン-4-カルボニル)デアセチル
コルヒチン、

N-ブチル-4-クロロデアセチルコルヒチン、

4-クロロ-N-イソブチルデアセチルコルヒチン、

4-クロロ-N-(シクロプロパンカルボニル)デアセチルコルヒチン、

4-クロロ-N-イソバレリルデアセチルコルヒチン、

4-クロロ-N-ヘプタノイルデアセチルコルヒチン、

4-クロロ-N-(シクロヘキサンカルボニル)デアセチルコルヒチン、

N-ベンゾイル-4-クロロデアセチルコルヒチン、

4-クロロ-N-(フェニルアセチル)デアセチルコルヒチン、

4-クロロ-N-(ピリジン-3-イルカルボニル)デアセチルコルヒチ
ン、

4-クロロ-N-(ピリジン-4-イルカルボニル)デアセチルコルヒチ
ン、

4-クロロ-N-(ピリジン-2-イルカルボニル)デアセチルコルヒチ
ン、

WO 2011/021397

19

PCT/JP2010/005136

4-クロロ-N-(トリフルオロアセチル)デアセチルコルヒチン、
4-クロロ-N-(メトキシアセチル)デアセチルコルヒチン、
4-クロロ-N-(フェノキシアセチル)デアセチルコルヒチン、
4-クロロ-N-[(メチルチオ)アセチル]デアセチルコルヒチン、
4-クロロ-N-[(4-ピリジルチオ)アセチル]デアセチルコルヒチン、
N-(4-ブロモブチリル)-4-クロロデアセチルコルヒチン、
4-クロロ-N-(4-フルオロベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(3, 5-ジフルオロベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(3, 4, 5-トリフルオロベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(4-メトキシベンゾイル)デアセチルコルヒチン、
[0033] 4-クロロ-N-(3, 4, 5-トリメトキシベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(2-メトキシベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(3-メトキシベンゾイル)デアセチルコルヒチン、
4-クロロ-N-[(フェニルチオ)アセチル]デアセチルコルヒチン、
4-クロロ-N-[3-(ジメチルアミノ)ベンゾイル]デアセチルコルヒチン、
4-クロロ-N-[4-(ジメチルアミノ)ベンゾイル]デアセチルコルヒチン、
4-クロロ-N-(2-フルオロベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(3-フルオロベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(2, 4-ジフルオロベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(ピリジン-3-イルオキシアセチル)デアセチルコルヒチン、

WO 2011/021397

20

PCT/JP2010/005136

4-クロロ-N-(ピリジン-2-イルアセチル)デアセチルコルヒチン、
、
4-クロロ-N-(ピリジン-3-イルアセチル)デアセチルコルヒチン、
、
4-クロロ-N-(メトキシカルボニル)デアセチルコルヒチン、
4-クロロ-N-(エトキシカルボニル)デアセチルコルヒチン、
4-クロロ-N-(イソプロピルオキシカルボニル)デアセチルコルヒチン、
、
4-クロロ-N-(イソブチルオキシカルボニル)デアセチルコルヒチン、
、
4-クロロ-N-(フェノキシカルボニル)デアセチルコルヒチン、
N-(ベンジルオキシカルボニル)-4-クロロデアセチルコルヒチン、
4-クロロ-N-(メタンスルホニル)デアセチルコルヒチン、
4-クロロ-N-(エタンスルホニル)デアセチルコルヒチン、
[0034] 4-クロロ-N-(イソプロパンスルホニル)デアセチルコルヒチン、
4-クロロ-N-(シクロプロパンスルホニル)デアセチルコルヒチン、
4-クロロ-N-(イソブタンスルホニル)デアセチルコルヒチン、
N-(ベンゼンスルホニル)-4-クロロデアセチルコルヒチン、
4-クロロ-N-(p-トルエンスルホニル)デアセチルコルヒチン、
4-クロロ-N-(2-チオフエンスルホニル)デアセチルコルヒチン、
4-クロロ-N-(トリフルオロメタンスルホニル)デアセチルコルヒチン、
、
4-クロロ-N-(エチルカルバモイル)デアセチルコルヒチン、
4-クロロ-N-(イソプロピルカルバモイル)デアセチルコルヒチン、
4-クロロ-N-(ヘキシルカルバモイル)デアセチルコルヒチン、
4-クロロ-N-(フェニルカルバモイル)デアセチルコルヒチン、
N-(ベンジルカルバモイル)-4-クロロデアセチルコルヒチン、
4-クロロ-N-(ジメチルカルバモイル)デアセチルコルヒチン、

WO 2011/021397

21

PCT/JP2010/005136

4-クロロ-N-(ジエチルカルバモイル)デアセチルコルヒチン、
4-クロロ-N-(モルフォリン-4-カルボニル)デアセチルコルヒチン、
4-クロロ-N-(ピロリジン-1-カルボニル)デアセチルコルヒチン、
4-クロロ-N-(ピペリジン-1-カルボニル)デアセチルコルヒチン、
4-クロロ-N-(トリクロロアセチルカルバモイル)デアセチルコルヒチン、
4-クロロ-N-(ジメチルアミノアセチル)デアセチルコルヒチン、
4-クロロ-N-(ジエチルアミノアセチル)デアセチルコルヒチン、
N-(t-ブチルオキシカルボニルアミノアセチル)-4-クロロデアセチルコルヒチン、

[0035] 4-クロロ-N-(アミノアセチル)デアセチルコルヒチン、
4-クロロ-N-(ピロリジン-1-イルアセチル)デアセチルコルヒチン、
4-クロロ-N-(ピペリジン-1-イルアセチル)デアセチルコルヒチン、
4-クロロ-N-(3-カルボキシプロピオニル)デアセチルコルヒチン、
4-クロロ-N-[3-(メトキシカルボニル)プロピオニル]デアセチルコルヒチン、
4-クロロ-N-(4-カルボキシブチリル)デアセチルコルヒチン、
4-クロロ-N-[4-(メトキシカルボニル)ブチリル]デアセチルコルヒチン、
N-(trans-1-カルボキシシクロヘキサン-4-イルオキシカルボニル)-4-クロロデアセチルコルヒチン、
4-クロロ-N-(ピリジン-3-イルオキシカルボニル)デアセチルコ

WO 2011/021397

22

PCT/JP2010/005136

ルヒチン、

4-クロロ-N-[1-(ジメチルアミノ)プロピル-3-イルオキシカルボニル]デアセチルコルヒチン、

[0036] 4-クロロ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチン、

4-クロロ-N-[4-(4-モルホリニルメチル)ベンゾイル]デアセチルコルヒチン、

4-クロロ-N-(ジエチルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(ピロリジン-1-チオカルボニル)デアセチルコルヒチン、

4-クロロ-N-(ピペリジン-1-チオカルボニル)デアセチルコルヒチン、

4-クロロ-N-(モルホリン-4-チオカルボニル)デアセチルコルヒチン、

4-クロロ-N-(チオモルホリン-4-チオカルボニル)デアセチルコルヒチン、

4-クロロ-N-(1,1-ジオキソチオモルホリン-4-チオカルボニル)デアセチルコルヒチン、

4-クロロ-N-(1-メチルピペラジン-4-チオカルボニル)デアセチルコルヒチン、

(S)-4-クロロ-N-(2-アセトキシプロピオニル)デアセチルコルヒチン、

(S)-4-クロロ-N-(2-ヒドロキシプロピオニル)デアセチルコルヒチン、

(R)-4-クロロ-N-(2-アセトキシプロピオニル)デアセチルコルヒチン、

(R)-4-クロロ-N-(2-ヒドロキシプロピオニル)デアセチルコルヒチン、

WO 2011/021397

23

PCT/JP2010/005136

4-クロロ-N-(2-ヒドロキシ-2-メチルプロピオニル)デアセチル
 コルヒチン、

4-クロロ-N-(3-ヒドロキシ-2, 2-ジメチルプロピオニル)デアセチル
 コルヒチン、

4-クロロ-N-[2, 2-ビス(ヒドロキシメチル)プロピオニル]デアセチル
 コルヒチン、

4-クロロ-N-(1-アセチルピペリジン-4-カルボニル)デアセチル
 コルヒチン、

4-クロロ-N-(1-ベンゾイルピペリジン-4-カルボニル)デアセチル
 コルヒチン、

4-クロロ-N-[1-(tert-ブチルオキシカルボニル)ピペリジン-4-カルボニル]
 デアセチルコルヒチン、

[0037] 4-クロロ-N-(ピペリジン-4-カルボニル)デアセチルコルヒチン
 、

4-クロロ-N-[3-(ジメチルアミノメチル)ベンゾイル]デアセチル
 コルヒチン、

4-クロロ-N-[3-(トリフェニルメチルオキシ)プロピオニル]デアセチル
 コルヒチン、

4-クロロ-N-(3-ヒドロキシプロピオニル)デアセチルコルヒチン
 、

4-クロロ-N-(メタンスルホニルアセチル)デアセチルコルヒチン、

4-クロロ-N-[3-(ジメチルアミノ)プロピオニル]デアセチルコル
 ヒチン、

4-クロロ-N-[3-(ジエチルアミノ)プロピオニル]デアセチルコル
 ヒチン、

4-クロロ-N-(3-ピペリジノプロピオニル)デアセチルコルヒチン
 、

N-[N'-(tert-ブチルオキシカルボニル)-N'-メチルアミノアセ

WO 2011/021397

24

PCT/JP2010/005136

チル] - 4-クロロデアセチルコルヒチン、

N - [N' - ベンジル - N' - (t-ブチルオキシカルボニル) アミノアセチル] - 4-クロロデアセチルコルヒチン、

4-クロロ-N - (1-メチルピペリジン-4-イルカルボニル) デアセチルコルヒチン、

4-クロロ-N - [1 - (ベンジルオキシカルボニル) ピペリジン-4-イルカルボニル] デアセチルコルヒチン、

[0038] 4-クロロ-N - (N' - メチルアミノアセチル) デアセチルコルヒチン、

N - (N' - ベンジルアミノアセチル) - 4-クロロデアセチルコルヒチン、

4-クロロ-N - [4 - (ジメチルアミノ) ブチリル] デアセチルコルヒチン、

4-クロロ-N - [2 - (ジメチルアミノ) ベンゾイル] デアセチルコルヒチン、

4-クロロ-N - (イソブチルチオカルバモイル) デアセチルコルヒチン、

N - (ベンジルチオカルバモイル) - 4-クロロデアセチルコルヒチン、

4-クロロ-N - (シクロヘキシルチオカルバモイル) デアセチルコルヒチン、

4-クロロ-N - [(2-ピペリジノエチル) チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N - {[2 - (4-モルフォリノ) エチル] チオカルバモイル} デアセチルコルヒチン、

4-クロロ-N - (3-ピリジルチオカルバモイル) デアセチルコルヒチン、

4-クロロ-N - [p - (ジメチルアミノ) フェニルチオカルバモイル] デアセチルコルヒチン、

WO 2011/021397

25

PCT/JP2010/005136

- [0039] 4-クロロ-N-(4-ニトロベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(2-ナフトイル)デアセチルコルヒチン、
4-クロロ-N-(2-ニトロベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(4-メチルベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(4-フェニルベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(3, 5-ジニトロベンゾイル)デアセチルコルヒチン
、
4-クロロ-N-(3, 5-ジメチルベンゾイル)デアセチルコルヒチン
、
4-クロロ-N-(3-ニトロベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(3, 5-ジメトキシベンゾイル)デアセチルコルヒチ
ン、
4-クロロ-N-(2, 4-ジメトキシベンゾイル)デアセチルコルヒチ
ン、
4-クロロ-N-(2, 4, 6-トリメチルベンゾイル)デアセチルコル
ヒチン、
4-クロロ-N-(4-シアノベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(3-ヒドロキシ-3-メチルブチリル)デアセチルコ
ルヒチン、
4-クロロ-N-[4-(ベンジルオキシ)ブチリル]デアセチルコルヒ
チン、
4-クロロ-N-(2-シアノベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(3-シアノベンゾイル)デアセチルコルヒチン、
4-クロロ-N-(2-エチル-2-ヒドロキシブチリル)デアセチルコ
ルヒチン、
4-クロロ-N-(1-ヒドロキシシクロプロパンカルボニル)デアセチ
ルコルヒチン、
4-クロロ-N-(3-フェニルベンゾイル)デアセチルコルヒチン、

WO 2011/021397

26

PCT/JP2010/005136

- 4-クロロ-N-(4-ヒドロキシブチル)デアセチルコルヒチン、
4-ブロモ-N-(t-ブチルオキシカルボニル)コルヒチン、
[0040] 4-ブロモ-N-(t-ブチルオキシカルボニル)デアセチルコルヒチン
、
4-ブロモデアセチルコルヒチン、
4-ブロモ-N-プロピオニルデアセチルコルヒチン、
4-ブロモ-N-(シクロヘキサンカルボニル)デアセチルコルヒチン、
4-ブロモ-N-ベンゾイルデアセチルコルヒチン、
4-ブロモ-N-(フェニルアセチル)デアセチルコルヒチン、
N-アリル-4-ブロモ-N-(t-ブチルオキシカルボニル)デアセチルコルヒチン、
4-ブロモ-N-(トリフルオロアセチル)デアセチルコルヒチン、
N-(アセトキシアセチル)-4-ブロモデアセチルコルヒチン、
4-ブロモ-N-(2-ピリジンカルボニル)デアセチルコルヒチン、
N-アリル-4-ブロモデアセチルコルヒチン、
[0041] N-(ベンジルオキシカルボニル)-4-ブロモデアセチルコルヒチン、
4-ブロモ-N-[(メチルチオ)アセチル]デアセチルコルヒチン、
4-ブロモ-N-[(N', N'-ジメチルアミノ)アセチル]デアセチルコルヒチン、
4-ブロモ-N-プロピルデアセチルコルヒチン、
4-ブロモ-N-(ヘキシルカルバモイル)デアセチルコルヒチン、
4-ブロモ-N-(フェニルカルバモイル)デアセチルコルヒチン、
4-ブロモ-N-(ヒドロキシアセチル)デアセチルコルヒチン、
4-ブロモ-N-(イソブタンスルホニル)デアセチルコルヒチン、
4-ブロモ-N-トシルデアセチルコルヒチン、
4-ブロモ-N-(2-チオフエンスルホニル)デアセチルコルヒチン、
4-ブロモ-N-(2, 4-ジフルオロベンゾイル)デアセチルコルヒチン、
ン、

WO 2011/021397

27

PCT/JP2010/005136

4-ブロモ-N-[3-(N', N'-ジメチルアミノ)ベンゾイル]デアセチルコルヒチン、

4-ブロモ-N-(2-フルオロベンゾイル)デアセチルコルヒチン、

4-ブロモ-N-(3-メトキシベンゾイル)デアセチルコルヒチン、

4-ブロモ-N-(エチルチオカルバモイル)デアセチルコルヒチン、

4-ブロモ-N-(フェニルチオカルバモイル)デアセチルコルヒチン、

[0042] 4-ブロモ-N-(N', N'-ジエチルカルバモイル)デアセチルコルヒチン、

4-ブロモ-N-(ピペリジン-1-イルカルボニル)デアセチルコルヒチン、

4-ブロモ-N-(3-ヒドロキシ-2, 2-ジメチルプロピオニル)デアセチルコルヒチン、

4-ブロモ-N-(2-ヒドロキシ-2-メチルプロピオニル)デアセチルコルヒチン、

4-ブロモ-N-(2-エチル-2-ヒドロキシブチリル)デアセチルコルヒチン、

4-ブロモ-N-(1-ヒドロキシシクロプロパンカルボニル)デアセチルコルヒチン、

4-ブロモ-N-[2, 2-ビス(ヒドロキシメチル)プロピオニル]デアセチルコルヒチン、

4-ブロモ-N-(3-ヒドロキシ-3-メチルブチリル)デアセチルコルヒチン、

4-ブロモ-N-[4-(ベンジルオキシ)ブチリル]デアセチルコルヒチン、

4-ブロモ-N-(3-ニトロベンゾイル)デアセチルコルヒチン、

4-ブロモ-N-(4-シアノベンゾイル)デアセチルコルヒチン、

4-ブロモ-N-(3, 5-ジメチルベンゾイル)デアセチルコルヒチン

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WO 2011/021397

28

PCT/JP2010/005136

4-ブロモ-N-(4-メチルベンゾイル)デアセチルコルヒチン、
4-ブロモ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチル
コルヒチン、

4-ブロモ-N-(イソブチルチオカルバモイル)デアセチルコルヒチン
、

4-ブロモ-N-(ベンジルチオカルバモイル)デアセチルコルヒチン、
4-ブロモ-N-[4-(N', N'-ジメチルアミノ)フェニルチオカル
バモイル]デアセチルコルヒチン、

[0043] 4-ブロモ-N-(ピリジン-3-イルチオカルバモイル)デアセチルコ
ルヒチン、

4-ブロモ-N-(N', N'-ジエチルチオカルバモイル)デアセチル
コルヒチン、

4-ブロモ-N-(ピロリジン-1-イルチオカルボニル)デアセチルコ
ルヒチン、

4-ブロモ-N-(ピペリジン-1-イルチオカルボニル)デアセチルコ
ルヒチン、

4-ブロモ-N-(4-ヒドロキシブチリル)デアセチルコルヒチン、
4-ブロモ-N-(チオモルホリン-4-イルチオカルボニル)デアセチ
ルコルヒチン、

4-ブロモ-N-(1, 1-ジオキソチオモルホリン-4-イルチオカル
ボニル)デアセチルコルヒチン、

4-ブロモ-N-(4-メチルピペラジン-1-イルチオカルボニル)デア
セチルコルヒチン、

4-ブロモ-N-(モルホリン-4-イルチオカルボニル)デアセチルコ
ルヒチン、

4-ブロモ-N-(エタンスルホニル)デアセチルコルヒチン、
4-クロロ-2, 3-ジメチルコルヒチン、
4-クロロ-2, 3-(メチレンジオキシ)-2, 3-ジデメトキシコル

WO 2011/021397

29

PCT/JP2010/005136

ヒチン、

4-クロロ-2, 3-(エチレンジオキシ)-2, 3-ジデメトキシコル

ヒチン、

4-クロロ-1, 2-(メチレンジオキシ)-1, 2-ジデメトキシコル

ヒチン、

4-クロロ-N-(エチルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(フェニルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(4-ピペリジノピペリジン-1-カルボキシアセチル)
デアセチルコルヒチン、

4-クロロ-N-(1-メチルピペラジン-4-カルボキシアセチル)デアセチルコルヒチン、

4-クロロ-N-(ジメチルアミノアセトキシアセチル)デアセチルコルヒチン、

4-クロロ-N-[3-(ジメチルアミノ)プロピオニルオキシアセチル]
デアセチルコルヒチン、

4-クロロ-N-[1-(ベンジルオキシカルボニル)ピペリジン-4-カルボキシアセチル]
デアセチルコルヒチン、

4-クロロ-N-(チオアセトキシアセチル)デアセチルコルヒチン、

4-クロロ-N-(メルカプトアセチル)デアセチルコルヒチン、

4-クロロ-N-[1-(エトキシカルボニル)ピペリジン-4-イルカルボニル]
デアセチルコルヒチン、

4-(フルオロメチル)コルヒチン、

4-(ジフルオロメチル)コルヒチン、

4-クロロ-N-(N', N'-ジメチルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(N', N'-ジプロピルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-[N'-エチル-N'-プロピルチオカルバモイル]デアセチルコルヒチン、

WO 2011/021397

30

PCT/JP2010/005136

アセチルコルヒチン、

4-クロロ-N-[(4-ヒドロキシピペリジン-1-イル)チオカルボニル]デアセチルコルヒチン、

4-クロロ-N-[(4-メチルピペリジン-1-イル)チオカルボニル]デアセチルコルヒチン、

4-クロロ-N-{N'-[2-(ジメチルアミノ)エチル]-N'-メチルチオカルバモイル}デアセチルコルヒチン、

4-クロロ-N-{[4-(ジメチルアミノ)ピペリジン-1-イル]チオカルバモイル}デアセチルコルヒチン、

4-クロロ-N-(N'-エチル-N'-メチルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-[1-アセチルピペラジン-4-イル)チオカルボニル]デアセチルコルヒチン、

4-クロロ-N-[N'-(2-ヒドロキシエチル)-N'-メチルチオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[N'-(3-ヒドロキシプロピル)-N'-メチルチオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[N', N'-ビス(2-ヒドロキシエチル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(3-ヒドロキシプロピル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(4, 4-ジフルオロピペリジン-1-イル)チオカルバモイル]デアセチルコルヒチン、

N-[(アゼチジン-1-イル)チオカルバモイル]-4-クロロデアセチルコルヒチン、

4-クロロ-N-[(インダゾール-5-イル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(cis-2, 6-ジメチルモルホリン-4-イル)

WO 2011/021397

31

PCT/JP2010/005136

チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-(2-トリルチオカルバモイル) デアセチルコルヒチン

、

4-クロロ-N-(3-トリルチオカルバモイル) デアセチルコルヒチン

、

4-クロロ-N-[3-(ジメチルアミノ) フェニルチオカルバモイル]

デアセチルコルヒチン、

4-クロロ-N-[4-(2-ヒドロキシエチル) フェニルチオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[3-(2-ヒドロキシエチル) フェニルチオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[(4-シアノフェニル) チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[2, 1, 3-ベンゾチアジアゾール-4-イル) チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[(2-メトキシフェニル) チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[(3-メトキシフェニル) チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[(3-シアノフェニル) チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[(3, 4-ジメトキシフェニル) チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[(2-クロロフェニル) チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[(3-クロロフェニル) チオカルバモイル] デアセチルコルヒチン、

4-クロロ-N-[(4-クロロフェニル) チオカルバモイル] デアセチ

WO 2011/021397

32

PCT/JP2010/005136

ルコルヒチン、

4-クロロ-N-[(2-シアノフェニル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(チアゾール-2-イル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-(チオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(メチルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(n-プロピルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(n-ブチルチオカルバモイル)デアセチルコルヒチン

、

4-クロロ-N-(tert-ブチルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(シクロペンチルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(イソプロピルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-(n-ヘキシルチオカルバモイル)デアセチルコルヒチン、

4-クロロ-N-[(trans-4-ヒドロキシシクロヘキシル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[2-(ジメチルアミノ)エチルチオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(2-ヒドロキシエチル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(4-メトキシフェニル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(p-トルイル)チオカルバモイル]デアセチルコル

WO 2011/021397

33

PCT/JP2010/005136

ヒチン、

4-クロロ-N-（シクロプロピルチオカルバモイル）デアセチルコルヒチン、

4-クロロ-N-（アリルチオカルバモイル）デアセチルコルヒチン、

4-クロロ-N-〔（1-メチルピペリジン-4-イル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-（シクロヘプチルチオカルバモイル）デアセチルコルヒチン、

4-クロロ-N-〔（2-メトキシエチル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（2, 2, 2-トリフルオロエチル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（インダン-2-イル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（4-スルファモイルフェニル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（3, 5-ジメチルイソオキサゾール-4-イル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（4-ヒドロキシフェニル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（5-メチルピラゾール-3-イル）チオカルバモイル〕デアセチルコルヒチン、

4-ブロモ-N-（プロピルチオカルバモイル）デアセチルコルヒチン、

4-ブロモ-N-（ブチルチオカルバモイル）デアセチルコルヒチン、

4-ブロモ-N-〔（2, 2, 2-トリフルオロエチル）チオカルバモイル〕デアセチルコルヒチン、

4-ブロモ-N-（シクロペンチルチオカルバモイル）デアセチルコルヒチン、

WO 2011/021397

34

PCT/JP2010/005136

4-ブロモ-N-（シクロヘキシルチオカルバモイル）デアセチルコルヒチン、

4-ブロモ-N-〔（4-メトキシフェニル）チオカルバモイル〕デアセチルコルヒチン、

4-ブロモ-N-〔〔2-（4-モルホリノ）エチル〕チオカルバモイル〕デアセチルコルヒチン、

4-ブロモ-N-（N'，N'-ジメチルチオカルバモイル）デアセチルコルヒチン、

4-ブロモ-N-（N'-エチル-N'-メチルチオカルバモイル）デアセチルコルヒチン、

4-ブロモ-N-〔（4-メチルピペリジン-1-イル）チオカルボニル〕デアセチルコルヒチン、

4-ブロモ-N-〔（4，4-ジフルオロピペリジン-1-イル）チオカルボニル〕デアセチルコルヒチン、

4-クロロ-N-〔（5-メチルイソオキサゾール-3-イル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（5-メチル-1，2，4-トリアゾール-3-イル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（イソオキサゾール-3-イル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（1，2，4-トリアゾール-3-イル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（3，4-ジメチルイソオキサゾール-5-イル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（4-シアノピラゾール-3-イル）チオカルバモイル〕デアセチルコルヒチン、

4-クロロ-N-〔（5-フェニルピラゾール-3-イル）チオカルバモイル〕デアセチルコルヒチン、

WO 2011/021397

35

PCT/JP2010/005136

N- (t-ブチルオキシカルボニル) -4-ヨードコルヒチン、
N- (t-ブチルオキシカルボニル) -4-ヨードデアセチルコルヒチン
、
4-ヨードデアセチルコルヒチン、
4-ヨード-N-プロピオニルデアセチルコルヒチン、
N-シクロプロピルカルボニル-4-ヨードデアセチルコルヒチン、
4-ヨード-N- (トリフルオロアセチル) デアセチルコルヒチン、
N-エチルスルホニル-4-ヨードデアセチルコルヒチン、
N- [3- (ジメチルアミノ) ベンゾイル] -4-ヨードデアセチルコル
ヒチン、
4-ヨード-N- (4-メトキシベンゾイル) デアセチルコルヒチン、
4-ヨード-N- (4-ニトロベンゾイル) デアセチルコルヒチン、
N- (3, 5-ジフルオロベンゾイル) -4-ヨードデアセチルコルヒチ
ン、
N- (3-シアノベンゾイル) -4-ヨードデアセチルコルヒチン、
N- (アセトキシアセチル) -4-ヨードデアセチルコルヒチン、
4-ヨード-N- (フェニルアセチル) デアセチルコルヒチン、
4-ヨード-N-プロピルデアセチルコルヒチン、
N- (ヒドロキシアセチル) -4-ヨードデアセチルコルヒチン、
N- [1- (ペンジルオキシカルボニル) ピペリジン-4-イルカルボニ
ル] -4-ヨードデアセチルコルヒチン、
4-ヨード-N- (ピリジン-4-イルカルボニル) デアセチルコルヒチ
ン、
N-フェニルスルホニル-4-ヨードデアセチルコルヒチン、
N- (シクロヘキシルチオカルバモイル) -4-ヨードデアセチルコルヒ
チン、
N- [4- (ジメチルアミノ) フェニルチオカルバモイル] -4-ヨード
デアセチルコルヒチン、

WO 2011/021397

36

PCT/JP2010/005136

N-（ベンジルカルバモイル）-4-ヨードデアセチルコルヒチン、
4-ヨード-N-〔（ピペリジン-1-イル）カルボニル〕デアセチルコルヒチン、
N-エチル-4-ヨードデアセチルコルヒチン、
4-ヨード-N-〔（ピペリジン-1-イル）チオカルボニル〕デアセチルコルヒチン、
4-ヨード-N-〔（チオモルホリン-4-イル）チオカルボニル〕デアセチルコルヒチン、
N-〔（ベンゾイミダゾール-2-イル）チオカルバモイル〕-4-クロロデアセチルコルヒチン、
N-〔（ベンゾチアゾール-2-イル）チオカルバモイル〕-4-クロロデアセチルコルヒチン、
4-クロロ-N-〔（ピリミジン-2-イル）チオカルバモイル〕デアセチルコルヒチン、
4-クロロ-N-〔（ピリダジン-3-イル）チオカルバモイル〕デアセチルコルヒチン、
4-クロロ-N-〔（ピリジン-2-イル）チオカルバモイル〕デアセチルコルヒチン、
4-クロロ-N-〔（ピラゾール-3-イル）チオカルバモイル〕デアセチルコルヒチン、
4-クロロ-N-〔（1-メチルピラゾール-3-イル）チオカルバモイル〕デアセチルコルヒチン、
4-クロロ-N-〔（4-メチルチアゾール-2-イル）チオカルバモイル〕デアセチルコルヒチン、
4-クロロ-N-〔（4, 5-ジメチルチアゾール-2-イル）チオカルバモイル〕デアセチルコルヒチン、
4-クロロ-N-〔（ピリジン-4-イル）チオカルバモイル〕デアセチルコルヒチン、

WO 2011/021397

37

PCT/JP2010/005136

4-クロロ-N-[3-(2-ヒドロキシエトキシ)ベンゾイル]デアセチルコルヒチン、

4-クロロ-N-[3-(2-ヒドロキシエトキシ)-4-メトキシベンゾイル]デアセチルコルヒチン、

4-クロロ-N-[(ピラジン-2-イル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(1, 3, 4-チアジアゾール-2-イル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(ピリミジン-5-イル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[3-(2-ヒドロキシエトキシ)フェニルチオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[4-(2-ヒドロキシエトキシ)フェニルチオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(1, 2-ジメチルイミダゾール-5-イル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-{[1-(2-ヒドロキシエチル)-2-メチルイミダゾール-5-イル]チオカルバモイル}デアセチルコルヒチン、

4-クロロ-N-{[1-(2-ヒドロキシエチル)ピラゾール-5-イル]チオカルバモイル}デアセチルコルヒチン、

4-クロロ-N-{[1-(2-ヒドロキシエチル)ピラゾール-3-イル]チオカルバモイル}デアセチルコルヒチン、

4-ブロモ-N-[(5-メチル-1, 2, 4-トリアゾール-3-イル)チオカルバモイル]デアセチルコルヒチン、

4-ブロモ-N-[(1-メチル-1, 2, 4-トリアゾール-3-イル)チオカルバモイル]デアセチルコルヒチン、

4-ブロモ-N-[(1, 2, 4-トリアゾール-3-イル)チオカルバモイル]デアセチルコルヒチン、

WO 2011/021397

38

PCT/JP2010/005136

4-ブロモ-N- [(4-シアノピラゾール-3-イル) チオカルバモイル] デアセチルコルヒチン、

4-ブロモ-N- [(5-メチルピラゾール-3-イル) チオカルバモイル] デアセチルコルヒチン、

4-ブロモ-N- [(1-メチルピラゾール-3-イル) チオカルバモイル] デアセチルコルヒチン、

4-ブロモ-N- [(ピラゾール-3-イル) チオカルバモイル] デアセチルコルヒチン、

4-ブロモ-N- [(3, 4-ジメチルイソオキサゾール-5-イル) チオカルバモイル] デアセチルコルヒチン、

4-ブロモ-N- [(5-メチルイソオキサゾール-3-イル) チオカルバモイル] デアセチルコルヒチン、

4-ブロモ-N- [(4-メチルチアゾール-2-イル) チオカルバモイル] デアセチルコルヒチン、

4-ブロモ-N- [(チアゾール-2-イル) チオカルバモイル] デアセチルコルヒチン、

4-ブロモ-N- [3-(2-ヒドロキシエチル) フェニルチオカルバモイル] デアセチルコルヒチン、

4-ブロモ-N- [(ピリダジン-3-イル) チオカルバモイル] デアセチルコルヒチン、

N- [(アゼチジン-1-イル) チオカルボニル] -4-ブロモデアセチルコルヒチン、

4-ブロモ-N- (メチルチオカルバモイル) デアセチルコルヒチン、

4-ブロモ-N- (チオカルバモイル) デアセチルコルヒチン、

4-ブロモ-N- { [1-(2-ヒドロキシエチル) -1, 2, 4-トリアゾール-3-イル] チオカルバモイル } デアセチルコルヒチン、

4-ブロモ-N- { [1-(2-ヒドロキシエチル) ピラゾール-3-イル] チオカルバモイル } デアセチルコルヒチン、

WO 2011/021397

39

PCT/JP2010/005136

4-ブロモ-N-[4-(2-ヒドロキシエトキシ)フェニルチオカルバモイル]デアセチルコルヒチン、

4-ブロモ-N-[3-(2-ヒドロキシエトキシ)フェニルチオカルバモイル]デアセチルコルヒチン、

4-ブロモ-N-[(4-シアノフェニル)チオカルバモイル]デアセチルコルヒチン、

4-ブロモ-N-[(2-ヒドロキシエチル)チオカルバモイル]デアセチルコルヒチン、

4-ブロモ-N-[N'-(2-ヒドロキシエチル)-N'-メチルチオカルバモイル]デアセチルコルヒチン、

4-ブロモ-N-[N'-(3-ヒドロキシプロピル)-N'-メチルチオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(1-メチル-1, 2, 4-トリアゾール-3-イル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-[(1-メチル-1, 2, 4-トリアゾール-5-イル)チオカルバモイル]デアセチルコルヒチン、

4-クロロ-N-{[1-(2-ヒドロキシエチル)-1, 2, 4-トリアゾール-3-イル]チオカルバモイル}デアセチルコルヒチン。

[0044] 本発明のコルヒチン誘導体の塩としては、有機酸塩及び無機酸塩が挙げられる。有機酸塩を形成する酸としては、酢酸、プロピオン酸、乳酸、リンゴ酸、クエン酸、酒石酸、フマル酸、マレイン酸、メシル酸等が挙げられる。無機酸塩を形成する無機酸としては、塩酸、硝酸、硫酸、リン酸、臭化水素酸等が挙げられる。

[0045] 本発明のコルヒチン誘導体、その塩又はそれらの溶媒和物は、7位が不斉炭素原子であることから、光学活性体である。また、この位置以外に不斉炭素原子がある場合は、その炭素原子に基づく光学異性体が存在し得る。本発明は、これらの光学異性体を全て含むものである。さらに、本発明化合物は、水和物等の溶媒和物の形態である場合も含まれる。

WO 2011/021397

40

PCT/JP2010/005136

- [0046] 本発明のコルヒチン誘導体の溶媒和物としては、水和物、エタノール溶媒和物等が挙げられる。
- [0047] 本発明のコルヒチン誘導体（１）は、コルヒチン化合物の４位にハロゲン原子、ヒドロキシ基、ニトロ基、アミノ基又はモノー、ジー若しくはトリフルオロメチル基を導入することにより製造することができる。例えばハロゲン原子の導入は、コルヒチン化合物に、Ｎ－フルオロベンゼンスルホンイミド、Ｎ－クロロコハク酸イミド、Ｎ－ブromoコハク酸イミド、Ｎ－ヨードコハク酸イミド等のハロゲン化試薬を反応させることにより行われる。当該ハロゲン化反応は、酢酸、アセトニトリル、Ｎ，Ｎ－ジメチルホルムアミド等の溶媒を用いて、０～１５０℃で１～５０時間行えばよい。
- [0048] ニトロ基の導入は、コルヒチン化合物に、硝酸二アンモニウムセリウムートリフルオロ酢酸無水物、硝酸、混酸、硝酸アセチル、ニトロニウム塩等のニトロ化試薬を反応させることにより行われる。この反応は、ジクロロメタン、クロロホルム、１，２－ジクロロエタン、四塩化炭素等のハロゲン化炭化水素系溶媒、酢酸、ニトロメタン、ニトロエタン等の溶媒中、－７８～１５０℃で１～５０時間行えばよい。
- [0049] ヒドロキシ基の導入は、コルヒチン化合物の４位にホルミル基を導入し、次いで酸化することにより行うことができる。まず、ホルミル基の導入は、コルヒチン化合物に、塩化スズ、塩化アルミニウム等のルイス酸とジクロロメチルメチルエーテル等を反応させることにより行われる。次いで、当該ホルミル基の酸化反応は、例えば、モノペルオキシフタル酸 マグネシウム等を反応させることにより行うことができる。
- [0050] アミノ基の導入は、４－ニトロコルヒチンを接触還元等により還元するか、又はコルヒチンの４位にカルボキシル基を導入し、次いでこのカルボキシル基をアミノ基に変換すればよい。まず、カルボキシル基の導入反応は、４－ホルミルコルヒチン化合物を酸化することにより行うことができ、例えば亜塩素酸ナトリウム、Jones 試薬、過マンガン酸塩、酸化銀等を反応させればよい。カルボキシル基のアミノ基への変換には、カルボン酸を直接ト

WO 2011/021397

41

PCT/JP2010/005136

リエチルアミンとジフェニルリン酸アジドと反応させるか、カルボン酸を酸塩化物又は酸ヒドラジドとしアジ化ナトリウム又は亜硝酸誘導体等を反応させればよい。

- [0051] モノー、ジー若しくはトリフルオロメチル基の導入は、モノフルオロメチル基の場合、コルヒチン化合物の4位にホルミル基を導入し、次いで還元により4位をヒドロキシメチル基とした後、ヒドロキシ部分をフッ素化することにより行うことができる。ジフルオロメチル基の場合、4位のホルミル基をジチオアセタールへと変換した後、フッ素化することにより行うことができる。フッ素化試薬としては、例えばフッ化水素-ピリジン錯体、(ジエチルアミノ)サルファートリフルオリド等を用いればよい。
- [0052] 本発明のコルヒチン誘導体、その塩又はそれらの溶媒和物は、後記実施例に示すように、*in vitro*及び*in vivo*において優れた癌細胞増殖阻害活性を示す。また、毒性も低い。従って、本発明のコルヒチン誘導体、その塩又はそれらの溶媒和物は、ヒトを含む哺乳類の抗癌剤として有用である。
- [0053] 本発明のコルヒチン誘導体、その塩又はそれらの溶媒和物を医薬として用いる場合、本発明の化合物は、そのままでも投与することができるが、他の薬学的に許容される担体、例えば分散補助剤、賦形剤等の担体と混合し、粉剤、液剤、カプセル剤、懸濁剤、乳剤、シロップ剤、エリキシル剤、顆粒剤、丸剤、錠剤、トローチ剤、リモネーデ剤等の経口剤又は注射剤等の剤形で使用することができる。これらの製剤は、公知の方法で製造することができる。
- [0054] 担体としては、例えば、マンニトール、乳糖、デキストラン等の水溶性の単糖類ないしオリゴ糖類もしくは多糖類；例えば、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロース、メチルセルロース等のゲル形成性又は水溶性のセルロース類；例えば、結晶性セルロース、 α -セルロース、架橋カルボキシメチルセルロースナトリウム、及びそれらの誘導体等の水吸収性でかつ水難溶性のセルロース類；例えば、ヒドロキシプロピル澱

粉、カルボキシメチル澱粉、架橋澱粉、アミロース、アミロペクチン、ペクチン及びそれらの誘導体等の水吸収性でかつ水難溶性の多糖類；例えば、アラビアガム、トラガントガム、グリコマンナン及びそれらの誘導体等の水吸収性でかつ水難溶性のガム類；例えば、ポリビニルピロリドン、架橋ポリアクリル酸及びその塩、架橋ポリビニルアルコール、ポリヒドロキシエチルメタクリレート及びそれらの誘導体等の架橋ビニル重合体類；リン脂質、コレステロール等のリポソーム等分子集合体を形成する脂質類等を挙げることができる。

[0055] 本発明の化合物の溶解性が低い場合には、可溶化処理を施すことができる。可溶化処理としては通常医薬に適用できる方法、例えば、ポリオキシエチレンアルコールエーテル類、ポリオキシエチレンアシルエステル類、ソルビタンアシルエステル類やポリオキシエチレンソルビタンアシルエステル類等の界面活性剤を添加する方法、ポリエチレングリコール等の水溶性高分子を使用する方法等が挙げられる。また、必要により、可溶性の塩にする方法、シクロデキストリン等を用いて包接化合物を形成させる方法等も使用できる。

[0056] 本発明の抗癌剤の対象となる癌種としては、頭頸部癌、食道癌、胃癌、結腸癌、直腸癌、肝臓癌、胆嚢・胆管癌、膵臓癌、肺癌、乳癌、卵巣癌、子宮頸癌、子宮体癌、腎癌、膀胱癌、前立腺癌、精巣腫瘍、骨・軟部肉腫、白血病、悪性リンパ腫、多発性骨髄腫、皮膚癌、脳腫瘍等が挙げられる。

[0057] 本発明の医薬の投与量は、投与方法や患者の症状等に合わせて適宜調整すればよいが、成人１日あたり１ｍｇ～１０ｇ、さらに１００ｍｇ～１０ｇ、特に５００ｍｇ～１０ｇ投与するのが好ましい。

実施例

[0058] 次に本発明を実施例を挙げてさらに詳細に説明するが、これは単に例示であって本発明を限定するものではない。

[0059] 参考例 1

４－ホルミルコルヒチンの合成

WO 2011/021397

43

PCT/JP2010/005136

コルヒチン100 mg (0.25 mmol) をジクロロメタン 2.5 mLに溶解し、氷冷下塩化スズ (IV) 87.8 μ L (0.75 mmol, 3 eq)、ジクロロメチルメチルエーテル0.3 mL (3.0 mmol, 12 eq) を順に滴下し、0°C、アルゴン雰囲気下で30分攪拌した後、室温まで昇温しさらに12.5時間攪拌した。反応液に氷、クロロホルムを加え1時間攪拌し、分液漏斗にあげて有機層を分取した後、10%水酸化ナトリウム次いで水で洗浄し、無水硫酸マグネシウムで乾燥後、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (10%→ 15%→ 20%メタノール/ 酢酸エチル) にて精製し、表題の化合物13 4.4 mg (収率 定量的) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 10.46 (1H, s, -CHO), 7.55 (1H, br-d, $J=6.6\text{Hz}$, -NH), 7.52 (1H, s, H-8), 7.23 (1H, d, $J=10.8\text{Hz}$, H-12), 6.85 (1H, d, $J=10.8\text{Hz}$, H-11), 4.54 (1H, ddd, $J=12.3, 6.2, 6.2\text{Hz}$, H-7), 4.06 (3H, s, -OMe), 4.02 (3H, s, -OMe), 3.98 (3H, s, -OMe), 3.87 (1H, dd, $J=13.3, 4.7\text{Hz}$, H-5 β), 3.69 (3H, s, -OMe), 2.34 (1H, dddd, $J=12.7, 12.7, 6.4, 6.4\text{Hz}$, H-6 β), 2.01 (3H, s, - NHCOCH_3), 2.00 (1H, ddd, $J=13.2, 13.2, 6.7\text{Hz}$, H-5 α), 1.81 (1H, ddd, $J=12.2, 12.2, 4.9\text{Hz}$, H-6 α).

EI-MS m/z (%) : 427 (M^+ , 50.0), 340 (100.0).

[0060] 実施例 1

4-ニトロコルヒチンの合成

硝酸ニアンモニウムセリウム (IV) (CAN) 14.3 mg (0.026 mmol, 1.05 eq) をジクロロメタン0.5 mLに溶解し、コルヒチン10 mg (0.025 mmol)、トリフルオロ酢酸無水物12.2 μ L (0.0875 mmol, 3.5 eq) を加え、室温で16時間攪拌した。反応液に飽和炭酸水素ナトリウム水溶液を加え、クロロホルムで4回抽出し、飽和食塩水で洗浄後、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (10%メタノール/ 酢酸エチル及び5%メタノール/ クロロホルム) にて精製し、表題の化合物1.6 mg (収率 15%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.47 (1H, s, H-8), 7.25 (1H, d, $J=11.0\text{Hz}$

WO 2011/021397

44

PCT/JP2010/005136

z, H-12), 7.10 (1H, br-d, J=6.7Hz, -NH), 6.84 (1H, d, J=10.8Hz, H-11), 4.61 (1H, ddd, J=12.1, 6.1, 6.1Hz, H-7), 4.04 (3H, s, -OMe), 4.03 (3H, s, -OMe), 4.01 (3H, s, -OMe), 3.68 (3H, s, -OMe), 2.56 (1H, dd, J=13.6, 5.0Hz, H-5 β), 2.34 (1H, dddd, J=12.8, 12.8, 6.4, 6.4Hz, H-6 β), 2.33 (1H, ddd, J=13.3, 13.3, 6.6Hz, H-5 α), 2.02 (3H, s, -NHC(=O)CH₃), 1.83 (1H, ddd, J=12.0, 12.0, 6.0Hz, H-6 α).

EI-MS m/z (%): 444 (M⁺, 35.5), 57 (100.0)

[0061] 実施例 2

4-ヒドロキシコルヒチンの合成

4-ホルミルコルヒチン15.7 mg (0.037 mmol) をメタノール0.37 mLに溶解し、80%モノペルオキシフタル酸 マグネシウム (MMPP) 22.3 mg (0.036 mmol, 3 eq) を加え、室温で4時間攪拌した。反応液に飽和炭酸水素ナトリウム水溶液を加えクエンチし、1N塩酸で酸性とし、クロロホルムで4回抽出した。有機層を分取し、飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (3%メタノール/クロロホルム) にて精製し、表題の化合物5.7 mg (収率 37%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.46 (1H, s, H-8), 7.32 (1H, d, J=10.6Hz, H-12), 6.86 (1H, br-d, J=8.4Hz, -NH), 6.83 (1H, d, J=11.2Hz, H-11), 5.68 (1H, br-s, -OH), 4.63 (1H, ddd, J=12.3, 6.3, 6.3Hz, H-7), 4.02 (3H, s, -OMe), 4.00 (3H, s, -OMe), 3.99 (3H, s, -OMe), 3.58 (3H, s, -OMe), 3.15 (1H, dd, J=13.7, 5.9Hz, H-5 β), 2.25 (1H, dddd, J=12.6, 12.6, 6.3, 6.3Hz, H-6 β), 2.00 (3H, s, -NHC(=O)CH₃), 1.97 (1H, ddd, J=13.4, 13.4, 6.6Hz, H-5 α), 1.78 (1H, ddd, J=11.9, 11.9, 5.9Hz, H-6 α).

EI-MS m/z (%): 415 (M⁺, 100.0), 328 (55.8), 313 (65.5).

[0062] 参考例 2

コルヒチン-4-カルボン酸の合成

4-ホルミルコルヒチン170 mg (0.4 mmol) をt-ブタノール2.0 mLと水1.0

WO 2011/021397

45

PCT/JP2010/005136

mLの混液に溶解し、氷冷下 2-メチルー 2-ブテン (2Mテトラヒドロフラン溶液) 3 mL (6.0 mmol, 15 eq)、亜塩素酸ナトリウム 181 mg (2.0 mmol, 5 eq)、リン酸二水素ナトリウム 480 mg (4.0 mmol, 10 eq) を順に加え、室温で3時間攪拌した。反応液に飽和塩化アンモニウム水溶液を加えてクエンチし、10%メタノール/ クロロホルムで4回抽出した。有機層を飽和食塩水で洗浄、MgSO₄乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (10% [10% 酢酸含有メタノール] / クロロホルム) にて精製し、表題の化合物 174.6 mg (収率 99%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.65 (1H, s, H-8), 7.44 (1H, br-s, -NH), 7.32 (1H, d, J=10.8Hz, H-12), 6.92 (1H, d, J=11.0Hz, H-11), 4.64 (1H, ddd, J=12.2, 6.3, 6.3Hz, H-7), 4.03 (3H, s, -OMe), 3.99 (3H, s, -OMe), 3.96 (3H, s, -OMe), 3.65 (3H, s, -OMe), 2.81 (1H, dd, J=13.7, 4.6Hz, H-5β), 2.33 (1H, dddd, J=12.4, 12.4, 6.2, 6.2Hz, H-6β), 2.18 (1H, ddd, J=13.1, 13.1, 6.2Hz, H-5α), 1.99 (3H, s, -NHC(=O)CH₃), 1.81 (1H, ddd, J=11.6, 11.6, 5.6Hz, H-6α).

EI-MS m/z (%): 443 (M⁺, 44.7), 370 (80.0), 338 (100.0).

[0063] 実施例 3

4-フルオロコルヒチンの合成

コルヒチン 30 mg (0.075 mmol) をギ酸 0.75 mL に溶解し、N-フルオロベンゼンスルホンイミド (NFSi) 47.3 mg (0.15 mmol, 2 eq) を加えて、アルゴン雰囲気下 70°C にて 12 時間攪拌した。その後、NFSi 47.3 mg (0.15 mmol, 2 eq) を加えて 6.5 時間攪拌した。さらに NFSi 47.3 mg (0.15 mmol, 2 eq)、ギ酸 0.5 mL を追加し、6.5 時間攪拌した。室温まで放冷し、反応液に飽和チオ硫酸ナトリウム水溶液を加えて数分間攪拌した後、水酸化ナトリウムを加えて塩基性にした。クロロホルムで4回抽出し、有機層を飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (10% → 15%メタノール/ 酢酸エチル) にて精製し、表題の化合物 4.5 mg (収率 15%) を得た。

WO 2011/021397

46

PCT/JP2010/005136

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.48 (1H, H-8), 7.31 (1H, d, $J=10.6$ Hz, H-12), 7.04 (1H, br-d, $J=4.8$ Hz, -NH), 6.84 (1H, d, $J=10.8$ Hz, H-11), 4.61 (1H, ddd, $J=12.2, 6.2, 6.2$ Hz, H-7), 4.01 (6H, s, -OMe), 4.00 (3H, s, -OMe), 3.61 (3H, s, -OMe), 3.07 (1H, dd, $J=13.9, 6.0$ Hz, H-5 β), 2.27 (1H, dddd, $J=12.6, 12.6, 6.3, 6.3$ Hz, H-6 β), 2.04 (1H, overlapped, H-5 α), 2.00 (3H, s, -NHCOCH₃), 1.84 (1H, ddd, $J=12.2, 12.2, 6.6$ Hz, H-6 α).

FAB-MS (NBA) m/z : 417 $[\text{M}+\text{H}]^+$.

[0064] 参考例 3

4-クロロコルヒチンの合成

コルヒチン200 mg (0.5 mmol) を酢酸0.25 mLに溶解し、N-クロロコハク酸イミド (NCS) 133.5 mg (1.0 mmol, 2 eq) を加えて、アルゴン雰囲気下70°Cにて3時間攪拌した。室温まで放冷し、反応液に飽和チオ硫酸ナトリウム水溶液を加えて数分間攪拌した後、飽和炭酸水素ナトリウム水溶液を加えて塩基性にした。クロロホルムで4回抽出し、有機層を飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (10%メタノール/酢酸エチル) にて精製し、表題の化合物177.2 mg (収率 82%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.60 (1H, br-d, $J=5.9$ Hz, -NH), 7.54 (1H, s, H-8), 7.30 (1H, d, $J=10.6$ Hz, H-12), 6.86 (1H, d, $J=11.0$ Hz, H-11), 4.55 (1H, ddd, $J=12.3, 6.3, 6.3$ Hz, H-7), 4.02 (3H, s, -OMe), 4.00 (3H, s, -OMe), 3.97 (3H, s, -OMe), 3.63 (3H, s, -OMe), 3.26 (1H, dd, $J=13.5, 4.9$ Hz, H-5 β), 2.30 (1H, dddd, $J=12.7, 12.7, 6.3, 6.3$ Hz, H-6 β), 2.16 (1H, ddd, $J=13.4, 13.4, 6.3$ Hz, H-5 α), 2.00 (3H, s, -NHCOCH₃), 1.85 (1H, ddd, $J=12.3, 12.3, 5.6$ Hz, H-6 α).

EI-MS m/z (%) : 433 (M^+ , 18.5), 435 ($[\text{M}+2]^+$, 6.7), 91 (100.0).

[0065] 参考例 4

4-ブロモコルヒチンの合成

WO 2011/021397

47

PCT/JP2010/005136

コルヒチン10 mg (0.025 mmol) を酢酸0.25 mLに溶解し、N-ブロモコハク酸イミド (NBS) 6.7 mg (0.05 mmol, 2 eq) を加えて、アルゴン雰囲気下70 °Cにて13時間攪拌した。室温まで放冷し、反応液に飽和チオ硫酸ナトリウム水溶液を加えて数分間攪拌した後、飽和炭酸水素ナトリウム水溶液を加えて塩基性にした。クロロホルムで4回抽出し、有機層を飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (5% → 10%メタノール/ クロロホルム) にて精製し、表題の化合物3.4 mg (収率 29%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm] : 7.99 (1H, br-s, -NH), 7.58 (1H, s, H-8), 7.29 (1H, d, J=10.6Hz, H-12), 6.87 (1H, d, J=10.8Hz, H-11), 4.52 (1H, ddd, J=12.2, 6.2, 6.2Hz, H-7), 4.02 (3H, s, -OMe), 3.98 (3H, s, -OMe), 3.95 (3H, s, -OMe), 3.62 (3H, s, -OMe), 3.25 (1H, dd, J=13.4, 5.2 Hz, H-5β), 2.31 (1H, dddd, J=12.6, 12.6, 6.3, 6.3Hz, H-6β), 2.24 (1H, ddd, J=13.4, 13.4, 6.1Hz, H-5α), 1.85 (3H, s, -NHC(=O)CH₃), 1.74 (1H, ddd, J=11.9, 11.9, 5.8Hz, H-6α).

EI-MS m/z (%) : 477 (M⁺, 4.3), 479 ([M+2]⁺).

[0066] 参考例 5

4-ヨードコルヒチンの合成

コルヒチン100 mg (0.25 mmol) を酢酸2.5 mLに溶解し、N-ヨードコハク酸イミド (NIS) 112.49 mg (0.5 mmol, 2 eq) を加えて、アルゴン雰囲気下70 °Cにて7時間攪拌した。室温まで放冷し、反応液に飽和チオ硫酸ナトリウム水溶液を加えて数分間攪拌した後、飽和炭酸水素ナトリウム水溶液を加えて塩基性にした。クロロホルムで4回抽出し、有機層を飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (5% → 10%メタノール/ クロロホルム) にて精製し、表題の化合物129.5 mg (収率 98.6%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm] : 7.49 (1H, s, H-8), 7.26 (1H, d, J=10.6 Hz, H-12), 7.15 (1H, br-d, J=6.8Hz, -NH), 6.84 (1H, d, J=11.2Hz, H-11),

WO 2011/021397

48

PCT/JP2010/005136

4.50 (1H, ddd, J=12.2, 6.2, 6.2Hz, H-7), 4.02 (3H, s, -OMe), 3.97 (3H, s, -OMe), 3.95 (3H, s, -OMe), 3.62 (3H, s, -OMe), 3.18 (1H, dd, J=13.5, 5.1Hz, H-5 β), 2.43 (1H, ddd, J=13.7, 13.7, 6.0Hz, H-5 α), 2.28 (1H, dddd, J=12.6, 12.6, 6.4, 6.4Hz, H-6 β), 2.00 (3H, s, -NHC(=O)CH₃), 1.75 (1H, ddd, J=11.8, 11.8, 5.7Hz, H-6 α).
EI-MS m/z (%): 525 (M⁺, 68.6), 438 (100.0).

[0067] 実施例 4

4-アミノコルヒチンの合成

コルヒチン-4-カルボン酸5.0 mg (0.011 mmol) をテトラヒドロフラン0.3 mLに溶解し、氷冷下トリエチルアミン2.3 μ L (0.0165 mmol, 1.5 eq)、ジフェニルリン酸アジド (DPPA) 2.8 μ L (0.013 mmol, 1.2 eq) を加え室温で4時間攪拌した。さらに水0.15 mLを加え、1時間煮沸還流した。反応液に飽和炭酸カリウム水溶液を加え、クロロホルムで4回抽出した。有機層を飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (10%メタノール/ クロロホルム) にて精製し、表題の化合物1.7 mg (収率 37%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.49 (1H, s, H-8), 7.33 (1H, d, J=10.8Hz, H-12), 7.09 (1H, br-s, -NH), 6.85 (1H, d, J=10.8Hz, H-11), 4.65 (1H, ddd, J=12.1, 6.1, 6.1Hz, H-7), 4.00 (3H, s, -OMe), 3.99 (3H, s, -OMe), 3.94 (3H, s, -OMe), 3.72 (2H, br-s, -NH₂), 3.55 (3H, s, -OMe), 2.63 (1H, dd, J=14.0, 5.1Hz, H-5 β), 2.26 (1H, dddd, J=12.6, 12.6, 6.3, 6.3Hz, H-6 β), 2.12 (1H, ddd, J=13.9, 13.9, 6.1Hz, H-5 α), 2.00 (3H, s, -NHC(=O)CH₃), 1.80 (1H, ddd, J=11.7, 11.7, 5.6Hz, H-6 α).

FAB-MS (NBA) m/z: 415 [M+H]⁺.

[0068] 参考例 6

グロリオサミンAの合成

4-ヒドロキシー-2, 3-(メチレンジオキシ)-2, 3-ジデメトキシコルヒチン2.3 mg (0.0057 mmol) をアセトン0.3 mLに溶解し、炭酸カリウム

WO 2011/021397

49

PCT/JP2010/005136

3.6 mg (0.026 mmol, 5 eq)、ヨウ化メチル3.5 μ L (0.057 mmol, 10 eq) を順に加え、50°Cで8.5時間攪拌した。反応液に水を加えてクエンチし、クロロホルムで4回抽出した。有機層を飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法(4%メタノール/クロロホルム)にて精製し、表題の化合物1.2 mg(収率 55%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.50 (1H, s, H-8), 7.77 (1H, br-d, $J=7.7$ Hz, -NH), 7.26 (1H, d, $J=10.6$ Hz, H-12), 6.85 (1H, d, $J=10.8$ Hz, H-11), 6.03 (1H, d, $J=1.5$ Hz, -OCH₂O-), 6.02 (1H, d, $J=1.5$ Hz, -OCH₂O-), 4.62 (1H, ddd, $J=12.1, 6.1, 6.1$ Hz, H-7), 4.01 (3H, s, -OMe), 3.92 (3H, s, -OMe), 3.69 (3H, s, -OMe), 3.11 (1H, dd, $J=13.5, 6.0$ Hz, H-5 β), 2.22 (1H, dddd, $J=12.5, 12.5, 6.2, 6.2$ Hz, H-6 β), 1.99 (3H, s, -NHCOCH₃), 1.90 (1H, ddd, $J=13.2, 13.2, 6.7$ Hz, H-5 α), 1.80 (1H, ddd, $J=11.9, 11.9, 6.4$ Hz, H-6 α).

EI-MS m/z (%): 413 (M^+ , 91.5).

[0069] 参考例 7

4-ヒドロキシ-2, 3-(メチレンジオキシ)-2, 3-ジデメトキシコルヒチンの合成

2, 3-ジデメチルコルヒチン38 mg (0.102 mmol) をアセトニトリル1 mL に懸濁し、炭酸カリウム112.8 mg (0.816 mmol, 8 eq)、ブロモクロロメタン0.5 mL (過剰量) を加え、60°Cで21時間攪拌した。反応液に水を加えてクエンチし、クロロホルムで3回抽出し、有機層を飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法(4%メタノール/クロロホルム)にて精製し、2, 3-(メチレンジオキシ)-2, 3-ジデメトキシコルヒチン(コルニゲリン)21.4 mg(収率 55%)を得た。コルニゲリン20 mg (0.052 mmol) をジクロロメタン0.52 mLに溶解し、氷冷下塩化すず(IV) 18.3 μ L (0.156 mmol, 3 eq)、ジクロロメチルメチルエーテル56.5 μ L (0.624 mmol, 12 eq

WO 2011/021397

50

PCT/JP2010/005136

）を順に滴下、0℃、アルゴン雰囲気下、30分攪拌した後、室温まで昇温しさらに7時間攪拌した。反応液に氷、クロロホルムを加え1時間攪拌し、分液漏斗にあけて有機層を分取した後、10%水酸化ナトリウム次いで、水で洗浄した。有機層を硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥し、得られた残渣をそのまま次の反応に用いた。4-ホルミル-2,3-(メチレンジオキシ)-ジデメトキシコルヒチン18 mg (0.044 mmol) をメタノール0.44 mLに溶解し、80%モノペルオキシフタル酸マグネシウム (MMPP) 81.9 mg (0.132 mmol, 3 eq) を加え室温で18時間攪拌した。反応液に飽和炭酸水素ナトリウム水溶液を加えクエンチし、1N塩酸で酸性とし、クロロホルムで4回抽出し、飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (4%メタノール/クロロホルム) にて精製し、表題の化合物1.3 mg (収率 7%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm] : 7.33 (1H, s, H-8), 7.32 (1H, d, J=9.0Hz, H-12), 6.92 (1H, d, J=11.0Hz, H-11), 6.02 (1H, d, J=1.3Hz, -OCH₂O-), 6.00 (1H, d, J=1.3Hz, -OCH₂O-), 4.63 (1H, ddd, J=12.7, 6.5, 6.5Hz, H-7), 4.01 (3H, s, -OMe), 3.64 (3H, s, -OMe), 3.14 (1H, dd, J=13.5, 6.1Hz, H-5β), 2.16 (1H, dddd, J=12.3, 12.3, 6.1, 6.1Hz, H-6β), 2.01 (3H, s, -NHCOCH₃), 1.87 (1H, ddd, J=13.0, 13.0, 6.7Hz, H-5α), 1.78 (1H, ddd, J=11.7, 11.7, 6.1Hz, H-6α).

EI-MS m/z (%) : 399 (M⁺, 75.3)

[0070] 参考例 8

4-メトキシ-1,2-(メチレンジオキシ)-1,2-ジデメトキシコルヒチンの合成

4-ヒドロキシ-1,2-(メチレンジオキシ)-1,2-ジデメトキシコルヒチン7.0 mg (0.018 mmol) をアセトン0.28 mLに溶解し、炭酸カリウム12.4 mg (0.09 mmol, 5 eq)、ヨウ化メチル22.6 μL (0.18 mmol, 10 eq) を順に加え、室温で8時間、55℃で12時間、次いで70℃で8時間攪拌した。反応液に水を加えてクエンチし、クロロホルムで4回抽出、飽和食塩水で洗浄、

WO 2011/021397

51

PCT/JP2010/005136

硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法（4%メタノール/ クロロホルム）にて精製し、表題の化合物2.0 mg（収率 27%）を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.41 (1H, s, H-8), 7.33 (1H, d, $J=10.6\text{ Hz}$, H-12), 6.80 (1H, d, $J=10.8\text{ Hz}$, H-11), 6.52 (1H, br-d, $J=7.3\text{ Hz}$, -NH), 6.03 (1H, d, $J=1.5\text{ Hz}$, - $\text{OCH}_2\text{O-}$), 5.89 (1H, d, $J=1.5\text{ Hz}$, - $\text{OCH}_2\text{O-}$), 4.71 (1H, ddd, $J=12.3, 6.3, 6.3\text{ Hz}$, H-7), 4.08 (3H, s, -OMe), 3.98 (3H, s, -OMe), 3.77 (3H, s, -OMe), 3.11 (1H, dd, $J=13.8, 5.9\text{ Hz}$, H-5 β), 2.28 (1H, dddd, $J=12.7, 12.7, 6.4, 6.4\text{ Hz}$, H-6 β), 2.02 (1H, ddd, $J=13.4, 13.4, 6.4\text{ Hz}$, H-5 α), 2.00 (3H, s, - NHCOCH_3), 1.83 (1H, ddd, $J=11.6, 11.6, 5.6\text{ Hz}$, H-6 α).

EI-MS m/z (%) : 413 (M^+ , 67).

[0071] 参考例 9

4-ヒドロキシ-1, 2-(メチレンジオキシ)-1, 2-ジデメトキシコルヒチンの合成

1, 2-ジデメチルコルヒチン100 mg (0.27 mmol) をアセトニトリル3 mL に懸濁し、炭酸カリウム298.5 mg (2.16 mmol, 8 eq)、ブロモクロロメタン1 mL (過剰量) を加え、60°Cで19時間攪拌した。反応液に水を加えてクエンチし、クロロホルムで3回抽出、飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。シリカゲルカラムクロマトグラフ法（5%メタノール/ クロロホルム）にて精製し、1, 2-(メチレンジオキシ)-1, 2-ジデメトキシコルヒチン110.5 mg（収率 定量的）を得た。

このもの60 mg (0.156 mmol) をジクロロメタン1.5 mLに溶解し、氷冷下塩化すず(IV) 55 μL (0.468 mmol, 3 eq)、ジクロロメチルメチルエーテル0.17 mL (1.872 mmol, 12 eq) を順に滴下、0°C、アルゴン雰囲気下、30分攪拌した後、室温まで昇温しさらに6.5時間攪拌した。反応液に氷、クロロホルムを加え1時間攪拌、分液漏斗にあけて有機層を分取した後、10%水酸化ナトリウム次いで水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真

WO 2011/021397

52

PCT/JP2010/005136

空乾燥し、得られた残渣をそのまま次の反応に用いた。4-ホルミル-1, 2-(メチレンジオキシ)-1, 2-ジデメトキシコルヒチン39 mg (0.095 mmol) をメタノール0.95 mLに溶解し、80%モノペルオキシフタル酸 マグネシウム (MMPP) 117.5 mg (0.19 mmol, 3 eq) を加え室温で18時間攪拌した。反応液に飽和炭酸水素ナトリウム水溶液を加えクエンチし、1N塩酸で酸性とし、クロロホルムで4回抽出し、飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (4%メタノール/ クロロホルム) にて精製し、表題の化合物8 mg (収率 21%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.32 (1H, s, H-8), 7.43 (1H, d, $J=10.8\text{ Hz}$, H-12), 7.43 (1H, d, $J=11.0\text{ Hz}$, H-11), 6.00 (1H, d, $J=1.5\text{ Hz}$, $-\text{OCH}_2\text{O}-$), 5.87 (1H, d, $J=1.3\text{ Hz}$, $-\text{OCH}_2\text{O}-$), 4.70 (1H, ddd, $J=12.6, 6.5, 6.5\text{ Hz}$, H-7), 4.09 (3H, s, $-\text{OMe}$), 4.00 (3H, s, $-\text{OMe}$), 3.15 (1H, dd, $J=13.8, 6.0\text{ Hz}$, H-5 β), 2.24 (1H, dddd, $J=12.7, 12.7, 6.4, 6.4\text{ Hz}$, H-6 β), 2.00 (3H, s, $-\text{NHCOCH}_3$), 1.96 (1H, ddd, $J=13.6, 13.6, 6.7\text{ Hz}$, H-5 α), 1.84 (1H, dd, $J=11.8, 11.8, 6.0\text{ Hz}$, H-6 α).

EI-MS m/z (%) : 399 (M^+ , 72.6).

[0072] 参考例 10

デアセチルコルヒチンの合成

コルヒチン100 mg (0.25 mmol) をアセトニトリル2.5 mLに溶解し、ジメチルアミノピリジン (DMAP) 30.5 mg (0.25 mmol, 1 eq) を加え、トリエチルアミン0.1 mL (0.75 mmol, 3 eq)、二炭酸ジ-*t*-ブチル0.29 mL (1.25 mmol, 5 eq) を順に滴下し、100°Cで7.5時間煮沸還流した。反応液をクロロホルムで希釈し、飽和クエン酸水溶液で3回洗浄、得られた水層をクロロホルムで3回抽出後、有機層を合わせて飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (1%メタノール/ 酢酸エチル) にて精製し、目的物のN-(*t*-ブトキシカルボニル) コルヒチン132.8 mg (収率 定量的) を得た。N-(*t*-ブ

WO 2011/021397

53

PCT/JP2010/005136

トキシカルボニル) コルヒチン570 mg (1.14 mmol) をメタノール5mLに溶解し、1Mナトリウムメトキシド2.28 mL (2.28 mmol, 2eq) を滴下して室温で1時間攪拌した。反応液に飽和食塩水を加えてクエンチし、クロロホルムで4回抽出し、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をそのまま次の反応に用いた。N-(*t*-ブトキシカルボニル) デアセチルコルヒチン551 mg (1.20 mmol) をトリフルオル酢酸1.7 mL (22.8 mmol, 19 eq) に溶解し、室温で40分攪拌した。反応液に5M水酸化ナトリウムを加えて塩基性にし、クロロホルムで3回抽出し、飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (10%メタノール/ クロロホルム) にて精製し、表題の化合物377 mg (収率 88%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.74 (1H, s, H-8), 7.18 (1H, d, $J=10.7\text{Hz}$, H-12), 6.79 (1H, d, $J=11.0\text{Hz}$, H-11), 6.53 (1H, s, H-4), 3.99 (3H, s, -OMe), 3.90 (6H, s, -OMe X 2), 3.70 (1H, dd, $J=11.0, 5.0\text{Hz}$, H-7), 3.65 (3H, s, -OMe), 2.47 (1H, dd, $J=13.7, 6.3\text{Hz}$, H-5 β), 2.39 (1H, ddd, $J=12.7, 12.7, 6.3\text{Hz}$, H-5 α), 2.30 (1H, dddd, $J=12.2, 12.2, 6.1, 6.1\text{Hz}$, H-6 β), 1.60 (1H, ddd, $J=11.5, 11.5, 4.8\text{Hz}$, H-6 α).

EI-MS m/z (%) : 357 (M^+ , 100.0), 312 (76.8), 298 (76.5).

[0073] 参考例 1 1

デアセチルコルヒチン塩酸塩の合成

メタノール2 mLに氷冷下、アセチル クロリド24 μL (0.34 mmol, 2 eq) を滴下して攪拌した。その溶液に、氷冷下、メタノール3 mLに溶解したデアセチルコルヒチン60 mg (0.17 mmol) を滴下し、1.5時間攪拌した。反応液を減圧留去、真空乾燥し、表題の化合物68.9 mgを得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.76 (1H, s, H-8), 7.32 (1H, d, $J=10.0\text{Hz}$, H-12), 6.89 (1H, d, $J=11.2\text{Hz}$, H-11), 6.55 (1H, s, H-4), 4.25 (1H, br-s, H-7), 3.96 (3H, s, -OMe), 3.93 (3H, s, -OMe), 3.92 (3H, s, -OMe), 3.70 (3H, s, -OMe), 2.72 (2H, br-s), 2.51 (2H, overlapped).

WO 2011/021397

54

PCT/JP2010/005136

[0074] 実施例 5

4-クロロ-N-(*t*-ブトキシカルボニル) コルヒチンの合成

4-クロロコルヒチン10 mg (0.023 mmol) をアセトニトリル0.5 mLに溶解し、ジメチルアミノピリジン2.8 mg (0.023 mmol, 1 eq) を加え、トリエチルアミン9.6 μ L (0.069 mmol, 3 eq)、二炭酸ジ-*t*-ブチル26.4 μ L (0.115 mmol, 5 eq) を順に滴下し、100°Cで10.5時間煮沸還流した。反応液をクロロホルムで希釈し、飽和クエン酸水溶液で3回洗浄、得られた水層をクロロホルムで3回抽出後、有機層を合わせて飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (1% メタノール/ 酢酸エチル) にて精製し、表題の化合物 9.2 mg (収率 75%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.56 (1H, s, H-8), 7.16 (1H, d, $J = 10.7$ Hz, H-12), 6.75 (1H, d, $J = 11.0$ Hz, H-11), 5.06 (1H, dd, $J = 12.2, 6.1$ Hz, H-7), 3.98 (3H, s, -OMe), 3.97 (3H, s, -OMe), 3.96 (3H, s, -OMe), 3.62 (3H, s, -OMe), 3.32 (1H, dd, $J = 13.9, 4.9$ Hz), 2.65–2.62 (1H, m), 2.30 (3H, s, $-\text{COCH}_3$), 2.28–2.21 (1H, m), 1.99–1.91 (1H, m), 1.57 (9H, s, $-\text{COOC}(\text{CH}_3)_3$).

ESI-MS m/z : 534 $[\text{M}+\text{H}]^+$.

[0075] 実施例 6

4-クロロ-N-(*t*-ブトキシカルボニル) デアセチルコルヒチン

4-クロロ-N-(*t*-ブトキシカルボニル) コルヒチン130 mg (0.24 mmol) をメタノール2.4 mLに溶解し、1M ナトリウムメトキシド 0.48 mL (0.48 mmol, 2 eq) を滴下して室温で6時間攪拌した。反応液に飽和食塩水を加えてクエンチし、クロロホルムで4回抽出、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (2% $\rightarrow$ 5% メタノール/ クロロホルム) にて精製し、表題の化合物105.6 mg (収率 89%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.51 (1H, s, H-8), 7.21 (1H, d, $J = 10$

WO 2011/021397

55

PCT/JP2010/005136

. 7 Hz, H-12), 6.79 (1H, d, J = 10.7 Hz, H-11), 4.95 (1H, d, J = 7.8 Hz, -NH), 4.34-4.28 (1H, m, H-7), 4.00 (3H, s, -OMe), 3.98 (3H, s, -OMe), 3.97 (3H, s, -OMe), 3.62 (3H, s, -OMe), 3.23 (1H, dd, J = 13.3, 4.5 Hz), 2.30-2.11 (2H, m), 1.65-1.61 (1H, m), 1.38 (9H, s, -COOC(CH₃)₃)

ESI-MS m/z : 492 [M+H]⁺.

[0076] 実施例 7

4-クロロデアセチルコルヒチンの合成

4-クロロ-N-(t-ブトキシカルボニル)デアセチルコルヒチン 95 mg (0.19 mmol) をトリフルオロ酢酸 0.27 mL (3.61 mmol, 19 eq) に溶解し、室温で 20 分撹拌した。反応液に 5N 水酸化ナトリウムを加えて塩基性にし、クロロホルムで 3 回抽出、飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (5% → 10% メタノール/クロロホルム) にて精製し、表題の化合物 65 mg (収率 87%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm] : 7.75 (1H, s, H-8), 7.15 (1H, d, J=10.6 Hz, H-12), 6.79 (1H, d, J=10.8 Hz, H-11), 4.01 (3H, s, -OMe), 3.96 (3H, s, -OMe), 3.95 (3H, s, -OMe), 3.62 (1H, overlapped, H-7), 3.61 (3H, s, -OMe), 3.19 (1H, dd, J=13.5, 4.9 Hz, H-5β), 2.29 (1H, dddd, J=12.6, 12.6, 4.2, 4.2 Hz, H-6β), 2.16 (1H, ddd, J=13.4, 13.4, 6.1 Hz, H-5α), 1.55 (1H, ddd, J=10.8, 10.8, 6.6 Hz, H-6α).

EI-MS m/z (%) : 391 (M⁺, 100.0), 393 ([M+2]⁺, 34.7).

[0077] 実施例 8

4-クロロデアセチルコルヒチン塩酸塩の合成

メタノール 2 mL に氷冷下、アセチルクロリド 20 μL (0.28 mmol, 2 eq) を滴下して撹拌した。その溶液に、氷冷下、メタノール 1.5 mL に溶解した 4-クロロデアセチルコルヒチン 55 mg (0.14 mmol) を滴下し、1.5 時間撹拌した。反応液を減圧留去、真空乾燥し、表題の化合物 61.5 mg を得た。

WO 2011/021397

56

PCT/JP2010/005136

¹H-NMR (400MHz, CDCl₃) δ [ppm] : 7.81 (1H, s, H-8), 7.35 (1H, d, J=10.6Hz, H-12), 6.96 (1H, d, J=10.8Hz, H-11), 4.21 (1H, br-s, H-7), 4.00 (6H, s, -OMe X 2), 3.98 (3H, s, -OMe), 3.65 (3H, s, -OMe), 3.29 (1H, br-dd, J=13.5, 5.5Hz), 2.62 (1H, m), 2.48 (1H, m), 2.23 (1H, m).

[0078] 実施例 9

4-フルオロ-N-(t-ブトキシカルボニル)コルヒチンの合成

4-フルオロコルヒチン12 mg (0.029 mmol) をアセトニトリル0.5 mLに溶解し、ジメチルアミノピリジン3.5 mg (0.029 mmol, 1 eq) を加え、トリエチルアミン12 μL (0.087 mmol, 3 eq)、二炭酸ジ-t-ブチル33.3 μL (0.145 mmol, 5 eq) を順に滴下し、100°Cで7.5時間煮沸還流した。反応液をクロロホルムで希釈し、飽和クエン酸水溶液で3回洗浄、得られた水層をクロロホルムで3回抽出後、有機層を合わせて飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (1% メタノール/ 酢酸エチル) にて精製し、表題の化合物 12.2 mg (収率 81%) を得た。

[0079] 実施例 10

4-フルオロ-N-(t-ブトキシカルボニル)デアセチルコルヒチンの合成

4-フルオロ-N-(t-ブトキシカルボニル)コルヒチン89 mg (0.17 mmol) をメタノール2mLに溶解し、1Mナトリウムメトキシド0.34 mL (0.34 mmol, 2 eq) を滴下して室温で1時間攪拌した。反応液に飽和食塩水を加えてクエンチし、クロロホルムで4回抽出、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥し、表題の化合物を得た。得られた化合物をそのまま次の反応に用いた。

[0080] 実施例 11

4-フルオロデアセチルコルヒチンの合成

4-フルオロ-N-(t-ブトキシカルボニル)デアセチルコルヒチン65 mg (0.14 mmol) をトリフルオロ酢酸0.2 mL (2.66 mmol, 19 eq) に溶解し、室温で40分攪拌した。反応液に5N水酸化ナトリウムを加えて塩基性にし、クロロホルム

WO 2011/021397

57

PCT/JP2010/005136

ムで3回抽出、飽和食塩水で洗浄、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法（10% メタノール/ クロロホルム）にて精製し、表題の化合物55 mg（収率 定量的）を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.75 (1H, s, H-8), 7.19 (1H, d, $J=10.6\text{ Hz}$, H-12), 6.79 (1H, d, $J=10.8\text{ Hz}$, H-11), 4.01 (3H, s, -OMe), 4.00 (3H, s, -OMe), 3.97 (3H, s, -OMe), 3.69 (1H, dd, $J=10.9, 6.3\text{ Hz}$, H-7), 3.59 (3H, s, -OMe), 3.00 (1H, dd, $J=13.7, 6.2\text{ Hz}$, H-5 β), 2.29 (1H, dddd, $J=12.8, 12.8, 6.4, 6.4\text{ Hz}$, H-6 β), 2.02 (1H, ddd, $J=13.5, 13.5, 6.9\text{ Hz}$, H-5 α), 1.59 (1H, ddd, $J=11.7, 11.7, 7.0\text{ Hz}$, H-6 α).

EI-MS m/z (%) : 375 (M^+ , 68.4), 83 (100).

[0081] 実施例 1 2

4-フルオロデアセチルコルヒチン塩酸塩の合成

メタノール1.5 mLに氷冷下、アセチル クロリド21.3 μL (0.3 mmol, 2 eq) を滴下して攪拌した。その溶液に、氷冷下、メタノール1.5 mLに溶解した4-フルオロデアセチルコルヒチン55 mg (0.15 mmol) を滴下し、1.5時間攪拌した。反応液を減圧留去、真空乾燥し、表題の化合物44.5 mgを得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.85 (1H, s, H-8), 7.42 (1H, d, $J=10.8\text{ Hz}$, H-12), 7.00 (1H, d, $J=10.8\text{ Hz}$, H-11), 4.27 (1H, br-s, H-7), 4.05 (3H, s, -OMe), 4.02 (3H, s, -OMe), 3.98 (3H, s, -OMe), 3.62 (3H, s, -OMe), 3.08 (1H, br-dd, $J=14.0, 6.0\text{ Hz}$), 2.65 (1H, m), 2.52 (1H, m), 2.08 (1H, m).

[0082] 実施例 1 3

4-クロロ-N-プロピオニルデアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン10 mg (0.026 mmol) をジクロロメタン0.5 mLに溶解し、氷冷下ピリジン2.3 μL (0.029 mmol, 1.1 eq)、塩化プロピオニル2.3 μL (0.026 mmol, 1 eq) を順に滴下し、室温で1.5時間攪拌した。反応液に水を加えてクエンチし、飽和炭酸水素ナトリウム水溶液で塩基性に

WO 2011/021397

58

PCT/JP2010/005136

した。クロロホルムで4回抽出し、有機層を飽和食塩水で洗浄後、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法（10%メタノール/ 酢酸エチル及び5%メタノール / クロロホルム）にて精製し、表題の化合物11.7 mg（収量 定量的）を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.49 (1H, s, H-8), 7.30 (1H, d, $J=11.0\text{Hz}$, H-12), 6.85 (1H, d, $J=10.8\text{Hz}$, H-11), 4.57 (1H, ddd, $J=12.0$, 6.1, 6.1Hz, H-7), 4.02 (3H, s, -OMe), 3.99 (3H, s, -OMe), 3.97 (3H, s, -OMe), 3.64 (3H, s, -OMe), 3.24 (1H, dd, $J=13.5$, 5.0Hz, H-5 β), 2.29 (3H, m), 2.15 (1H, ddd, $J=13.4$, 13.4, 6.2Hz, H-5 α), 1.80 (1H, ddd, $J=11.9$, 11.9, 5.9Hz, H-6 α), 1.10 (3H, dd, $J=7.6$, 7.6Hz, $-\text{NHCOCH}_2\text{CH}_3$).

EI-MS m/z (%) : 446 (M^+ , 45.6), 448 ($[\text{M}+2]^+$, 17.3), 55 (100.0).

[0083] 実施例 1 4

4-クロロ-N-ホルミルデアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン11.8 mg (0.030 mmol) をN,N-ジメチルホルムアミド0.4mLに溶解し、ギ酸エチル0.2 mLを加え80°Cで3時間攪拌した。その後120°Cに昇温し、22時間攪拌した。反応液を減圧留去し、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法（5%メタノール/ クロロホルム及び7%メタノール/ 酢酸エチル）にて精製し、表題の化合物12.5 mg（収量 99%）を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 8.20 (1H, s, $-\text{NHCOH}$), 7.57 (1H, br-d, $J=6.8\text{Hz}$, -NH), 7.53 (1H, s, H-8), 7.30 (1H, d, $J=10.7\text{Hz}$, H-12), 6.86 (1H, d, $J=11.0\text{Hz}$, H-11), 4.64 (1H, ddd, $J=12.4$, 6.3, 6.3Hz, H-7), 4.02 (3H, s, -OMe), 4.00 (3H, s, -OMe), 3.98 (3H, s, -OMe), 3.63 (3H, s, -OMe), 3.27 (1H, dd, $J=13.3$, 5.0Hz, H-5 β), 2.30 (1H, dddd, $J=12.6$, 12.6, 6.2, 6.2Hz, H-6 β), 2.18 (1H, ddd, $J=13.3$, 13.3, 6.1Hz, H-5 α), 1.87 (1H, ddd, $J=11.9$, 11.9, 5.2Hz, H-6 α).

EI-MS m/z (%) : 418 (M^+ , 30.1), 420 ($[\text{M}+2]^+$, 12.8), 55 (100.0).

WO 2011/021397

59

PCT/JP2010/005136

[0084] 実施例 1 5

4-クロロ-N-(クロロアセチル)デアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン24 mg (0.061 mmol) をジクロロメタン0.8 mLに溶解し、氷冷下トリエチルアミン13 μ L (0.092 mmol, 1.5 eq)、塩化クロロアセチル5.8 μ L (0.073 mmol, 1.2 eq) を順に加えて室温で30分間攪拌した。反応液に飽和炭酸水素ナトリウム水溶液を加えてクエンチし、クロロホルムで4回抽出し、有機層を飽和食塩水で洗浄後、無水硫酸マグネシウムで乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルカラムクロマトグラフ法 (1% $\rightarrow$ 5% $\rightarrow$ 10%メタノール/ クロロホルム) にて精製し、表題の化合物31.3 mg (収量 定量的) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm] : 7.70 (1H, br-d, $J=6.8\text{Hz}$, -NH), 7.40 (1H, s, H-8), 7.28 (1H, d, $J=10.7\text{Hz}$, H-12), 6.84 (1H, d, $J=10.7\text{Hz}$, H-11), 4.55 (1H, ddd, $J=12.1, 6.2, 6.2\text{Hz}$, H-7), 4.09 (1H, d, $J=14.9\text{Hz}$, - CH_2Cl), 4.02 (1H, d, $J=14.9\text{Hz}$, - CH_2Cl), 4.01 (3H, s, -OMe), 3.99 (3H, s, -OMe), 3.97 (3H, s, -OMe), 3.62 (3H, s, -OMe), 3.28 (1H, dd, $J=13.2, 4.4\text{Hz}$, H-5 β), 2.29 (1H, dddd, $J=12.1, 12.1, 6.0, 6.0\text{Hz}$, H-6 β), 2.19 (1H, ddd, $J=13.1, 13.1, 5.9\text{Hz}$, H-5 α), 1.89 (1H, ddd, $J=11.6, 11.6, 5.0\text{Hz}$, H-6 α).

FAB-MS (NBA) m/z : 468 $[\text{M}+\text{H}]^+$.

[0085] 実施例 1 6

4-クロロ-N,N-ジメチルデアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン(90 mg, 0.230 mmol)のギ酸(690 μ L)溶液に、室温でホルムアルデヒド(37% in water, 460 μ L)を加えた後、アルゴンガス雰囲気下、2.5時間煮沸環流した。室温まで放冷後、クロロホルムと飽和重曹水を加えた。有機層を取り、水層はさらにクロロホルムにて抽出した。有機層を合わせて、無水硫酸ナトリウムにて乾燥後、ろ過、ろ液を濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製、目的物画分を濃縮乾固して標記化合物(

WO 2011/021397

60

PCT/JP2010/005136

淡褐色固体, 55 mg, 0.130 mmol, 57%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.09 (1H, s), 7.18 (1H, d, $J=10.7$ Hz), 6.76 (1H, d, $J=10.7$ Hz), 3.99 (6H, s), 3.98 (3H, s), 3.57 (3H, s), 3.21–3.16 (1H, m), 2.59 (1H, dd, $J=11.0, 5.9$ Hz), 2.21–2.03 (2H, m), 2.16 (6H, br-s), 1.73–1.66 (1H, m). ESI-MS m/z : 420 $[\text{M}+\text{H}]^+$.

[0086] 実施例 17

4-クロロ-N,N-ジエチルデアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) のメタノール-酢酸混液 (100:1, 1.9 mL) に氷冷、アルゴンガス雰囲気下にてアセトアルデヒド (25 μL , 0.128×3.5 mmol) 及びシアノ水素化ホウ素ナトリウム (18 mg, 0.128×2.2 mmol) を加えた。本混合液を室温で3時間攪拌した後、飽和重曹水と酢酸エチルを加えた。有機層を取り、ブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、次いで減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法 (装置: Biotage社 Isolera One, クロロホルム/メタノール) にて精製し、標記化合物 (淡黄色固体, 30 mg, 0.067 mmol, 53%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.11 (1H, s), 7.19 (1H, d, $J=10.7$ Hz), 6.76 (1H, d, $J=10.7$ Hz), 3.99 (6H, s), 3.97 (3H, s), 3.58 (3H, s), 3.23–3.09 (2H, m), 2.82–2.31 (3H, m), 2.22–2.03 (2H, m), 1.81–1.60 (2H, m), 0.90 (6H, t, $J=7.1$ Hz).

ESI-MS m/z : 448 $[\text{M}+\text{H}]^+$.

[0087] 実施例 18

4-クロロ-7-(ピペラジン-1-イル)-7-デ(アセチルアミノ)コルヒチンの合成

4-クロロ-N,N-ジエチルデアセチルコルヒチンの合成と同様の方法にて、標記化合物 (淡褐色固体, 30 mg, 0.065 mmol, 51%) を得た。アセトアルデヒドの代わりにグルタルアルデヒドを用いた。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.20 (1H, s), 7.19 (1H, d, $J=10.7$ Hz), 6.75 (1H, d, $J=10.7$ Hz), 3.99 (6H, s), 3.97 (3H, s), 3.57 (3H, s), 3.21–3.16 (1H, m), 2.75–2.64 (1H, m), 2.22–2.02 (2H, m), 1.70–1.49 (11H,

WO 2011/021397

61

PCT/JP2010/005136

m).

ESI-MS m/z : 460 $[M+H]^+$.

[0088] 実施例 19

N-(tert-ブチルオキシカルボニル)-4-クロロ-N-メチルデアセチルコルヒチンの合成

N-(tert-ブチルオキシカルボニル)-4-クロロデアセチルコルヒチン (0.100 g, 0.203 mmol) のN,N-ジメチルホルムアミド (有機合成用, 2 mL) 溶液に氷冷、アルゴンガス雰囲気下にて水素化ナトリウム (60% in oil, 16 mg, 0.203×2 mmol) を添加して、20分間攪拌した。さらにヨウ化メチル (86 mg, 0.203×3 mmol) を加えて、氷冷下30分間、次いで室温で一晩攪拌した。反応液に酢酸エチルと飽和塩化アンモニウム溶液を加えて、有機層を取った。有機層はブラインにて洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法 (装置: Biotage社 Isolera One, クロロホルムメタノール) にて精製、目的物画分を濃縮乾固して標記化合物 (淡褐色個体, 67 mg, 0.132 mmol, 65%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.27 (1H, s), 7.17 (1H, d, $J=10.8\text{Hz}$), 6.79 (1H, d, $J=10.8\text{Hz}$), 4.68–4.57 (1H, m), 4.00 (3H, s), 3.97 (6H, s), 3.66 (3H, br-s), 3.34–3.29 (1H, m), 3.12 (3H, s), 2.24–2.10 (3H, m), 1.35 (9H, br-s).

ESI-MS m/z : 506 $[M+H]^+$.

[0089] 実施例 20

N-(tert-ブチルオキシカルボニル)-4-クロロ-N-エチルデアセチルコルヒチンの合成

N-(tert-ブチルオキシカルボニル)-4-クロロ-N-メチルデアセチルコルヒチンの合成と同様の方法にて、標記化合物 (淡褐色固体, 81 mg, 0.155 mmol, 51%) を得た。ヨウ化メチルの代わりにヨウ化エチルを用いた。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.25 (1H, s), 7.17 (1H, d, $J=10.7\text{Hz}$), 6.78 (1H, d, $J=10.7\text{Hz}$), 4.73–4.65 (1H, m), 3.99 (3H, s), 3.97 (6H, s)

WO 2011/021397

62

PCT/JP2010/005136

, 3.64 (3H, br-s), 3.34-3.20 (3H, m), 2.19-2.08 (3H, m), 1.35 (9H, br-s), 1.15 (3H, t, J=7.0 Hz).

ESI-MS m/z: 520 [M+H]⁺.

[0090] 実施例 2 1

N-アリル-4-クロロ-N-(tert-ブチルオキシカルボニル)デアセチルコルヒチンの合成

N-(tert-ブチルオキシカルボニル)-4-クロロ-N-メチルデアセチルコルヒチンの合成と同様の方法にて、標記化合物(淡褐色固体, 117 mg, 0.219 mmol, 54%)を得た。ヨウ化メチルの代わりに臭化アリルを用いた。

¹H-NMR(400MHz, CDCl₃) δ [ppm]: 7.27 (1H, s), 7.18 (1H, d, J=10.8 Hz), 6.79 (1H, d, J=10.8 Hz), 5.87-5.77 (1H, m), 5.15-5.07 (2H, m), 4.80-4.68 (1H, m), 4.52 (1H, dd, J=16.8, 4.3 Hz), 4.00 (3H, s), 3.97 (6H, s), 3.84 (1H, dd, J=16.8, 5.5 Hz), 3.65 (3H, s), 3.32-3.24 (1H, m), 2.20-2.02 (3H, m), 1.34 (9H, s).

ESI-MS m/z: 532 [M+H]⁺.

[0091] 実施例 2 2

N-(tert-ブチルオキシカルボニル)-4-クロロ-N-(2-メチルアリル)デアセチルコルヒチンの合成

N-(tert-ブチルオキシカルボニル)-4-クロロ-N-メチルデアセチルコルヒチンの合成と同様の方法にて、標記化合物(淡褐色固体, 59 mg, 0.108 mmol, 27%)を得た。ヨウ化メチルの代わりに3-ブロモ-2-メチルプロペンを用いた。

¹H-NMR(400MHz, CDCl₃) δ [ppm]: 7.32 (1H, s), 7.20 (1H, d, J=10.7 Hz), 6.80 (1H, d, J=10.7 Hz), 4.86-4.73 (1H, m), 4.82 (1H, s), 4.67 (1H, s), 4.48 (1H, d, J=18.0 Hz), 4.00 (3H, s), 3.98 (6H, s), 3.73 (1H, d, J=18.0 Hz), 3.66 (3H, br-s), 3.29-3.20 (1H, m), 2.20-1.96 (3H, m), 1.72 (3H, s), 1.32 (9H, s).

ESI-MS m/z: 644 [M+H]⁺.

[0092] 実施例 2 3

WO 2011/021397

63

PCT/JP2010/005136

N-ベンジル-N-(tert-ブチルオキシカルボニル)-4-クロロデアセチルコルヒチンの合成

N-(tert-ブチルオキシカルボニル)-4-クロロ-N-メチルデアセチルコルヒチンの合成と同様の方法にて、標記化合物(乳白色固体, 91 mg, 0.156 mmol, 51%)を得た。ヨウ化メチルの代わりに臭化ベンジルを用いた。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.40 (1H, s), 7.33–7.16 (6H, m), 6.81 (1H, d, $J=10.7$ Hz), 5.26 (1H, d, $J=17.1$ Hz), 4.92–4.82 (1H, m), 4.48 (1H, d, $J=17.1$ Hz), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.67 (3H, s), 3.20–3.15 (1H, m), 2.16–1.86 (3H, m), 1.27 (9H, s).

ESI-MS m/z : 582 $[\text{M}+\text{H}]^+$.

[0093] 実施例 2 4

4-クロロ-N-メチルデアセチルコルヒチンの合成

N-(tert-ブチルオキシカルボニル)-4-クロロ-N-メチルデアセチルコルヒチン(47 mg, 0.093 mmol)のジクロロメタン(750 μL)溶液に氷冷、アルゴンガス雰囲気下してトリフルオロ酢酸(300 μL)を添加した。本溶液を氷冷下にて5分間、次いで室温で2時間攪拌した。反応液を飽和重曹水で塩基性とした後、クロロホルムとブラインを加えた。有機層を取り、水層はクロロホルムで再び抽出した。有機層は合わせて、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社Isolera One, クロロホルム/メタノール)にて精製、目的物画分を濃縮乾固して標記化合物(淡褐色固体, 36 mg, 0.088 mmol, 95%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.71 (1H, s), 7.18 (1H, d, $J=10.7$ Hz), 6.78 (1H, d, $J=10.7$ Hz), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.57 (3H, s), 3.20–3.16 (2H, m), 2.26 (3H, s), 2.23–2.09 (2H, m), 1.67–1.60 (1H, m).

ESI-MS m/z : 406 $[\text{M}+\text{H}]^+$.

[0094] 実施例 2 5

4-クロロ-N-エチルデアセチルコルヒチンの合成

WO 2011/021397

64

PCT/JP2010/005136

4-クロロ-N-メチルデアセチルコルヒチンの合成と同様の方法にて、標記化合物(淡黄褐色固体, 44 mg, 0.104 mmol, 76%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.75 (1H, s), 7.17 (1H, d, $J=10.7$ Hz), 6.78 (1H, d, $J=10.7$ Hz), 4.00 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.56 (3H, s), 3.32–3.27 (1H, m), 3.20–3.14 (1H, m), 2.60–2.51 (1H, m), 2.35–2.27 (1H, m), 2.22–2.08 (2H, m), 1.64–1.55 (1H, m), 1.04 (3H, t, $J=7.1$ Hz).

ESI-MS m/z : 420 $[\text{M}+\text{H}]^+$.

[0095] 実施例 2 6

4-クロロ-N-エチルデアセチルコルヒチンの別途合成法

4-クロロデアセチルコルヒチン(0.150 g, 0.383 mmol)のジクロロメタン-酢酸混液(50:1, 7.7 mL)溶液に、室温、アルゴンガス雰囲気下にて、アセトアルデヒド(20.4 μL , 0.383×0.95 mmol)を加えて10分間攪拌した。本溶液にトリアセトキシ水素化ホウ素ナトリウム(0.179 mg, 0.383×2.2 mmol)を加えて、室温で一晩攪拌した後、酢酸エチルと飽和重曹水を添加した。有機層を取り、ブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社Isolera One, クロロホルム/メタノール)にて精製し、標記化合物(乳白色固体, 97 mg, 0.231 mmol, 60%)を得た。

[0096] 実施例 2 7

4-クロロ-N-エチルデアセチルコルヒチン塩酸塩の合成法

メタノール(2 mL)に氷冷、アルゴンガス雰囲気下にてアセチルクロリド(24.6 μL , 0.231×1.5 mmol)を添加して、氷冷下1時間攪拌した。本溶液に、4-クロロ-N-エチルデアセチルコルヒチン(97 mg)のメタノール(2 mL)溶液を加えた後、減圧下濃縮乾固した。残留物を少量のクロロホルムに溶かし、ヘキサンを加えて固体化した。析出物をろ取、ヘキサンで洗浄後、減圧乾燥して標記化合物(淡黄色固体, 0.105 g, 定量的)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 10.68 (1H, br-s), 10.57 (1H, br-s), 8.04

WO 2011/021397

65

PCT/JP2010/005136

(1H, s), 7.48 (1H, d, J=11.0 Hz), 7.04 (1H, d, J=11.0 Hz), 4.08 (3H, s), 3.99 (3H, s), 3.99 (3H, s), 3.90–3.83 (1H, m), 3.71 (3H, s), 3.38–3.11 (3H, m), 2.57–2.48 (2H, m), 2.25–2.14 (1H, m), 1.54 (3H, t, J=7.2 Hz).

[0097] 実施例 28

N-アリル-4-クロロデアセチルコルヒチンの合成

4-クロロ-N-メチルデアセチルコルヒチンの合成と同様の方法にて、標記化合物(淡褐色固体, 44 mg, 0.104 mmol, 76%)を得た。

¹H-NMR(400MHz, CDCl₃) δ [ppm]: 7.78 (1H, s), 7.18 (1H, d, J=10.7 Hz), 6.78 (1H, d, J=10.7 Hz), 5.85–5.75 (1H, m), 5.07–4.97 (2H, m), 4.01 (3H, s), 3.97 (3H, s), 3.97 (3H, s), 3.55 (3H, s), 3.34 (1H, dd, J=11.0, 5.9 Hz), 3.26–3.17 (2H, m), 2.95 (1H, dd, J=14.2, 7.0 Hz), 2.24–2.10 (2H, m), 1.71–1.63 (1H, m).

ESI-MS m/z: 432 [M+H]⁺.

[0098] 実施例 29

4-クロロ-N-(2-メチルアリル)デアセチルコルヒチンの合成

4-クロロ-N-メチルデアセチルコルヒチンの合成と同様の方法にて、標記化合物(褐色固体, 30 mg, 0.068 mmol, 71%)を得た。

¹H-NMR(400MHz, CDCl₃) δ [ppm]: 7.81 (1H, s), 7.17 (1H, d, J=10.7 Hz), 6.77 (1H, d, J=10.7 Hz), 4.75 (1H, s), 4.72 (1H, s), 4.00 (3H, s), 3.97 (3H, s), 3.97 (3H, s), 3.56 (3H, s), 3.30–3.26 (1H, m), 3.21–3.16 (1H, m), 3.09 (1H, d, J=14.2 Hz), 2.87 (1H, d, J=14.2 Hz), 2.23–2.10 (2H, m), 1.70–1.63 (1H, m), 1.68 (3H, s).

ESI-MS m/z: 446 [M+H]⁺.

[0099] 実施例 30

N-ベンジル-4-クロロデアセチルコルヒチンの合成

4-クロロ-N-メチルデアセチルコルヒチンの合成と同様の方法にて、標記化合物(乳白色固体, 40 mg, 0.084 mmol, 73%)を得た。

WO 2011/021397

66

PCT/JP2010/005136

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.68 (1H, s), 7.25–7.13 (5H, m), 7.11 (1H, d, $J=10.7$ Hz), 7.02 (1H, d, $J=10.7$ Hz), 3.90 (3H, s), 3.86 (3H, s), 3.85 (3H, s), 3.57 (1H, dd, $J=13.8, 7.4$ Hz), 3.41 (3H, s), 3.39–3.34 (1H, m), 3.15–2.91 (3H, m), 2.12–2.01 (2H, m), 1.61–1.55 (1H, m).
ESI-MS m/z : 482 $[\text{M}+\text{H}]^+$.

[0100] 実施例 3 1

N-ベンジル-4-クロロデアセチルコルヒチン塩酸塩の合成

N-ベンジル-4-クロロデアセチルコルヒチン (0.132 g, 0.274 mmol) のメタノール (1 mL) 溶液に、氷冷、アルゴンガス雰囲気下にて 10% HCl-メタノール (166 μL , 0.274×1.5 mmol) を添加した。本溶液を減圧下、濃縮乾固して標記化合物 (乳白色固体, 142 mg, 0.274 mmol, 定量的) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 10.13 (1H, s), 9.61 (1H, s), 7.45–7.29 (6H, m), 7.19 (2H, d, $J=10.7$ Hz), 7.10 (2H, d, $J=10.7$ Hz), 4.30–4.13 (2H, m), 3.93 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.72–3.64 (1H, m), 3.52 (3H, s), 3.17 (1H, dd, $J=13.9, 4.9$ Hz), 2.20–1.97 (3H, m).

[0101] 実施例 3 2

4-クロロ-N-プロピルデアセチルコルヒチンの合成

N-アリル-4-クロロデアセチルコルヒチン (36 mg, 0.0833 mmol) の酢酸エチル (860 μL) 溶液に、氷冷、アルゴンガス雰囲気下にて、10%パラジウム炭素 (8 mg) 及び臭化亜鉛 (11 mg) を加えた。反応容器内を脱気した後、水素ガスで置換した。本懸濁液を室温、水素ガス雰囲気下にて一晩攪拌した。不要物をマイクロフィルター (0.45 μm) により除去、ろ液を減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法 (装置: Biotage 社 Isolera One, クロロホルム/メタノール) にて精製、目的物画分を濃縮乾固して標記化合物 (淡黄褐色固体, 16 mg, 0.037 mmol, 44%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.77 (1H, s), 7.18 (1H, d, $J=10.7$ Hz), 6.78 (1H, d, $J=10.7$ Hz), 4.00 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.57 (3H, s), 3.29–3.25 (1H, m), 3.20–3.15 (1H, m), 2.50–2.43 (1H, m), 2.

WO 2011/021397

67

PCT/JP2010/005136

26–2.09 (3H, m), 1.66–1.59 (1H, m), 1.50–1.39 (2H, m), 0.85 (3H, t, $J=7.4$ Hz).

ESI-MS m/z : 434 $[M+H]^+$.

[0102] 実施例 3 3

N-イソブチル-4-クロロデアセチルコルヒチンの合成

N-プロピルデアセチルコルヒチンの合成と同様の方法にて、4-クロロ-N-(2-メチルアリル)デアセチルコルヒチンから、標記化合物(乳白色固体, 4 mg, 0.0090 mmol, 17%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.79 (1H, s), 7.17 (1H, d, $J=10.7$ Hz), 6.76 (1H, d, $J=10.7$ Hz), 4.00 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.57 (3H, s), 3.26–3.14 (2H, m), 2.32–2.07 (4H, m), 1.69–1.60 (1H, m), 1.30–1.25 (1H, m), 0.89 (3H, d, $J=6.6$ Hz), 0.85 (3H, d, $J=6.6$ Hz).

ESI-MS m/z : 448 $[M+H]^+$.

[0103] 実施例 3 4

N-(アセトキシアセチル)-4-クロロデアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン(0.150 g, 0.383 mmol)のジクロロメタン(5.7 mL)溶液に氷冷、アルゴンガス雰囲気下にてトリエチルアミン(64.1 μL , 0.3831×1.2 mmol)次いでアセトキシアセチルクロリド(45.3 μL , 0.383×1.1 mmol)を添加した。本溶液を氷冷下、10分間次いで室温で3時間攪拌した。酢酸エチルと10%クエン酸を加えた後、有機層を取った。有機層を飽和重曹水次いでブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 IsoleraOne, クロロホルム/メタノール)にて精製し、標記化合物(乳白色固体, 0.142 g, 0.238 mmol, 76%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.45 (1H, s), 7.31 (1H, d, $J=10.7$ Hz), 7.12 (1H, d, $J=7.1$ Hz), 6.86 (1H, d, $J=10.7$ Hz), 4.63–4.58 (1H, m), 4.60 (1H, d, $J=15.7$ Hz), 4.54 (1H, d, $J=15.7$ Hz), 4.02 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.30–3.25 (1H, m), 2.32–2.14 (2H,

WO 2011/021397

68

PCT/JP2010/005136

m), 2.18 (3H, s), 1.90–1.83 (1H, m).

ESI-MS m/z : 492 $[M+H]^+$.

[0104] 実施例 3 5

4-クロロ-N-(ヒドロキシアセチル)デアセチルコルヒチンの合成

N-(アセトキシアセチル)-4-クロロデアセチルコルヒチン (0.161 g, 0.327 mmol) のメタノール (3.3 mL) 溶液に、氷冷、アルゴンガス雰囲気下にてナトリウムメトキシド (5M in MeOH, 65.4 μ L, 0.327 mmol) を添加した後、氷冷下5分間、次いで室温で30分間撹拌した。反応液にブラインと10%クエン酸を加え、クロロホルム ($\times 2$) で抽出した。有機層は合わせて、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法 (装置: Biotage社 Isolera One, クロロホルム/メタノール) にて精製し、標記化合物 (乳白色固体, 0.147 g, 0.327 mmol, 定量的) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.68 (1H, s), 7.56 (1H, d, $J=7.3$ Hz), 7.34 (1H, d, $J=10.7$ Hz), 6.90 (1H, d, $J=10.7$ Hz), 4.66–4.59 (1H, m), 4.20 (1H, d, $J=16.3$ Hz), 4.04 (1H, d, $J=16.3$ Hz), 4.02 (3H, s), 3.99 (3H, s), 3.98 (3H, s), 3.62 (3H, s), 3.31–3.26 (1H, m), 2.33–2.14 (2H, m), 1.94–1.87 (1H, m).

ESI-MS m/z : 450 $[M+H]^+$.

[0105] 参考例 1 2

Trans-4-ヒドロキシ(シクロヘキサン)カルボン酸ベンジルの合成

Trans-4-ヒドロキシ(シクロヘキサン)カルボン酸 (1.00 g, 6.94 mmol) のN,N-ジメチルホルムアミド (69 mL) 溶液に、室温、アルゴンガス雰囲気下にて炭酸カリウム (0.959 g, 6.94 mmol) 及び臭化ベンジル (825 μ L, 6.94 mmol) を添加した。本懸濁液を室温で一晩撹拌した後、酢酸エチル-ヘキサン混液 (1:1) を添加して不要物をろ去、次いで酢酸エチル-ヘキサン (1:1) で洗いこんだ。ろ液と洗浄液は合わせて、水 ($\times 3$) 次いでブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法 (装置: Biotage社 Isolera One, ヘキサン/酢酸エチル) にて精製し、標

WO 2011/021397

69

PCT/JP2010/005136

記化合物(白色固体, 1.567 g, 6.69 mmol, 96%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.39–7.30 (5H, m), 5.11 (2H, s), 3.61 (1H, tt, $J=10.9, 4.0$ Hz), 2.31 (1H, tt, $J=12.0, 3.5$ Hz), 2.08–2.00 (4H, m), 1.58–1.47 (3H, m), 1.34–1.23 (2H, m).

[0106] 参考例 1 3

1-メチルピペリジン-4-イルオキシカルボニルクロリド塩酸塩の合成

4-ヒドロキシ-1-メチルピペリジン(1 mL, 8.50 mmol)のアセトニトリル(17 mL)溶液に、氷冷、アルゴンガス雰囲気下にてトリホスゲン(2.52 g, 8.50 mmol)を加えて、室温に戻しながら一晩撹拌した。析出物をろ取、エーテル次いでヘキサンで洗浄した後、減圧乾燥して表記化合物(白色固体, 1.135 g, 62%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 12.94 (1H, br-s), 5.27–5.24 (1H, m), 3.47–3.41 (2H, m), 3.08–2.98 (2H, m), 2.83 (3H, d, $J=4.9$ Hz), 2.78–2.69 (2H, m), 2.24–2.18 (2H, m).

[0107] 参考例 1 4

1-(ベンジルオキシカルボニル)ピペリジン-4-イルオキシカルボニルクロリドの合成

4-ヒドロキシ-1-(ベンジルオキシカルボニル)ピペリジン(2.00 g, 8.50 mmol)のアセトニトリル(17 mL)溶液に、氷冷、アルゴンガス雰囲気下にてトリホスゲン(2.52 g, 8.50 mmol)を加えて、室温に戻しながら一晩撹拌した。反応液を濃縮乾固して、表記化合物(微褐色澄明の粘性液, 2.47 g, 8.29 mmol, 98%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.40–7.30 (5H, m), 5.13 (2H, s), 5.06–5.00 (1H, m), 3.79–3.73 (2H, m), 3.43–3.37 (2H, m), 2.01–1.92 (2H, m), 1.84–1.74 (2H, m).

[0108] 参考例 1 5

Trans-4-(クロロカルボニルオキシ)シクロヘキサンカルボン酸ベンジルの合成

WO 2011/021397

70

PCT/JP2010/005136

Trans-4-ヒドロキシ(シクロヘキサン)カルボン酸ベンジルエステル(1.00 g, 4.27 mmol)のアセトニトリル(8.5 mL)溶液に、氷冷、アルゴンガス雰囲気下にてトリホスゲン(1.27g, 4.27 mmol)を加えて、室温に戻しながら一晩撹拌した。反応液を濃縮乾固して、標記化合物(白濁粘性液, 1.27 g, 定量的)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.39–7.31 (5H, m), 5.12 (2H, s), 4.83–4.76 (1H, m), 2.42–2.35 (1H, m), 2.18–2.08 (4H, m), 1.66–1.49 (4H, m).

[0109] 参考例 1 6

チオモルホリン-4-カルボニルクロリドの合成

チオモルホリン(500 μL , 5.28 mmol)のアセトニトリル(11 mL)溶液に、氷冷、アルゴンガス雰囲気下にてトリホスゲン(1.57 g, 5.28 mmol)を加えて、室温に戻しながら一晩撹拌した。反応液を濃縮乾固して、標記化合物(淡褐色澄明液, 0.860 g, 5.19 mmol, 98%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 4.01–3.98 (2H, m), 3.91–3.88 (2H, m), 2.70–2.68 (4H, m).

[0110] 参考例 1 7

チオモルホリン-4-カルボニルクロリド 1,1-ジオキシドの合成

チオモルホリン 1,1-ジオキシド(0.500 g, 3.70 mmol)のアセトニトリル(7.4 mL)溶液に、氷冷、アルゴンガス雰囲気下にてトリホスゲン(1.10 g, 3.70 mmol)を加えて、室温に戻しながら一晩撹拌した。反応液を濃縮乾固して得られた固体にヘキサンを添加して超音波処理した。固体をろ取、ヘキサンで洗浄、減圧乾燥して、標記化合物(白色個体, 0.731 g, 3.70 mmol, 定量的)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 4.27–4.22 (2H, m), 4.15–4.10 (2H, m), 3.15–3.12 (4H, m).

[0111] 参考例 1 8

1-メチルピペラジン-4-カルボニルクロリド塩酸塩の合成

1-メチルピペラジン(500 μL , 4.54 mmol)のアセトニトリル(9 mL)溶液に

WO 2011/021397

71

PCT/JP2010/005136

、氷冷、アルゴンガス雰囲気下にてトリホスゲン(1.35 g, 4.54 mmol)を加えて、室温に戻しながら一晩攪拌した。反応液を濃縮乾固して得られた固体にジエチルエーテルを添加して超音波処理した。固体をろ取、ジエチルエーテル次いでヘキサンで洗浄、減圧乾燥して、標記化合物(白色固体, 0.904 g, 4.54 mmol, 定量的)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 13.7 (1H, br-s), 4.45 (2H, d, $J=15.1$ Hz), 4.12 (1H, t, $J=12.6$ Hz), 3.88 (1H, t, $J=12.6$ Hz), 3.60–3.52 (2H, m), 2.98–2.89 (2H, m), 2.87 (3H, d, $J=4.6$ Hz).

[0112] 実施例 3 6

4-クロロ-N-(1-メチルピペラジン-4-イルオキシカルボニル)デアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン(30 mg, 0.0766 mmol)のジクロロメタン(1.5 mL)溶液に室温で、ピリジン(12.4 μL , 0.0766 $\times$ 2 mmol)及び1-メチルピペリジン-4-イルオキシカルボニルクロリド塩酸塩(25 mg, 0.0766 $\times$ 1.5 mmol)を添加した。本混合液を室温で一晩攪拌した後、クロロホルムと飽和重曹水を添加した。有機層を取り、水層はさらにクロロホルムにて抽出した。有機層は合わせて無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社Isolera One, クロロホルム/メタノール)にて精製し、標記化合物(淡黄色固体, 35 mg, 0.0654 mmol, 85%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.48 (1H, s), 7.24 (1H, d, $J=10.7$ Hz), 6.81 (1H, d, $J=10.7$ Hz), 5.43 (1H, d, $J=6.6$ Hz), 4.68–4.60 (1H, m), 4.37–4.30 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.59 (3H, s), 3.26 (1H, dd, $J=13.4, 4.6$ Hz), 2.85–2.73 (2H, m), 2.58–2.39 (2H, m), 2.43 (3H, s), 2.33–2.13 (2H, m), 2.08–1.98 (2H, m), 1.89–1.69 (3H, m).

ESI-MS m/z : 533 $[\text{M}+\text{H}]^+$.

[0113] 実施例 3 7

WO 2011/021397

72

PCT/JP2010/005136

4-クロロ-N-[1-(ベンジルオキシカルボニル)ピペリジン-4-イルオキシカルボニル]デアセチルコルヒチンの合成

4-クロロ-N-(1-メチルピペラジン-4-イルオキシカルボニル)デアセチルコルヒチンの合成と同様の方法にて、標記化合物(乳白色固体, 62 mg, 0.0952 mmol, 83%)を得た。1-メチルピペリジン-4-イルオキシカルボニルクロリド塩酸塩の代わりに1-(ベンジルオキシカルボニル)ピペリジン-4-イルオキシカルボニルクロリド(0.9 eq.)を用いた。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.53 (1H, d, $J=8.3$ Hz), 7.38–7.30 (5H, m), 7.26 (1H, d, $J=10.7$ Hz), 6.82 (1H, d, $J=10.7$ Hz), 5.31 (1H, d, $J=7.8$ Hz), 5.11 (2H, s), 4.73–4.67 (1H, m), 4.37–4.30 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.79–3.69 (2H, m), 3.59 (3H, s), 3.28–3.20 (3H, m), 2.33–2.12 (2H, m), 1.86–1.50 (5H, m).

ESI-MS m/z : 653 [M+H] $^+$.

[0114] 実施例 3 8

4-クロロ-N-(ピペリジン-4-イルオキシカルボニル)デアセチルコルヒチンの合成

4-クロロ-N-[1-(ベンジルオキシカルボニル)ピペリジン-4-イルオキシカルボニル]デアセチルコルヒチン(47 mg, 0.072 mmol)のメタノール-テトラヒドロフラン混液(2:1, 3 mL)に、氷冷、アルゴンガス雰囲気下にて20%水酸化パラジウム-炭素(10 mg)を添加した。反応容器内を減圧にて脱気、続く水素ガスによって置換した(本操作は3回繰り返した)。反応混合液を室温、水素ガス雰囲気下にて3時間激しく攪拌した。不要物をマイクロフィルター(0.45 μ m)により除去し、ろ液を濃縮乾固して標記化合物(褐色固体, 37 mg, quant.)を得た。

ESI-MS m/z : 519 [M+H] $^+$.

[0115] 実施例 3 9

N-[trans-1-(ベンジルオキシカルボニル)シクロヘキサン-4-イルオキシカルボニル]-4-クロロデアセチルコルヒチンの合成

WO 2011/021397

73

PCT/JP2010/005136

4-クロロ-N-[1-(ベンジルオキシカルボニル)ピペリジン-4-イルオキシカルボニル]デアセチルコルヒチンの合成と同様の方法にて、標記化合物(淡黄色固体, 74 mg, 0.113 mmol, 82%)を得た。1-(ベンジルオキシカルボニル)ピペリジン-4-イルオキシカルボニルクロリドの代わりに、Trans-4-(クロロカルボニルオキシ)シクロヘキサンカルボン酸ベンジルエステル(0.9 eq.)を用いた。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.50 (1H, s), 7.37–7.29 (5H, m), 7.25 (1H, d, $J=10.7$ Hz), 6.81 (1H, d, $J=10.7$ Hz), 5.12–5.09 (1H, m), 5.08 (2H, s), 4.50–4.43 (1H, m), 4.36–4.29 (1H, m), 4.00 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.59 (3H, s), 3.25 (1H, dd, $J=13.3, 4.5$ Hz), 2.34–2.12 (3H, m), 2.05–1.95 (4H, m), 1.71–1.64 (1H, m), 1.57–1.45 (2H, m), 1.41–1.28 (2H, m).

ESI-MS m/z : 652 $[\text{M}+\text{H}]^+$.

[0116] 実施例 4 O

4-クロロ-N-(チオモルホリン-4-カルボニル)デアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン(50 mg, 0.128 mmol)のジクロロメタン(3.8 mL)溶液に、室温でトリエチルアミン(89 μL , 0.128×5.0 mmol)及びチオモルホリン-4-カルボニルクロリド(106 mg, 0.128×5.0 mmol)を添加した。本溶液をアルゴンガス雰囲気下、一晚煮沸環流した。反応液を室温まで放冷後、酢酸エチル及び10%クエン酸を添加して有機層を取った。有機層はさらに10%クエン酸次いでブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記化合物(微褐色固体, 47 mg, 0.090 mmol, 70%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.80 (1H, s), 7.43 (1H, d, $J=11.0$ Hz), 6.96 (1H, d, $J=11.0$ Hz), 5.72 (1H, d, $J=5.9$ Hz), 4.57–4.51 (1H, m), 4.04 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.80–3.65 (4H, m), 3.64 (3H, s), 3.25 (1H, dd, $J=13.3, 5.2$ Hz), 2.64–2.53 (4H, m), 2.33–2.24 (1H,

WO 2011/021397

74

PCT/JP2010/005136

m), 2.15–2.07 (1H, m), 1.94–1.86 (1H, m).

ESI-MS m/z : 521 $[M+H]^+$.

[0117] 実施例 4 1

4-クロロ-N-(1,1-ジオキソチオモルホリン-4-カルボニル) デアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) のジクロロメタン (有機合成用, 3.8 mL) 溶液に、室温でトリエチルアミン (89 μ L, 0.128 $\times$ 5.0 mmol) 及びチオモルホリン-4-カルボニルクロリド 1,1-ジオキシド (0.202 g, 0.128 $\times$ 8.0 mmol) を添加した。本溶液をアルゴンガス雰囲気下、一晩煮沸環流した。反応液を室温まで放冷後、酢酸エチル及び10%クエン酸を添加して有機層を取った。有機層はさらに10%クエン酸次いでブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法 (装置: Biotage社 Isolera One, クロロホルム/メタノール) にて精製し、標記化合物 (淡褐色固体, 48 mg, 0.087 mmol, 68%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.76 (1H, s), 7.43 (1H, d, $J=10.7\text{Hz}$), 7.04 (1H, s), 6.98 (1H, d, $J=10.7\text{Hz}$), 4.55–4.49 (1H, m), 4.04 (3H, s), 4.03–3.93 (4H, m), 4.00 (3H, s), 3.98 (3H, s), 3.64 (3H, s), 3.25 (1H, dd, $J=13.5, 5.5\text{Hz}$), 3.02–2.97 (4H, m), 2.34–2.26 (1H, m), 2.17–2.06 (1H, m), 1.99–1.91 (1H, m).

ESI-MS m/z : 553 $[M+H]^+$.

[0118] 実施例 4 2

4-クロロ-N-(1-メチルピペラジン-4-カルボニル) デアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) のジクロロメタン (3.8 mL) 溶液に、室温でトリエチルアミン (54 μ L, 0.128 $\times$ 3.0 mmol) 及び1-メチルピペラジン-4-カルボニルクロリド塩酸塩 (0.127 g, 0.128 $\times$ 5.0 mmol) を添加した。本溶液をアルゴンガス雰囲気下、6時間沸環流した。反応液を室温まで放冷後、クロロホルム及び飽和重曹水を添加して有機層を取った。水層

WO 2011/021397

75

PCT/JP2010/005136

はさらにクロロホルムで抽出した。有機層合わせてブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記化合物(淡褐色固体, 30 mg, 0.058 mmol, 45%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.52 (1H, s), 7.25 (1H, d, $J=10.7$ Hz), 6.80 (1H, d, $J=10.7$ Hz), 4.47–4.41 (1H, m), 3.98 (6H, s), 3.95 (3H, s), 3.79–3.66 (4H, m), 3.63 (3H, s), 3.24 (1H, dd, $J=13.3, 4.3$ Hz), 2.94–2.76 (4H, m), 2.60 (3H, s), 2.27–2.12 (2H, m), 1.96–1.86 (1H, m).
ESI-MS m/z : 518 $[\text{M}+\text{H}]^+$.

[0119] 実施例 4 3

N-ブチリル-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、 0°C に冷却した。そこへトリエチルアミン (19 μl , 0.128×1.1 mmol)、ブチリルクロリド (13 μl , 0.128×1 mmol) を加え、室温に戻して40分撹拌した。反応液に水を加えをクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。

得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SN AP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 52 mg, 0.113 mmol, 88%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.52 (1H, s), 7.30 (1H, d, $J = 10.7$ Hz), 6.85 (1H, d, $J = 10.7$ Hz), 6.79 (1H, d, $J = 6.8$ Hz), 4.61–4.55 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.63 (3H, s), 3.25 (1H, dd, $J = 12.8, 4.3$ Hz), 2.25–2.23 (3H, m), 2.21–2.14 (1H, m), 1.81–1.77 (1H, m), 1.70–1.58 (3H, m), 0.92 (3H, t, $J = 7.4$ Hz)

ESI-MS m/z : 462 $[\text{M}+\text{H}]^+$.

[0120] 実施例 4 4

4-クロロ-N-イソブチリルデアセチルコルヒチンの合成

WO 2011/021397

76

PCT/JP2010/005136

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、イソ酪酸クロリド (13 μ l, 0.128 $\times$ 1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 9 mg, 0.019 mmol, 15%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.48 (1H, s), 7.31 (1H, d, $J = 10.7$ Hz), 6.84 (1H, d, $J = 10.7$ Hz), 6.38 (1H, d, $J = 6.6$ Hz), 4.58–4.54 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.63 (3H, s), 3.25 (1H, dd, $J = 12.6, 4.5$ Hz), 2.48–2.47 (1H, m), 2.24–2.16 (2H, m), 1.82–1.77 (1H, m), 1.20 (3H, d, $J = 6.8$ Hz), 1.15 (3H, d, $J = 6.6$ Hz)

ESI-MS m/z : 462 $[\text{M}+\text{H}]^+$.

[0121] 実施例 4 5

4-クロロ-N- (シクロプロパンカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、シクロプロパンカルボニルクロリド (12 μ l, 0.128 $\times$ 1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。

得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄褐色固体, 58 mg, 0.126 mmol, 99%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.71 (1H, s), 7.36 (1H, d, $J = 10.7$ Hz), 6.91 (1H, d, $J = 10.7$ Hz), 6.83 (1H, br. s), 4.64–4.58 (1H, m), 4.0

WO 2011/021397

77

PCT/JP2010/005136

3 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.60 (3H, s), 3.26 (1H, dd, $J = 13.5, 5.0$ Hz), 2.30–2.27 (1H, m), 2.18–2.10 (1H, m), 1.88–1.84 (1H, m), 1.56–1.52 (1H, m), 0.88–0.86 (2H, m), 0.75–0.73 (2H, m).

ESI-MS m/z : 460 $[M+H]^+$.

[0122] 実施例 4 6

4-クロロ-N-イソバレリルデアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128×1.1 mmol)、イソバレリルクロリド (16 μ l, 0.128×1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。

得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SN AP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 57 mg, 0.120 mmol, 94%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.47 (1H, s), 7.29 (1H, d, $J = 10.7$ Hz), 6.83 (1H, d, $J = 10.7$ Hz), 6.24 (1H, d, $J = 7.3$ Hz), 4.61–4.57 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.25 (1H, dd, $J = 13.4, 4.4$ Hz), 2.27–2.04 (5H, m), 1.77–1.74 (2H, m), 0.95 (3H, d, $J = 6.3$ Hz), 0.92 (3H, d, $J = 6.1$ Hz).

ESI-MS m/z : 476 $[M+H]^+$.

[0123] 実施例 4 7

4-クロロ-N-ヘプタノイルデアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128×1.1 mmol)、ヘプタノイルクロリド (20 μ l, 0.128×1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で

WO 2011/021397

78

PCT/JP2010/005136

洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。

得られた残渣をシリカゲルクロマトグラフィー (Biorage Isorela One, SN AP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 59 mg, 0.117 mmol, 92%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.48 (1H, s), 7.29 (1H, d, $J = 10.7$ Hz), 6.84 (1H, d, $J = 10.7$ Hz), 6.38 (1H, br. s), 4.58–4.55 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 2.26–2.11 (2H, m), 1.78–1.75 (1H, m), 1.62–1.54 (2H, m), 1.29–1.27 (8H, m), 0.88–0.84 (3H, m).

ESI-MS m/z : 504 $[\text{M}+\text{H}]^+$.

[0124] 実施例 4 8

4-クロロ-N- (シクロヘキサンカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、 0°C に冷却した。そこへトリエチルアミン (19 μl , 0.128×1.1 mmol)、シクロヘキサンカルボニルクロリド (17 μl , 0.128×1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 18 mg, 0.036 mmol, 28%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.47 (1H, s), 7.29 (1H, d, $J = 10.7$ Hz), 6.83 (1H, d, $J = 10.7$ Hz), 6.23 (1H, d, $J = 6.6$ Hz), 4.58–4.52 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.25 (1H, dd, $J = 12.8, 4.5$ Hz), 2.26–2.12 (3H, m), 1.94–1.90 (2H, m), 1.82–1.76 (1H, m), 1.66–1.63 (2H, m), 1.50–1.15 (5H, m).

ESI-MS m/z : 502 $[\text{M}+\text{H}]^+$.

[0125] 実施例 4 9

N-ベンゾイル-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (150 mg, 0.383 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (57 μ l, 0.383 $\times$ 1.1 mmol)、ベンゾイルクロリド (44 μ l, 0.383 $\times$ 1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。

有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 146 mg, 0.294 mmol, 77%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.80–7.79 (2H, m), 7.63 (1H, s), 7.49–7.47 (1H, m), 7.41–7.37 (2H, m), 7.35 (1H, d, $J = 10.7$ Hz), 6.93 (1H, br. s), 6.88 (1H, d, $J = 11.0$ Hz), 4.80–4.78 (1H, m), 4.01 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.70 (3H, s), 3.32 (1H, dd, $J = 13.5, 5.2$ Hz), 2.37–2.32 (1H, m), 2.25–2.22 (1H, m), 1.98–1.94 (1H, m).

ESI-MS m/z : 496 $[\text{M}+\text{H}]^+$.

[0126] 実施例 50

4-クロロ-N-(フェニルアセチル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (21 μ l, 0.128 $\times$ 1.5 mmol)、フェニルアセチルクロリド (25 μ l, 0.128 $\times$ 1.2 mmol) を加え、室温に戻して40分攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 44 mg, 0.086 mmol, 67%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.45 (1H, s), 7.38–7.26 (6H, m), 6.86

WO 2011/021397

80

PCT/JP2010/005136

(1H, d, $J = 10.7$ Hz), 6.39 (1H, br. s), 4.57–4.51 (1H, m), 4.02 (3H, s), 3.98 (3H, s), 3.96 (3H, s), 3.65–3.56 (2H, m), 3.61 (3H, s), 3.21–3.19 (1H, m), 2.16–2.08 (1H, m), 1.97–1.86 (1H, m), 1.74–1.70 (1H, m).

ESI-MS m/z : 510 $[M+H]^+$.

[0127] 実施例 5 1

4-クロロ-N- (ピリジン-3-イルカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (47 μ l, 0.128×2.7 mmol)、ニコチン酸クロリド塩酸塩 (27 mg, 0.128×1.2 mmol) を加え、室温に戻して2時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (45 mg) を得たが、これには原料が20%含まれていた。そこでこのうち20 mg をジクロロメタン (0.8 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l)、ニコチン酸クロリド塩酸塩 (11 mg) を加え、室温に戻して3時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 20 mg, 0.040 mmol, 71%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 9.27 (1H, s), 8.71 (1H, d, $J = 3.7$ Hz), 8.24 (1H, d, $J = 8.3$ Hz), 7.98 (1H, br. s), 7.54 (1H, s), 7.42 (1H, dd, $J = 7.4, 5.2$ Hz), 7.32 (1H, d, $J = 10.7$ Hz), 6.86 (1H, d, $J = 11.0$ Hz), 4.82–4.76 (1H, m), 4.01 (3H, s), 4.00 (3H, s), 3.98 (3H, s), 3.70 (3H, s), 3.32 (1H, dd, $J = 13.4, 4.9$ Hz), 2.38–2.33 (1H, m), 2.25

WO 2011/021397

81

PCT/JP2010/005136

-2.22 (1H, m), 2.15-2.08 (1H, m).

ESI-MS m/z : 497 $[M+H]^+$.

[0128] 実施例 5 2

4-クロロ-N- (ピリジン-4-イルカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (47 μ l, 0.128 $\times$ 2.7 mmol)、イソニコチン酸クロリド塩酸塩 (27 mg, 0.128 $\times$ 1.2 mmol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 67 mg, 定量的) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.65 (2H, d, $J = 5.9$ Hz), 8.28 (1H, br s), 7.74 (2H, d, $J = 5.9$ Hz), 7.54 (1H, s), 7.36 (1H, d, $J = 10.7$ Hz), 6.90 (1H, d, $J = 11.0$ Hz), 4.78-4.72 (1H, m), 4.03 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.71 (3H, s), 3.31 (1H, dd, $J = 13.2, 4.6$ Hz), 2.39-2.34 (1H, m), 2.24-2.20 (1H, m), 2.10-2.02 (1H, m).

ESI-MS m/z : 497 $[M+H]^+$.

[0129] 実施例 5 3

4-クロロ-N- (ピリジン-2-イルカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (160 mg, 0.408 mmol) をジクロロメタン (4 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (86 μ l, 0.408 $\times$ 1.5 mmol)、ピコリン酸クロリド塩酸塩 (88 mg, 0.408 $\times$ 1.2 mmol) を加え、室温に戻して終夜撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 25 g,

WO 2011/021397

82

PCT/JP2010/005136

メタノール/クロロホルム)で精製し、標記の化合物(黄色固体, 136 mg, 0.274 mmol, 67%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.60–8.58 (1H, m), 8.05 (1H, d, $J = 7.8$ Hz), 7.83 (1H, dd, $J = 7.7, 6.5$ Hz), 7.48–7.46 (1H, m), 7.45 (1H, s), 7.26 (1H, d, $J = 10.2$ Hz), 6.79 (1H, d, $J = 10.7$ Hz), 4.75–4.69 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.69 (3H, s), 3.33 (1H, dd, $J = 12.7, 4.4$ Hz), 2.41–2.23 (2H, m), 2.01–1.97 (1H, m).

ESI-MS m/z : 497 $[\text{M}+\text{H}]^+$.

[0130] 実施例 5 4

4-クロロ-N-(トリフルオロアセチル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (6 mL) に溶解し、 0°C に冷却した。そこへトリフルオロ酢酸無水物 (38 μl) を加え、 0°C で30分撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物(白色固体, 56 mg, 0.115 mmol, 90%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.64 (1H, br. s), 7.44 (1H, s), 7.34 (1H, d, $J = 10.5$ Hz), 7.34 (1H, d, $J = 11.0$ Hz), 4.64–4.58 (1H, m), 4.03 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.63 (3H, s), 3.31 (1H, dd, $J = 13.8, 5.7$ Hz), 2.41–2.33 (1H, m), 2.20–2.17 (1H, m), 2.09–2.01 (1H, m).

ESI-MS m/z : 488 $[\text{M}+\text{H}]^+$.

[0131] 実施例 5 5

4-クロロ-N-(メトキシアセチル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、 0°C に冷却した。そこへトリエチルアミ

WO 2011/021397

83

PCT/JP2010/005136

ン (26 μ l, 0.128 $\times$ 1.5 mmol)、メトキシアセチルクロリド (14 μ l, 0.128 $\times$ 1.2 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 41 mg, 0.088 mmol, 69%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.40 (1H, s), 7.26–7.24 (1H, m), 7.00 (1H, d, $J = 7.3$ Hz), 6.81 (1H, d, $J = 10.7$ Hz), 4.60–4.54 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.91 (1H, d, $J = 15.1$ Hz), 3.83 (1H, d, $J = 15.1$ Hz), 3.61 (3H, s), 3.46 (3H, s), 3.29–3.26 (1H, m), 2.25–2.18 (2H, m), 1.86–1.81 (1H, m).

ESI-MS m/z : 464 $[\text{M}+\text{H}]^+$.

[0132] 実施例 5 6

4-クロロ-N- (フェノキシアセチル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (26 μ l, 0.128 $\times$ 1.5 mmol)、フェノキシアセチルクロリド (17 μ l, 0.128 $\times$ 1.2 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 36 mg, 0.068 mmol, 54%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.39 (1H, s), 7.35 (2H, dd, $J = 8.1, 7.4$ Hz), 7.26 (1H, d, $J = 10.7$ Hz), 7.06 (2H, t, $J = 7.3$ Hz), 6.95 (2H, d, $J = 8.1$ Hz), 6.81 (1H, d, $J = 10.7$ Hz), 4.65–4.59 (1H, m), 4.54 (1H, d, $J = 15.1$ Hz), 4.43 (1H, d, $J = 14.9$ Hz), 4.00 (3H, s), 4.00

WO 2011/021397

84

PCT/JP2010/005136

(3H, s), 3.97 (3H, s), 3.64 (3H, s), 3.29–3.27 (1H, m), 2.25–2.20 (2H, m), 1.90–1.82 (1H, m).

ESI-MS m/z : 526 $[M+H]^+$.

[0133] 実施例 5 7

4-クロロ-N-[(メチルチオ)アセチル]デアセチルコルヒチンの合成

アルゴン雰囲気下、(メチルチオ)酢酸 (13 μ l, 0.128 $\times$ 1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 $\times$ 1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物 (21 mg, 0.128 $\times$ 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 31 mg, 0.065 mmol, 51%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.43 (1H, s), 7.29 (1H, d, $J = 10.7$ Hz), 6.83 (1H, d, $J = 10.7$ Hz), 4.59–4.53 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.30–3.28 (1H, m), 3.25 (1H, d, $J = 16.3$ Hz), 3.17 (1H, d, $J = 16.3$ Hz), 2.28–2.20 (2H, m), 2.17 (3H, s), 1.89–1.86 (1H, m).

ESI-MS m/z : 480 $[M+H]^+$.

[0134] 実施例 5 8

4-クロロ-N-[(4-ピリジルチオ)アセチル]デアセチルコルヒチンの合成

アルゴン雰囲気下、(4-ピリジルチオ)酢酸 (26 mg, 0.128 $\times$ 1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 $\times$ 1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物 (21 mg, 0.128 $\times$

WO 2011/021397

85

PCT/JP2010/005136

1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 45 mg, 0.083 mmol, 65%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 8.50 (2H, d, J = 4.9 Hz), 7.94 (1H, d, J = 6.6 Hz), 7.36 (1H, s), 7.34 (2H, d, J = 4.9 Hz), 7.25 (1H, d, J = 11.0 Hz), 6.80 (1H, d, J = 11.0 Hz), 4.57–4.51 (1H, m), 3.99 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.96 (1H, d, J = 16.3 Hz), 3.77 (1H, d, J = 16.3 Hz), 3.61 (3H, s), 3.28–3.19 (1H, m), 2.16–2.11 (2H, m), 1.88–1.85 (1H, m).

ESI-MS m/z: 543 [M+H]⁺.

[0135] 実施例 59

N-(4-ブロモブチリル)-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、4-ブロモ酪酸 (43 mg, 0.128 × 1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水和物 (21 mg, 0.128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mol) を加え、室温に戻して1時間撹拌した。反応液に水を加えてクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 27 mg, 0.070 mmol, 39%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.47 (1H, s), 7.30 (1H, d, J = 11.0 Hz

WO 2011/021397

86

PCT/JP2010/005136

), 6.85 (1H, d, $J = 11.0$ Hz), 6.65–6.64 (1H, br. s), 4.59–4.53 (1H, m), 4.02 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.55 (1H, t, $J = 6.2$ Hz), 3.42 (1H, t, $J = 6.5$ Hz), 3.26 (1H, dd, $J = 13.2, 4.4$ Hz), 2.49–2.39 (1H, m), 2.29–2.11 (3H, m), 2.11–2.06 (1H, m), 1.80–1.75 (1H, m).

ESI-MS m/z : 540 $[M+H]^+$.

[0136] 実施例 6 O

4-クロロ-N- (4-フルオロベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128×1.1 mmol)、4-フルオロベンゾイルクロリド (15 μ l, 0.128×1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。

有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 45 mg, 0.088 mmol, 68%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.83–7.79 (2H, m), 7.57 (1H, s), 7.36 (1H, br. s), 7.34 (1H, d, $J = 10.7$ Hz), 7.01–6.99 (2H, m), 6.88 (1H, d, $J = 11.0$ Hz), 4.78–4.72 (1H, m), 4.02 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.70 (3H, s), 3.31 (1H, dd, $J = 13.4, 5.4$ Hz), 2.37–2.33 (1H, m), 2.24–2.20 (1H, m), 1.97–1.94 (1H, m) ESI-MS m/z : 514 $[M+H]^+$.

[0137] 実施例 6 1

4-クロロ-N- (3,5-ジフルオロベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128×1.1 mmol)、3,5-ジフルオロベンゾイルクロリド (16 μ l, 0.128×1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を

WO 2011/021397

87

PCT/JP2010/005136

加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (赤褐色固体, 50 mg, 0.094 mmol, 73%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.92 (1H, br. s), 7.63 (1H, s), 7.37 (1H, d, $J = 11.0$ Hz), 7.34–7.30 (2H, m), 6.92 (1H, d, $J = 11.0$ Hz), 6.85–6.83 (1H, m), 4.79–4.73 (1H, m), 4.03 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.69 (3H, s), 3.31 (1H, dd, $J = 13.4, 5.1$ Hz), 2.42–2.33 (1H, m), 2.23–2.20 (1H, m), 2.09–2.04 (1H, m).

ESI-MS m/z : 532 $[\text{M}+\text{H}]^+$.

[0138] 実施例 6 2

4-クロロ-N- (3,4,5-トリフルオロベンゾイル) デアセチルコルヒチンの合成
アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μl , 0.128×1.1 mmol)、3,4,5-トリフルオロベンゾイルクロリド (17 μl , 0.128×1 mmol) を加え、室温に戻して30分撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (赤褐色固体, 38 mg, 0.069 mmol, 54%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.26 (1H, br. s), 7.65 (1H, s), 7.49–7.47 (2H, m), 7.40 (1H, d, $J = 10.7$ Hz), 6.95 (1H, d, $J = 11.0$ Hz), 4.78–4.72 (1H, m), 4.05 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.68 (3H, s), 3.31 (1H, dd, $J = 13.5, 5.6$ Hz), 2.39–2.35 (1H, m), 2.20–2.15 (2H, m).

ESI-MS m/z : 550 $[\text{M}+\text{H}]^+$.

[0139] 実施例 6 3

4-クロロ-N-(4-メトキシベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、4-メトキシベンゾイルクロリド (26 mg, 0.128 $\times$ 1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage isorela One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 56 mg, 0.106 mmol, 83%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.75 (2H, d, $J = 8.5$ Hz), 7.54 (1H, s), 7.31 (1H, d, $J = 10.7$ Hz), 6.87 (2H, d, $J = 9.0$ Hz), 6.85 (1H, br. s), 6.84 (1H, d, $J = 11.0$ Hz), 4.78-4.72 (1H, m), 4.00 (3H, s), 4.00 (3H, s), 3.97 (3H, s), 3.83 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, $J = 13.3, 4.5$ Hz), 2.38-2.29 (1H, m), 2.25-2.21 (1H, m), 1.92-1.89 (1H, m).

ESI-MS m/z : 526 $[\text{M}+\text{H}]^+$.

[0140] 実施例 6 4

4-クロロ-N-(3,4,5-トリメトキシベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、3,4,5-トリメトキシベンゾイルクロリド (30 mg, 0.128 $\times$ 1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄褐色固体,

WO 2011/021397

89

PCT/JP2010/005136

68 mg, 0.116 mmol, 91%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.75 (1H, s), 7.63 (1H, br. s), 7.40 (1H, d, $J = 10.7$ Hz), 7.07 (2H, s), 6.93 (1H, d, $J = 11.0$ Hz), 4.79–4.72 (1H, m), 4.03 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.84 (3H, s), 3.81 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, $J = 13.5, 5.5$ Hz), 2.42–2.33 (1H, m), 2.22–2.16 (1H, m), 2.09–2.03 (1H, m).

ESI-MS m/z : 586 $[\text{M}+\text{H}]^+$.

[0141] 実施例 6 5

4-クロロ-N- (2-メトキシベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μl , 0.128 $\times$ 1.1 mmol)、2-メトキシベンゾイルクロリド (17 μl , 0.128 $\times$ 1 mmol) を加え、室温に戻して30分攪拌した。反応液に水を加えてクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 49 mg, 0.093mmol, 73%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.35 (1H, d, $J = 6.6$ Hz), 8.01 (1H, dd, $J = 7.7, 1.8$ Hz), 7.51 (1H, s), 7.48–7.44 (1H, m), 7.28 (1H, d, $J = 10.7$ Hz), 7.04–7.00 (2H, m), 6.80 (1H, d, $J = 10.7$ Hz), 4.79–4.73 (1H, m), 4.05 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, $J = 13.2, 4.6$ Hz), 2.40–2.31 (1H, m), 2.27–2.23 (1H, m), 1.89–1.85 (1H, m).

ESI-MS m/z : 526 $[\text{M}+\text{H}]^+$.

[0142] 実施例 6 6

4-クロロ-N- (3-メトキシベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol)

WO 2011/021397

90

PCT/JP2010/005136

をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、3-メトキシベンゾイルクロリド (17 μ l, 0.128 $\times$ 1 mmol) を加え、室温に戻して30分撹拌した。0分後、反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 53 mg, 0.101 mmol, 79%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.54 (1H, s), 7.34–7.31 (3H, m), 7.27 (1H, d, J = 10.7 Hz), 7.02–7.00 (1H, m), 6.96 (1H, d, J = 5.4 Hz), 6.85 (1H, d, J = 10.7 Hz), 4.01 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.80 (3H, s), 3.70 (3H, s), 3.31 (1H, dd, J = 13.3, 5.0 Hz), 2.39–2.30 (1H, m), 2.25–2.21 (1H, m), 1.95–1.89 (1H, m).

ESI-MS m/z: 526 [M+H]⁺.

[0143] 実施例 67

4-クロロ-N-[(フェニルチオ)アセチル]デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (26 μ l, 0.128 $\times$ 1.2 mmol)、(フェニルチオ)アセチルクロリド (23 μ l, 0.128 $\times$ 1.5 mmol) を加え、室温に戻して30分撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄褐色固体, 41 mg, 0.076 mmol, 59%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.48 (1H, s), 7.35–7.32 (6H, m), 7.25–7.23 (1H, m), 6.87 (1H, d, J = 11.0 Hz), 4.54–4.49 (1H, m), 4.02 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.72 (1H, d, J = 16.8 Hz), 3.63 (1H,

WO 2011/021397

91

PCT/JP2010/005136

d, $J = 16.8$ Hz), 3.60 (3H, s), 3.23 (1H, dd, $J = 12.7, 4.6$ Hz), 2.17–2.02 (2H, m), 1.79–1.76 (1H, m).

ESI-MS m/z : 542 $[M+H]^+$.

[0144] 実施例 6 8

4-クロロ-N- [3-(ジメチルアミノ)ベンゾイル]デアセチルコルヒチンの合成
アルゴン雰囲気下、3-(ジメチルアミノ)安息香酸 (25 mg, 0.128×1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、 0°C に冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128×1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水和物 (21 mg, 0.128×1.2 mmol) を加え、 0°C で30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 37 mg, 0.069 mmol, 54%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.96 (1H, d, $J = 7.3$ Hz), 7.28 (1H, t, $J = 7.9$ Hz), 7.20–7.17 (3H, m), 7.13 (1H, d, $J = 2.0$ Hz), 7.05 (1H, d, $J = 11.2$ Hz), 6.89 (1H, dd, $J = 8.1, 2.4$ Hz), 4.51–4.45 (1H, m), 3.94 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.60 (3H, s), 3.16 (1H, dd, $J = 12.1, 5.5$ Hz), 2.92 (6H, s), 2.21–2.10 (2H, m), 2.07–2.03 (1H, m).

ESI-MS m/z : 539 $[M+H]^+$.

[0145] 実施例 6 9

4-クロロ-N- [4-(ジメチルアミノ)ベンゾイル]デアセチルコルヒチンの合成
アルゴン雰囲気下、4-ジメチルアミノ安息香酸 (25 mg, 0.128×1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、 0°C に冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.1

28 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (21 mg, 0.128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 38 mg, 0.070 mmol, 55%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 7.71 (2H, d, J = 8.8 Hz), 7.50 (1H, s), 7.28 (1H, d, J = 10.7 Hz), 6.83 (1H, d, J = 7.8 Hz), 6.80 (1H, d, J = 10.7 Hz), 6.73 (2H, d, J = 8.3 Hz), 4.78-4.72 (1H, m), 4.00 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, J = 12.9, 4.1 Hz), 3.01 (6H, s), 2.32-2.23 (2H, m), 1.95-1.88 (1H, m).

ESI-MS m/z: 539 [M+H]⁺.

[0146] 実施例 7 O

4-クロロ-N- (2-フルオロベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μl, 0.128 × 1.1 mmol)、2-フルオロベンゾイルクロリド (15 μl, 0.128 × 1 mmol) を加え、室温に戻して1時間撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄褐色固体, 46 mg, 0.090mmol, 70%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.96-7.94 (1H, m), 7.55 (1H, s), 7.52-7.48 (1H, m), 7.33 (1H, d, J = 10.7 Hz), 7.26-7.14 (4H, m), 6.85 (1H, d, J = 10.7 Hz), 4.79-4.73 (1H, d, J = 10.7 Hz), 4.01 (3H, s), 4.01

WO 2011/021397

93

PCT/JP2010/005136

(3H, s), 3.98 (3H, s), 3.70 (3H, s), 3.32 (1H, dd, $J = 13.5, 5.2$ Hz), 2.42–2.32 (1H, m), 2.27–2.21 (1H, m), 1.95–1.87 (1H, m).

ESI-MS m/z : 514 $[M+H]^+$.

[0147] 実施例 7 1

4-クロロ-N-(3-フルオロベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128×1.1 mmol)、3-フルオロベンゾイルクロリド (15 μ l, 0.128×1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP10 g, メタノール/クロロホルム) で精製し、標記の化合物 (赤褐色固体, 21 mg, 0.041mmol, 32%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.82 (1H, s), 7.66 (1H, d, $J = 7.6$ Hz), 7.55–7.53 (2H, m), 7.47 (1H, d, $J = 10.7$ Hz), 7.35–7.33 (1H, m), 7.15–7.13 (1H, m), 6.99 (1H, d, $J = 11.0$ Hz), 4.82–4.77 (1H, m), 4.05 (3H, s), 4.01 (3H, s), 3.99 (3H, s), 3.70 (3H, s), 3.32 (1H, dd, $J = 13.1, 5.7$ Hz), 2.41–2.32 (1H, m), 2.29–2.19 (1H, m), 2.16–2.08 (1H, m).

ESI-MS m/z : 514 $[M+H]^+$.

[0148] 実施例 7 2

4-クロロ-N-(2,4-ジフルオロベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、2,4-ジフルオロ安息香酸 (24 mg, 0.128×1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128×1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物 (21 mg, 0.128×1.2 mmol) を加え、0°Cで30分攪拌した。そこへ4-クロロデアセチルコル

ヒチン (50 mg, 0.128 mol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 25 mg, 0.047 mmol, 37%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.02–7.96 (1H, m), 7.64 (1H, s), 7.38 (1H, d, $J = 10.7$ Hz), 7.20–7.17 (1H, m), 7.00–6.89 (3H, m), 4.78–4.71 (1H, m), 4.02 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.69 (3H, s), 3.32 (1H, dd, $J = 14.0, 4.8$ Hz), 2.42–2.32 (1H, m), 2.24–2.21 (1H, m), 1.98–1.91 (1H, m).

ESI-MS m/z : 532 $[\text{M}+\text{H}]^+$.

[0149] 実施例 7 3

4-クロロ-N- (ピリジン-3-イルオキシアセチル) デアセチルコルヒチンの合成
アルゴン雰囲気下、ピリジン-3-イルオキシ酢酸 (24 mg, 0.128 $\times$ 1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 $\times$ 1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物 (21 mg, 0.128 $\times$ 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 36 mg, 0.068 mmol, 53%) を得た。

$^1\text{H-NMR}$ (400MHz, $\text{DMSO}-d_6$) δ [ppm]: 8.47 (1H, s), 8.35 (1H, s), 7.47 (1H, d, $J = 5.9$ Hz), 7.39 (1H, s), 7.36–7.32 (2H, m), 7.26 (1H, d, $J = 10.7$ Hz), 6.81 (1H, d, $J = 10.7$ Hz), 4.65–4.62 (1H, m), 4.60 (1H, d, J

WO 2011/021397

95

PCT/JP2010/005136

= 14.9 Hz), 4.53 (1H, d, J = 14.6 Hz), 4.00 (3H, s), 4.00 (3H, s), 3.97 (3H, s), 3.63 (3H, s), 3.29 (1H, dd, J = 12.4, 4.1 Hz), 2.32–2.20 (2H, m), 1.97–1.94 (1H, m).

ESI-MS m/z: 527 [M+H]⁺.

[0150] 実施例 7 4

4-クロロ-N- (ピリジン-2-イルアセチル) デアセチルコルヒチンの合成

アルゴン雰囲気下、2-ピリジル酢酸塩酸塩 (27 mg, 0.128 × 1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (21 μ l, 0.128 × 1.2 mmol)、1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物 (21 mg, 0.128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して4時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、10% クエン酸水溶液、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 15 mg, 0.029 mmol, 23%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 9.01 (1H, d, J = 7.3 Hz), 8.52 (1H, d, J = 4.4 Hz), 7.81 (1H, t, J = 7.1 Hz), 7.38–7.32 (2H, m), 7.24 (1H, s), 7.12 (1H, d, J = 10.7 Hz), 7.02 (1H, d, J = 10.7 Hz), 4.27–4.21 (1H, m), 3.89 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.75 (2H, s), 3.49 (3H, s), 3.13 (1H, dd, J = 13.3, 5.0 Hz), 2.15–2.11 (1H, m), 2.02–1.98 (1H, m), 1.91–1.88 (1H, m).

ESI-MS m/z: 511 [M+H]⁺.

[0151] 実施例 7 5

4-クロロ-N- (ピリジン-3-イルアセチル) デアセチルコルヒチンの合成

アルゴン雰囲気下、3-ピリジル酢酸塩酸塩 (27 mg, 0.128 × 1.2 mmol)

をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (21 μ l, 0.128 $\times$ 1.2 mmol)、1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 $\times$ 1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物 (21 mg, 0.128 $\times$ 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して4時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、10% クエン酸水溶液、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 8 mg, 0.016 mmol, 11%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO- d_6) δ [ppm]: 9.06 (1H, d, J = 7.3 Hz), 8.70 (2H, s), 8.22 (1H, d, J = 7.6 Hz), 7.81 (1H, t, J = 6.7 Hz), 7.13 (1H, d, J = 10.5 Hz), 7.12 (1H, s), 7.03 (1H, d, J = 10.7 Hz), 4.27-4.20 (1H, m), 3.89 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.78-3.77 (2H, m), 3.48 (3H, s), 3.12 (1H, dd, J = 13.2, 5.1 Hz), 2.18-2.09 (1H, m), 2.06-1.97 (1H, m), 1.92-1.85 (1H, m).

ESI-MS m/z : 511 $[\text{M}+\text{H}]^+$.

[0152] 実施例 7 6

4-クロロ-N- (メトキシカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、クロロギ酸メチル (10 μ l, 0.128 $\times$ 1 mmol) を加え、室温に戻して1時間撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 48 mg, 0.107 mmol,

WO 2011/021397

97

PCT/JP2010/005136

83%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.50 (1H, s), 7.24 (1H, d, J = 10.7 Hz), 6.80 (1H, d, J = 10.7 Hz), 5.27 (1H, d, J = 6.1 Hz), 4.37–4.30 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.60 (3H, s), 3.60 (3H, s), 3.25 (1H, dd, J = 14.1, 5.1 Hz), 2.31–2.13 (2H, m), 1.73–1.65 (1H, m).

ESI-MS m/z: 450 [M+H]⁺.

[0153] 実施例 77

4-クロロ-N- (エトキシカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (260 mg, 0.664 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (109 μl, 0.664 × 1.2 mmol)、クロロギ酸エチル (95 μl, 0.664 × 1.5 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。

得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SN AP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 106 mg, 0.228 mmol, 34%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.53 (1H, s), 7.25 (1H, d, J = 10.7 Hz), 6.81 (1H, d, J = 10.7 Hz), 5.24 (1H, d, J = 7.3 Hz), 4.37–4.31 (1H, m), 4.01 (3H, s), 4.06–4.01 (2H, m), 3.99 (3H, s), 3.97 (3H, s), 3.60 (3H, s), 3.25 (1H, dd, J = 14.0, 5.2 Hz), 2.31–2.13 (2H, m), 1.72–1.68 (1H, m), 1.20 (3H, t, J = 7.1 Hz).

ESI-MS m/z: 464 [M+H]⁺.

[0154] 実施例 78

4-クロロ-N- (イソプロピルオキシカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミ

ン (19 μ l, 0.128 $\times$ 1.1 mmol)、クロロギ酸イソプロピル (15 μ l, 0.128 $\times$ 1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 53 mg, 0.111 mmol, 87%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.52 (1H, s), 7.24 (1H, d, J = 10.7 Hz), 6.80 (1H, d, J = 10.7 Hz), 5.07 (1H, d, J = 7.3 Hz), 4.81–4.75 (1H, m), 4.37–4.31 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.60 (3H, s), 3.25 (1H, dd, J = 13.2, 4.1 Hz), 2.25–2.16 (2H, m), 1.71–1.65 (1H, m), 1.21 (3H, d, J = 6.3 Hz), 1.18 (3H, d, J = 6.1 Hz).

ESI-MS m/z : 478 $[\text{M}+\text{H}]^+$.

[0155] 実施例 79

4-クロロ-N- (イソブチルオキシカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、クロロギ酸イソブチル (17 μ l, 0.128 $\times$ 1 mmol) を加え、室温に戻して2時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。

得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 58 mg, 0.118 mmol, 92%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.57 (1H, s), 7.27 (1H, d, J = 10.5 Hz), 6.83 (1H, d, J = 10.7 Hz), 5.26 (1H, d, J = 6.8 Hz), 4.38–4.32 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.77–3.72 (2H, m), 3.60 (3H, s), 3.26 (1H, dd, J = 13.4, 4.9 Hz), 2.32–2.23 (1H, m), 2.18–

WO 2011/021397

99

PCT/JP2010/005136

2.15 (1H, m), 1.91–1.81 (1H, m), 1.76–1.68 (1H, m), 0.89 (6H, d, $J = 6.6$ Hz).

ESI-MS m/z : 492 $[M+H]^+$.

[0156] 実施例 80

4-クロロ-N- (フェノキシカルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128×1.1 mmol)、クロロギ酸フェニル (16 μ l, 0.128×1 mmol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。

有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 20 mg, 0.039 mmol, 31%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.64 (1H, s), 7.31–7.27 (3H, m), 7.18–7.15 (1H, m), 7.06–7.05 (2H, m), 6.83 (1H, d, $J = 10.7$ Hz), 5.63 (1H, d, $J = 6.8$ Hz), 4.45–4.39 (1H, m), 4.02 (3H, s), 3.97 (3H, s), 3.97 (3H, s), 3.52 (3H, s), 3.30 (1H, dd, $J = 13.5, 5.2$ Hz), 2.41–2.32 (1H, m), 2.23–2.19 (1H, m), 1.83–1.78 (1H, m).

ESI-MS m/z : 512 $[M+H]^+$.

[0157] 実施例 81

N-(ベンジルオキシカルボニル)-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (300 mg, 0.766 mmol) をジクロロメタン (6 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (115 μ l, 0.766×1.1 mmol)、クロロギ酸ベンジル (108 μ l, 0.766×1 mmol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。

WO 2011/021397

100

PCT/JP2010/005136

得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SN AP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 105 mg, 0.200 mmol, 26%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.57 (1H, s), 7.34–7.30 (5H, m), 7.26 (1H, d, $J = 10.5$ Hz), 6.82 (1H, d, $J = 10.5$ Hz), 5.38 (1H, d, $J = 6.8$ Hz), 5.08 (1H, d, $J = 12.0$ Hz), 4.94 (1H, d, $J = 12.2$ Hz), 4.40–4.34 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.61 (3H, s), 3.25 (1H, dd, $J = 12.7, 4.9$ Hz), 2.32–2.25 (1H, m), 2.21–2.13 (1H, m), 1.76–1.69 (1H, m).

ESI-MS m/z : 526 $[\text{M}+\text{H}]^+$.

[0158] 実施例 8 2

4-クロロ-N-(メタンスルホニル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、 0°C に冷却した。そこへトリエチルアミン (26 μl , 0.128×1.5 mmol)、メタンスルホニルクロリド (12 μl , 0.128×1.2 mmol) を加え、 0°C で1時間攪拌した。反応液に水を加えクエンチし、クロロホルムで抽出した。有機層を飽和重曹水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 57 mg, 0.121 mmol, 95%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.77 (1H, s), 7.31 (1H, d, $J = 10.7$ Hz), 6.88 (1H, d, $J = 10.7$ Hz), 5.52 (1H, br. s), 4.36–4.30 (1H, m), 4.03 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.58 (3H, s), 3.27 (1H, dd, $J = 14.1, 5.4$ Hz), 2.89 (3H, s), 2.40–2.31 (1H, m), 2.15–2.12 (1H, m), 1.89–1.85 (1H, m).

ESI-MS m/z : 470 $[\text{M}+\text{H}]^+$.

[0159] 実施例 8 3

4-クロロ-N-(エタンスルホニル)デアセチルコルヒチンの合成

WO 2011/021397

101

PCT/JP2010/005136

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (198 mg, 0.505 mmol) をジクロロメタン (4 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (142 μ l, 0.487 $\times$ 2 mmol)、エタンスルホニルクロリド (72 μ l, 0.487 $\times$ 1.5 mmol) を加え、室温まで昇温させながら終夜撹拌した。反応液に水を加えクエンチし、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 243 mg, 0.502 mmol, 99%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.70 (1H, s), 7.26 (1H, d, $J = 10.7$ Hz), 6.84 (1H, d, $J = 11.0$ Hz), 5.36 (1H, br. s), 4.33–4.27 (1H, m), 4.02 (3H, s), 3.99 (3H, s), 3.99 (3H, s), 3.58 (3H, s), 3.27 (1H, dd, $J = 13.8, 5.2$ Hz), 2.99–2.89 (2H, m), 2.36–2.31 (1H, m), 2.17–2.13 (1H, m), 1.91–1.83 (1H, m), 1.32 (3H, t, $J = 7.4$ Hz).

ESI-MS m/z : 484 $[\text{M}+\text{H}]^+$.

[0160] 実施例 8 4

4-クロロ-N-(イソプロパンスルホニル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (26 μ l, 0.128 $\times$ 1.5 mmol)、イソプロパンスルホニルクロリド (17 μ l, 0.128 $\times$ 1.2 mmol) を加え、0°Cで2時間撹拌した。反応液に水を加えクエンチし、0.1 N mol/L塩酸を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 6 mg, 0.012 mmol, 9%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.65 (1H, s), 7.26–7.23 (1H, m), 6.81 (1H, d, $J = 11.0$ Hz), 5.08 (1H, br. s), 4.30–4.24 (1H, m), 4.01 (3H, s

WO 2011/021397

102

PCT/JP2010/005136

), 3.99 (3H, s), 3.97 (3H, s), 3.57 (3H, s), 3.26 (1H, dd, J = 13.5, 5.2 Hz), 3.04–2.97 (1H, m), 2.38–2.33 (1H, m), 2.22–2.11 (1H, m), 1.88–1.83 (1H, m), 1.32 (3H, d, J = 6.8 Hz), 1.27 (3H, d, J = 6.8 Hz).
ESI-MS m/z: 498 [M+H]⁺.

[0161] 実施例 8 5

4-クロロ-N-(シクロプロパンスルホニル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (26 μ l, 0.128 $\times$ 1.5 mmol)、シクロプロパンスルホニルクロリド (12 μ l, 0.128 $\times$ 1.2 mmol) を加え、0°Cで1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 36 mg, 0.073 mmol, 57%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.83 (1H, s), 7.28 (1H, d, J = 10.5 Hz), 6.85 (1H, d, J = 10.5 Hz), 5.18 (1H, br. s), 4.33–4.27 (1H, m), 4.02 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.61 (3H, s), 3.27 (1H, dd, J = 13.7, 5.6 Hz), 2.42–2.30 (2H, m), 2.16–2.13 (1H, m), 1.85–1.81 (1H, m), 1.09–1.05 (1H, m), 0.99–0.96 (1H, m), 0.87–0.79 (2H, m).

ESI-MS m/z: 496 [M+H]⁺.

[0162] 実施例 8 6

4-クロロ-N-(イソブタンスルホニル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (26 μ l, 0.128 $\times$ 1.5 mmol)、イソブタンスルホニルクロリド (12 μ l, 0.128 $\times$ 1.2 mmol) を加え、0°Cで1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水

WO 2011/021397

103

PCT/JP2010/005136

で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 56 mg, 0.109 mmol, 85%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.70 (1H, s), 7.26 (1H, d, $J = 10.7$ Hz), 6.84 (1H, d, $J = 10.7$ Hz), 5.18 (1H, d, $J = 5.1$ Hz), 4.33–4.27 (1H, m), 4.02 (3H, s), 3.99 (3H, s), 3.99 (3H, s), 3.57 (3H, s), 3.26 (1H, dd, $J = 14.0, 5.0$ Hz), 2.88 (1H, dd, $J = 14.0, 6.0$ Hz), 2.74 (1H, dd, $J = 14.0, 7.0$ Hz), 2.38–2.29 (1H, m), 2.25–2.10 (2H, m), 1.88–1.82 (1H, m), 1.03 (3H, d, $J = 6.8$ Hz), 1.00 (3H, d, $J = 6.8$ Hz).

ESI-MS m/z : 512 $[\text{M}+\text{H}]^+$.

[0163] 実施例 87

N-(ベンゼンスルホニル)-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、 0°C に冷却した。そこへトリエチルアミン (26 μl , 0.128×1.5 mmol)、ベンゼンスルホニルクロリド (20 μl , 0.128×1.2 mmol) を加え、 0°C で30分撹拌した。反応液に水を加えクエンチし、クロロホルムで抽出、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 30 mg, 0.056 mmol, 44%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.65–7.63 (2H, m), 7.47 (1H, t, $J = 7.4$ Hz), 7.37 (2H, t, $J = 7.6$ Hz), 7.28 (1H, s), 7.17 (1H, d, $J = 10.5$ Hz), 6.72 (1H, d, $J = 11.0$ Hz), 5.41 (1H, d, $J = 7.8$ Hz), 4.16–4.10 (1H, m), 4.00 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.17 (1H, dd, $J = 13.2, 4.6$ Hz), 2.18–2.03 (2H, m), 1.74–1.71 (1H, m).

ESI-MS m/z : 532 $[\text{M}+\text{H}]^+$.

[0164] 実施例 88

WO 2011/021397

104

PCT/JP2010/005136

4-クロロ-N- (p-トルエンスルホニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (26 μ l, 0.128 $\times$ 1.5 mmol)、p-トルエンスルホニルクロリド (29 mg, 0.128 $\times$ 1.2 mmol) を加え、0°Cで2時間攪拌した。反応液に水を加えクエンチし、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄褐色固体, 36 mg, 0.066 mmol, 52%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.51 (2H, d, J = 8.1 Hz), 7.31 (1H, s), 7.18 (1H, d, J = 10.5 Hz), 7.15 (2H, d, J = 7.8 Hz), 6.74 (1H, d, J = 10.7 Hz), 5.30 (1H, d, J = 7.1 Hz), 4.13–4.07 (1H, m), 4.00 (3H, s), 4.00 (3H, s), 3.98 (3H, s), 3.63 (3H, s), 3.17–3.15 (1H, m), 2.35 (3H, s), 2.11–2.06 (2H, m), 1.77–1.71 (1H, m).

ESI-MS m/z : 546 $[\text{M}+\text{H}]^+$.

[0165] 実施例 89

4-クロロ-N- (2-チオフェンスルホニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (172 mg, 0.439 mmol) をジクロロメタン (3.5 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (92 μ l, 0.439 $\times$ 1.5 mmol)、2-チオフェンスルホニルクロリド (121 mg, 0.439 $\times$ 1.5 mmol) を加え、室温で終夜攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP25 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 209 mg, 0.388 mmol, 88%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.46–7.43 (3H, m), 7.22 (1H, d, J = 10

WO 2011/021397

105

PCT/JP2010/005136

.5 Hz), 6.96 (1H, dd, J = 4.9, 3.7Hz), 6.78 (1H, d, J = 10.5 Hz), 5.54 (1H, br. s), 4.23-4.17 (1H, m), 4.01 (3H, s), 4.00 (3H, s), 4.00 (3H, s), 3.63 (3H, s), 3.21-3.19 (1H, m), 2.14-2.09 (2H, m), 1.83-1.75 (1H, m).

ESI-MS m/z: 538 [M+H]⁺.

[0166] 実施例 90

4-クロロ-N-(トリフルオロメタンスルホニル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへピリジン (15 μ l, 0.128 $\times$ 1.5 mmol)、トリフルオロメタンスルホニルクロリド (16 μ l, 0.128 $\times$ 1.2 mmol) を加え、0°Cで3時間攪拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 8 mg, 0.015 mmol, 12%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 8.00 (1H, br. s), 7.80 (1H, s), 7.35 (1H, d, J = 10.7 Hz), 6.93 (1H, d, J = 11.0 Hz), 4.31-4.28 (1H, m), 4.04 (3H, s), 3.99 (3H, s), 3.99 (3H, s), 3.53 (3H, s), 3.29 (1H, dd, J = 12.7, 5.6 Hz), 2.44-2.37 (1H, m), 2.17-2.11 (2H, m).

ESI-MS m/z: 524 [M+H]⁺.

[0167] 実施例 91

4-クロロ-N-(エチルカルバモイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をメタノール-水 (2:1, 1.5 mL) に溶解し、0°Cに冷却した。そこへイソシアン酸エチル (20 μ l, 0.128 $\times$ 2 mmol) を加え、0°Cで75分攪拌した。反応液をクロロホルムで抽出、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (B

WO 2011/021397

106

PCT/JP2010/005136

biotage isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄褐色固体, 36 mg, 0.078 mmol, 61%) を得た。

$^1\text{H-NMR}$ (500MHz, CDCl_3) δ [ppm]: 7.79 (1H, s), 7.36 (1H, d, $J = 10.6$ Hz), 6.92 (1H, d, $J = 10.6$ Hz), 6.67 (1H, br. s), 4.54–4.49 (1H, m), 4.03 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.63 (3H, s), 3.24–3.15 (2H, m), 3.10–3.06 (1H, m), 2.30–2.27 (1H, m), 2.14–2.07 (1H, m), 1.72–1.65 (1H, m), 1.04 (3H, t, $J = 7.2$ Hz).

ESI-MS m/z : 463 $[\text{M}+\text{H}]^+$.

[0168] 実施例 9 2

4-クロロ-N- (イソプロピルカルバモイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をメタノール-水 (2:1, 1.5 mL) に溶解し、0°Cに冷却した。そこへイソシアン酸イソプロピル (25 μl , 0.128 $\times$ 2 mmol) を加え、0°Cで75分撹拌した。反応液をクロロホルムで抽出、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 59 mg, 0.124 mmol, 97%) を得た。

$^1\text{H-NMR}$ (500MHz, CDCl_3) δ [ppm]: 7.94 (1H, s), 7.46 (1H, d, $J = 10.9$ Hz), 7.02 (1H, d, $J = 10.9$ Hz), 6.28 (1H, br. s), 4.57–4.53 (1H, m), 4.06 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.84–3.80 (1H, m), 3.62 (3H, s), 3.23 (1H, dd, $J = 13.5, 4.9$ Hz), 2.34–2.28 (1H, m), 2.12–2.05 (1H, m), 1.79–1.76 (1H, m), 1.08 (3H, d, $J = 6.3$ Hz), 1.05 (3H, d, $J = 6.6$ Hz).

ESI-MS m/z : 477 $[\text{M}+\text{H}]^+$.

[0169] 実施例 9 3

4-クロロ-N- (ヘキシルカルバモイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をメタノール-水 (2:1, 1.5 mL) に溶解し、0°Cに冷却した。そこへイソシア

WO 2011/021397

107

PCT/JP2010/005136

ン酸ヘキシル (37 μ l, 0.128 $\times$ 2 mmol) を加え、0°Cで75分撹拌した。反応液をクロロホルムで抽出、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isorela One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 53 mg, 0.102 mmol, 80%) を得た。

$^1\text{H-NMR}$ (500MHz, CDCl_3) δ [ppm]: 8.21 (1H, s), 7.61 (1H, d, $J = 10.9$ Hz), 7.15 (1H, d, $J = 10.9$ Hz), 6.78 (1H, br. s), 4.59–4.57 (1H, m), 4.10 (3H, s), 3.99 (3H, s), 3.98 (3H, s), 3.62 (3H, s), 3.25 (1H, dd, $J = 14.3, 5.7$ Hz), 3.18–3.12 (1H, m), 3.04–2.98 (1H, m), 2.40–2.32 (1H, m), 2.07–2.02 (1H, m), 1.92–1.84 (1H, m), 1.42–1.39 (2H, m), 1.26–1.20 (4H, m), 0.88–0.86 (2H, m), 0.82 (3H, t, $J = 6.9$ Hz).

ESI-MS m/z : 519 $[\text{M}+\text{H}]^+$.

[0170] 実施例 9 4

4-クロロ-N- (フェニルカルバモイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (200 mg, 0.510 mmol) をジクロロメタン (4 mL) に溶解し、0°Cに冷却した。そこへイソシアン酸フェニル (110 μ l, 0.510 $\times$ 2 mmol) を加え、0°Cで2時間撹拌した。反応液を濃縮し、得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 168 mg, 0.329 mmol, 65%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.19 (1H, s), 7.48 (1H, d, $J = 10.7$ Hz), 7.29–7.26 (2H, m), 7.16–7.14 (2H, m), 7.07 (1H, d, $J = 8.8$ Hz), 7.03 (1H, d, $J = 11.2$ Hz), 6.92–6.90 (1H, m), 4.73–4.67 (1H, m), 4.03 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.65 (3H, s), 3.22 (1H, dd, $J = 13.5, 5.2$ Hz), 2.43–2.33 (1H, m), 2.12–2.04 (1H, m), 1.84–1.80 (1H, m).

ESI-MS m/z : 511 $[\text{M}+\text{H}]^+$.

[0171] 実施例 9 5

N-(ベンジルカルバモイル)-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をメタノール-水 (2:1, 1.5 mL) に溶解し、0°Cに冷却した。そこへイソシアン酸ベンジル (32 μ l, 0.128 $\times$ 2 mmol) を加え、0°Cで75分撹拌した。反応液をクロロホルムで抽出、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 37 mg, 0.070 mmol, 55%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.01 (1H, s), 7.39 (1H, d, $J = 10.7$ Hz), 7.21-7.20 (4H, m), 7.17-7.12 (1H, m), 6.87 (1H, d, $J = 10.7$ Hz), 6.54 (1H, br. s), 4.64-4.60 (1H, m), 4.59 (1H, d, $J = 15.1$ Hz), 4.25 (1H, d, $J = 15.1$ Hz), 3.99 (3H, s), 3.98 (3H, s), 3.85 (3H, s), 3.65 (3H, s), 3.21 (1H, dd, $J = 13.8, 5.2$ Hz), 2.37-2.28 (1H, m), 2.11-2.03 (1H, m), 1.80-1.72 (1H, m).

ESI-MS m/z : 525 ($[\text{M}+\text{H}]^+$).

[0172] 実施例 9 6

4-クロロ-N-(ジメチルカルバモイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解した。そこへトリエチルアミン (26 μ l, 0.128 $\times$ 1.5 mmol)、ジメチルカルバモイルクロリド (14 μ l, 0.128 $\times$ 1.2 mmol) を加え、加熱還流した。4時間後、トリエチルアミン (26 μ l, 0.128 $\times$ 1.5 mmol)、ジメチルカルバモイルクロリド (14 μ l, 0.128 $\times$ 1.2 mmol) を追加した。18時間後、さらにトリエチルアミン (26 μ l, 0.128 $\times$ 1.5 mmol)、ジメチルカルバモイルクロリド (14 μ l, 0.128 $\times$ 1.2 mmol) を追加した。20時間後、水を加え反応をクエンチした。混合物に飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で

WO 2011/021397

109

PCT/JP2010/005136

精製し、標記の化合物（淡黄色固体，58 mg，0.125 mmol，98%）を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.58 (1H, s), 7.29 (1H, d, $J = 10.7$ Hz), 6.83 (1H, d, $J = 11.0$ Hz), 5.03 (1H, d, $J = 5.9$ Hz), 4.51–4.45 (1H, m), 4.00 (3H, s), 3.98 (3H, s), 3.95 (3H, s), 3.64 (3H, s), 3.25 (1H, dd, $J = 13.1, 4.3$ Hz), 2.92 (6H, s), 2.31–2.22 (1H, m), 2.18–2.14 (1H, m), 1.77–1.74 (1H, m).

ESI-MS m/z : 463 $[\text{M}+\text{H}]^+$.

[0173] 実施例 97

4-クロロ-N-（ジエチルカルバモイル）デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン（301 mg，0.768 mmol）をジクロロエタン（6 mL）に溶解した。そこへトリエチルアミン（215 μl ， 0.768×2 mmol）、ジエチルカルバミン酸クロリド（146 μl ， 0.768×1.5 mmol）を加え、3時間加熱還流した。反応液を放冷し、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー（Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム）で精製し、標記の化合物（黄色固体，168 mg，0.342 mmol，44%）を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.60 (1H, s), 7.31 (1H, d, $J = 10.5$ Hz), 6.84 (1H, d, $J = 11.0$ Hz), 4.85 (1H, d, $J = 6.3$ Hz), 4.55–4.49 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.96 (3H, s), 3.64 (3H, s), 3.36–3.32 (2H, m), 3.28–3.19 (3H, m), 2.28–2.23 (1H, m), 2.17–2.14 (1H, m), 1.76–1.74 (1H, m), 1.14 (6H, t, $J = 7.1$ Hz).

ESI-MS m/z : 491 $[\text{M}+\text{H}]^+$.

[0174] 実施例 98

4-クロロ-N-（モルフォリン-4-カルボニル）デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン（50 mg，0.128 mmol）をジクロロメタン（2 mL）に溶解した。そこへトリエチルアミン（87 μl ， 0.128×5 mmol）、モルフォリン-4-カルボニルクロリド（73 μl ， $0.128 \times$

WO 2011/021397

110

PCT/JP2010/005136

5 mmol) を加え、5時間加熱還流した。反応液を放冷し、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 30 mg, 0.059 mmol, 46%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.61 (1H, s), 7.33 (1H, d, $J = 10.7$ Hz), 6.87 (1H, d, $J = 11.0$ Hz), 5.81 (1H, br. s), 4.55–4.49 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.96 (3H, s), 3.66 (4H, t, $J = 4.9$ Hz), 3.64 (3H, s), 3.41 (4H, t, $J = 4.5$ Hz), 3.24 (1H, dd, $J = 13.3, 5.2$ Hz), 2.32–2.23 (1H, m), 2.15–2.12 (1H, m), 1.82–1.79 (1H, m).

ESI-MS m/z : 505 $[\text{M}+\text{H}]^+$.

[0175] 実施例 99

4-クロロ-N- (ピロリジン-1-カルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解した。そこへトリエチルアミン (87 μl , 0.128×5 mmol)、ピロリジン-1-カルボニルクロリド (73 μl , 0.128×5 mmol) を加え、終夜加熱還流した。反応液を放冷し、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 23 mg, 0.047 mmol, 36%) を得た。

$^1\text{H-NMR}$ (400MHz, $\text{DMSO}-d_6$) δ [ppm]: 7.24 (1H, s), 7.12 (1H, d, $J = 10.5$ Hz), 7.02 (1H, d, $J = 10.7$ Hz), 6.73 (1H, d, $J = 7.3$ Hz), 4.24–4.18 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.52 (3H, s), 3.29–3.24 (2H, m), 3.21–3.15 (2H, m), 3.10 (1H, dd, $J = 13.5, 5.0$ Hz), 2.10–2.06 (1H, m), 1.97–1.91 (2H, m), 1.79–1.78 (4H, m).

ESI-MS m/z : 489 $[\text{M}+\text{H}]^+$.

[0176] 実施例 100

WO 2011/021397

111

PCT/JP2010/005136

4-クロロ-N- (ピペリジン-1-カルボニル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解した。そこへトリエチルアミン (87 μ l, 0.128 $\times$ 5 mmol)、ピペリジン-1-カルボニルクロリド (80 μ l, 0.128 $\times$ 5 mmol) を加え、終夜加熱還流した。反応液を放冷し、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 36 mg, 0.072 mmol, 56%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.18 (1H, s), 7.11 (1H, d, $J = 10.7$ Hz), 7.02 (1H, d, $J = 11.0$ Hz), 7.00 (1H, d, $J = 7.1$ Hz), 4.20–4.17 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.29–3.28 (4H, m), 3.10 (1H, dd, $J = 13.5, 4.3$ Hz), 2.09–2.06 (1H, m), 2.00–1.89 (2H, m), 1.56–1.50 (2H, m), 1.41–1.37 (4H, m).

ESI-MS m/z : 503 $[\text{M}+\text{H}]^+$.

[0177] 実施例 101

4-クロロ-N- (トリクロロアセチルカルバモイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (100 mg, 0.255 mmol) をジクロロメタン (5 mL) に溶解し、 0°C に冷却した。そこへイソシアン酸トリクロロアセチル (48 mL, 0.255 $\times$ 1.6 mmol) を加え、 0°C で20分撹拌した。反応液を濃縮し、得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (赤褐色固体, 19 mg, 0.033 mmol, 13%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.40 (1H, br. s), 8.30 (1H, d, $J = 6.3$ Hz), 7.42 (1H, s), 7.25 (1H, d, $J = 10.7$ Hz), 6.81 (1H, d, $J = 10.7$ Hz), 4.50–4.44 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.61 (3H, s), 3.31 (1H, dd, $J = 12.8, 4.8$ Hz), 2.37–2.19 (2H, m), 1.90–1.

WO 2011/021397

112

PCT/JP2010/005136

87 (1H, m).

ESI-MS m/z : 579 $[M+H]^+$.

[0178] 実施例 102

4-クロロ-N- (ジメチルアミノアセチル) デアセチルコルヒチンの合成

アルゴン雰囲気下、N,N-ジメチルグリシン (41 mg, 0.332 × 1.2 mmol) をN,N-ジメチルホルムアミド (2 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (76 mg, 0.332 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (54 mg, 0.332 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 107 mg, 0.224 mmol, 68%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.58 (1H, d, $J = 7.3$ Hz), 7.16 (1H, s), 7.12 (1H, d, $J = 10.7$ Hz), 7.03 (1H, d, $J = 10.7$ Hz), 4.31-4.28 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.10 (1H, dd, $J = 13.2, 5.6$ Hz), 2.89 (2H, d, $J = 8.3$ Hz), 2.21 (6H, s), 2.10-2.06 (1H, m), 1.93-1.92 (1H, m).

ESI-MS m/z : 477 $[M+H]^+$.

[0179] 実施例 103

4-クロロ-N- (ジメチルアミノアセチル) デアセチルコルヒチン塩酸塩の合成

氷冷下、メタノール (2 mL) にアセチルクロライド (24 mL, 0.224 × 1.5 mmol) を滴下して1時間撹拌した。そこへ4-クロロ-N-ジメチルアミノアセチルデアセチルコルヒチン (107 mg, 0.224 mmol) のメタノール溶液 (2 mL) を滴下し、0°Cで150分撹拌した。反応液を濃縮乾固し、真空乾燥して標記の化合物 (黄褐色固体, 106 mg, 0.206 mmol, 92%) を得た。

WO 2011/021397

113

PCT/JP2010/005136

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.71 (1H, br. s), 9.40 (1H, d, $J = 6.8$ Hz), 7.16 (1H, d, $J = 10.5$ Hz), 7.10 (1H, s), 7.05 (1H, d, $J = 10.7$ Hz), 4.33–4.27 (1H, m), 4.10 (1H, dd, $J = 15.4, 4.9$ Hz), 3.96–3.88 (1H, m), 3.92 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.14 (1H, dd, $J = 13.1, 5.0$ Hz), 2.75 (6H, s), 2.18–2.13 (1H, m), 2.08–1.99 (1H, m), 1.93–1.83 (1H, m).

[0180] 実施例 104

4-クロロ-N- (ジエチルアミノアセチル) デアセチルコルヒチンの合成

アルゴン雰囲気下、N,N-ジエチルグリシン (66.9 mg, 0.255 $\times$ 2 mmol) をN,N-ジメチルホルムアミド (1.5 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (98 mg, 0.255 $\times$ 2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (69 mg, 0.255 $\times$ 2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (100 mg, 0.255 mol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 51 mg, 0.101 mmol, 40%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.42 (1H, d, $J = 7.8$ Hz), 7.13 (1H, s), 7.12 (1H, d, $J = 10.7$ Hz), 7.02 (1H, d, $J = 10.7$ Hz), 4.33–4.26 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.32–3.30 (2H, m), 3.10 (1H, dd, $J = 13.1, 5.5$ Hz), 2.98 (2H, d, $J = 8.5$ Hz), 2.53 (2H, d, $J = 7.1$ Hz), 2.15–2.01 (2H, m), 1.94–1.89 (1H, m), 0.99 (6H, t, $J = 7.1$ Hz).

ESI-MS m/z : 505 $[\text{M}+\text{H}]^+$.

[0181] 実施例 105

N- (t-ブチルオキシカルボニルアミノアセチル)-4-クロロデアセチルコルヒ

チンの合成

アルゴン雰囲気下、N-(*t*-ブチルオキシカルボニル)グリシン (64 mg, 0.306 × 1.2 mmol) をN,N-ジメチルホルムアミド (1.5 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (70 mg, 0.306 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (50 mg, 0.306 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (120 mg, 0.306 mol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 153 mg, 0.279 mmol, 94%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.61 (1H, d, J = 7.3 Hz), 7.13 (1H, d, J = 10.7 Hz), 7.10 (1H, s), 7.03 (1H, d, J = 10.7 Hz), 6.89 (1H, t, J = 6.0 Hz), 4.25-4.24 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.63 (1H, dd, J = 16.8, 6.3 Hz), 3.56-3.54 (1H, m), 3.54 (3H, s), 3.11 (1H, dd, J = 12.9, 5.4 Hz), 2.13-2.09 (1H, m), 1.96-1.89 (1H, m), 1.35 (9H, s).

ESI-MS m/z: 549 [M+H]⁺.

[0182] 実施例 106

4-クロロ-N-(アミノアセチル)デアセチルコルヒチン トリフルオロ酢酸塩の合成

アルゴン雰囲気下、N-(*t*-ブチルオキシカルボニルアミノアセチル)-4-クロロデアセチルコルヒチン (50 mg, 0.091 mmol) をジクロロメタン (750 μl) に溶解し、0°Cに冷却した。そこへトリフルオロ酢酸 (291 μl, 3.2 mL/mm ol) を加え、室温に戻して1時間撹拌した。反応液を濃縮乾固し、真空乾燥して、標記の化合物 (褐色固体, 88 mg, 定量的) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 9.10 (1H, d, J = 7.1 Hz), 7.95-7.92

WO 2011/021397

115

PCT/JP2010/005136

(3H, m), 7.16 (1H, d, $J = 10.5$ Hz), 7.11 (1H, s), 7.05 (1H, d, $J = 11.0$ Hz), 4.26–4.22 (1H, m), 3.92 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.69–3.61 (2H, m), 3.53 (3H, s), 3.14 (1H, dd, $J = 13.3, 5.0$ Hz), 2.17–2.13 (1H, m), 2.05–1.95 (1H, m), 1.88–1.83 (1H, m).

ESI-MS m/z : 449 $[M+H]^+$.

[0183] 実施例 107

4-クロロ-N- (ピロリジン-1-イルアセチル) デアセチルコルヒチンの合成

アルゴン雰囲気下、ピロリジン-1-イル酢酸 (15.8 mg, 0.128×1.2 mmol) をN,N-ジメチルホルムアミド (1.5 mL) に溶解し、 0°C に冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水合物 (21 mg, 0.128×1.2 mmol) を加え、 0°C で30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 20 mg, 0.040 mmol, 31%) を得た。

$^1\text{H-NMR}$ (400MHz, $\text{DMSO}-d_6$) δ [ppm]: 8.52 (1H, d, $J = 7.8$ Hz), 7.15 (1H, s), 7.12 (1H, d, $J = 10.5$ Hz), 7.02 (1H, d, $J = 10.7$ Hz), 4.29–4.26 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.35–3.31 (1H, m), 3.10 (1H, dd, $J = 13.7, 6.3$ Hz), 3.07 (2H, s), 2.51–2.49 (3H, m), 2.11–2.03 (2H, m), 1.93–1.91 (1H, m), 1.71 (4H, br. s).

ESI-MS m/z : 503 $[M+H]^+$.

HPLC測定条件

[0184] 実施例 108

4-クロロ-N- (ピペリジン-1-イルアセチル) デアセチルコルヒチンの合成

アルゴン雰囲気下、ピペリジン-1-イル酢酸 (15.8 mg, 0.128×1.2 mmol

WO 2011/021397

116

PCT/JP2010/005136

）をN,N-ジメチルホルムアミド（1.5 mL）に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩（29 mg, 0.128 × 1.2 mmol）、1-ヒドロキシベンゾトリアゾール水和物（21 mg, 0.128 × 1.2 mmol）を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン（50 mg, 0.128 mol）を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー（Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム）で精製し、標記の化合物（白色固体, 48 mg, 0.093 mmol, 73%）を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.45 (1H, br. s), 7.14 (1H, s), 7.12 (1H, d, J = 10.5 Hz), 7.02 (1H, d, J = 10.7 Hz), 4.32-4.27 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.38 (1H, dd, J = 13.7, 6.6 Hz), 3.11 (1H, dd, J = 13.5, 5.2 Hz), 2.90 (2H, br. s), 2.37 (3H, br. s), 2.13-2.07 (1H, m), 2.01-1.91 (2H, m), 1.55-1.51 (4H, m), 1.38-1.37 (2H, m).

ESI-MS m/z: 517 [M+H]⁺.

[0185] 実施例 109

4-クロロ-N-(3-カルボキシプロピオニル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン（80 mg, 0.204 mmol）をジメチルスルホキシド（510 μl）に溶解した。そこへN-メチルモルフォリン（88 μl, 0.204 × 5 mmol）、こはく酸無水物（24 mg, 0.204 × 1.1 mmol）を加え、室温で1時間撹拌した。反応液に酢酸エチルを加え、飽和クエン酸水溶液、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー（Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム）で精製し、標記の化合物（淡黄色固体, 60 mg, 0.122 mmol, 60%）を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.75 (1H, d, J = 7.1 Hz), 7.16 (1H,

WO 2011/021397

117

PCT/JP2010/005136

s), 7.13 (1H, d, $J = 10.5$ Hz), 7.03 (1H, d, $J = 11.0$ Hz), 4.25–4.19 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.10 (1H, dd, $J = 13.4, 5.4$ Hz), 2.35 (2H, t, $J = 6.5$ Hz), 2.26 (2H, t, $J = 6.6$ Hz), 2.12–2.08 (1H, m), 1.97–1.92 (1H, m), 1.87–1.78 (1H, m).
ESI-MS m/z : 492 $[M+H]^+$.

[0186] 実施例 1 1 0

4-クロロ-N- [3-(メトキシカルボニル)プロピオニル] デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロ-N-(4-カルボキシプロピオニル)-デアセチルコルヒチン (56 mg, 0.114 mmol) をトルエン (2.3 mL) に溶解した。そこへメタノール (570 μ l, 5ml/mmol)、トリメチルシリルジアゾメタン (949 μ l, 0.096×5 mmol, 0.6 M ヘキサン溶液) を加え、室温で1時間撹拌した。反応液に水を加え、クロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isorela One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄褐色固体, 34 mg, 0.067 mmol, 59%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.52 (1H, s), 7.31 (1H, d, $J = 10.7$ Hz), 6.86 (1H, d, $J = 10.7$ Hz), 6.68 (1H, d, $J = 7.8$ Hz), 4.59–4.53 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.69 (3H, s), 3.61 (3H, s), 3.26 (1H, dd, $J = 12.7, 4.6$ Hz), 2.69–2.52 (4H, m), 2.30–2.11 (2H, m), 1.85–1.78 (1H, m).

ESI-MS m/z : 506 $[M+H]^+$.

[0187] 実施例 1 1 1

4-クロロ-N- (4-カルボキシブチリル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (100 mg, 0.255 mmol) をジメチルスルホキシド (638 μ l) に溶解した。そこへN-メチルモルフォリン (110 μ l, 0.225×5 mmol)、グルタル酸無水物 (32 mg, 0.255×1.1

WO 2011/021397

118

PCT/JP2010/005136

mmol) を加え、室温で40分撹拌した。反応液に酢酸エチルを加え、飽和クエン酸水溶液、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 25 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 112 mg, 0.241 mmol, 95%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.87 (1H, s), 7.77 (1H, br. s), 7.44 (1H, d, $J = 10.7$ Hz), 7.02 (1H, d, $J = 10.5$ Hz), 4.58–4.52 (1H, m), 4.04 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.60 (3H, s), 3.27 (1H, dd, $J = 13.1, 4.8$ Hz), 2.53–2.34 (6H, m), 2.26–2.23 (1H, m), 2.18–2.10 (1H, m), 2.01–2.00 (1H, m).

ESI-MS m/z : 506 $[\text{M}+\text{H}]^+$.

[0188] 実施例 1 1 2

4-クロロ-N- [4-(メトキシカルボニル) ブチリル] デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロ-N-(1-カルボキシブチリル)-デアセチルコルヒチン (87 mg, 0.172 mmol) をトルエン (3.4 mL) に溶解した。そこへメタノール (860 μl , 5 mL/mmol)、トリメチルシリルジメタノール (1.43 mL, 0.172 $\times$ 5 mmol, 0.6 M ヘキサン溶液) を加え、室温で20分撹拌した。反応液に水を加え、クロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 67 mg, 0.129 mmol, 75%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.54 (1H, s), 7.33 (1H, d, $J = 10.7$ Hz), 6.88 (1H, d, $J = 10.7$ Hz), 6.55 (1H, d, $J = 6.6$ Hz), 4.59–4.53 (1H, m), 4.02 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.67 (3H, s), 3.62 (3H, s), 3.26 (1H, dd, $J = 13.3, 4.3$ Hz), 2.40–2.27 (4H, m), 2.25–2.11 (2H, m), 1.95–1.88 (3H, m).

WO 2011/021397

119

PCT/JP2010/005136

ESI-MS m/z : 520 $[M+H]^+$.

[0189] 実施例 1 1 3

N-(trans-1-カルボキシシクロヘキサン-4-イルオキシカルボニル)-4-クロロ
デアセチルコルヒチンの合成

N-[trans-1-(ベンジルオキシカルボニル)シクロヘキサン-4-イルオキシカル
ボニル]-4-クロロデアセチルコルヒチン(66 mg, 0.101 mmol)の酢酸エチル
(2 mL)溶液に氷冷、アルゴンガス雰囲気下にて10%パラジウム-炭素(7 mg)を
添加した。本反応液を室温、水素ガス雰囲気下にて一晩撹拌した後、不溶物
をろ去、次いで酢酸エチルにて洗いこんだ。ろ液と洗浄液は合わせて濃縮乾
固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One,
クロロホルム/メタノール)にて精製し、標記の化合物(乳白色固体, 20 mg,
0.0354 mmol, 35%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.50 (1H, s), 7.25 (1H, d, $J=6.81$ (1H, d,
 , $J=10.7$ Hz), 5.10 (1H, d, $J=7.6$ Hz), 4.51-4.45 (1H, m), 4.36-4.30 (1
H, m), 4.00 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.59 (3H, s), 3.25 (1
H, dd, $J=13.3, 4.3$ Hz), 2.34-2.12 (3H, m), 2.08-1.96 (4H, m), 1.71-1.
.64 (1H, m), 1.56-1.46 (2H, m), 1.43-1.28 (2H, m).

ESI-MS m/z : 562 $[M+H]^+$.

[0190] 実施例 1 1 4

4-クロロ-N-(ピリジン-3-イルオキシカルボニル)デアセチルコルヒチンの合
成

3-ヒドロキシピリジン(50 mg, 0.526 mmol)のジクロロメタン溶液(1.1 mL)
に氷冷、アルゴンガス雰囲気下にてピリジン(55 μL , 0.526×1.3 mmol)及び
トリホスゲン(156 mg, 0.526 mmol)を添加して、氷冷下、10分間、次いで室
温にて3時間撹拌した。反応液を減圧下、濃縮乾固して得た残留物をジクロロ
メタン(2 mL)に溶かし、ピリジン(18.6 μL , 0.0766×3 mmol)及び4-クロロデ
アセチルコルヒチン(30 mg, 0.0766 mmol)を添加した。本混合液を室温で一
晩撹拌した後、クロロホルムと飽和重曹水を添加した。有機層を取り、水層

WO 2011/021397

120

PCT/JP2010/005136

はさらにクロロホルムにて抽出した。有機層は合わせて無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(乳白色固体, 32 mg, 0.0629 mmol, 82%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.34 (2H, br-s), 7.58 (1H, s), 7.48 (1H, d, $J=7.3\text{ Hz}$), 7.26 (2H, br-s), 7.21 (2H, d, $J=10.7\text{ Hz}$), 6.78 (1H, d, $J=10.7\text{ Hz}$), 6.57 (1H, d, $J=6.6\text{ Hz}$), 4.38-4.32 (1H, m), 3.95 (3H, s), 3.91 (3H, s), 3.91 (3H, s), 3.49 (3H, s), 3.24 (1H, dd, $J=13.8, 5.5\text{ Hz}$), 2.37-2.29 (1H, m), 2.19-2.11 (1H, m), 1.89-1.81 (1H, m).

ESI-MS m/z : 513 $[\text{M}+\text{H}]^+$.

[0191] 参考例 1 9

クロロギ酸-3-(ジメチルアミノ)プロピル塩酸塩の合成

3-(ジメチルアミノ)プロピル(500 μL , 4.27 mmol)のアセトニトリル(9 mL)溶液に氷冷、アルゴンガス雰囲気下にてトリホスゲン(1.27 g, 4.27 mmol)を添加した。本反応液を徐々に室温に戻しながら、一晩撹拌した後、エーテルとヘキサンを加えた。析出物をろ取、エーテル次いでヘキサンで洗浄した後、減圧乾燥して標記の化合物(白色固体, 0.649 g, 3.21 mmol, 75%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 10.82 (1H, s), 4.17 (2H, t, $J=6.1\text{ Hz}$), 3.12-3.07 (2H, m), 2.73 (3H, s), 2.72 (3H, s), 2.08-2.01 (2H, m).

[0192] 実施例 1 1 5

4-クロロ-N-[1-(ジメチルアミノ)プロピル-3-イルオキシカルボニル]デアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン(30 mg, 0.0766 mmol)のジクロロメタン(2 mL)溶液に、クロロギ酸-3-(ジメチルアミノ)プロピル塩酸塩(100 mg, 0.0766 $\times$ 6.5 mmol)及びピリジン(211 μL , 0.0766 $\times$ 16 mmol)を添加して、室温で一晩撹拌した。反応液に酢酸エチル及び飽和重曹水を加えて有機層を取った。有機層は、飽和重曹水次いでブラインにて洗浄、無水硫酸ナトリウムにて乾

WO 2011/021397

121

PCT/JP2010/005136

燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(乳白色固体, 13 mg, 0.0249 mmol, 33%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.48 (1H, s), 7.22 (1H, d, $J=10.7$ Hz), 6.81 (1H, d, $J=10.7$ Hz), 6.60 (1H, br-s), 4.30–4.22 (2H, m), 4.02–3.95 (1H, m), 4.00 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.58 (3H, s), 3.26 (1H, dd, $J=13.7, 4.9$ Hz), 3.17–3.10 (2H, m), 2.89 (6H, s), 2.34–2.13 (4H, m), 1.90–1.82 (1H, m).

ESI-MS m/z : 521 $[\text{M}+\text{H}]^+$.

[0193] 実施例 116

4-クロロ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチンの合成

3-(ピロリジン-1-イル)安息香酸(22 mg, 0.0766×1.5 mmol)のジメチルホルムアミド(1.5 mL)溶液に室温、アルゴンガス雰囲気下にて1-エチル-3-(3-ジメチルアミノプロピル)カルボジイミド塩酸塩(22 mg, 0.0766×1.5 mmol)次いでヒドロキシベンゾトリアゾール-水合物(18 mg, 0.0766×1.5 mmol)を添加した。本溶液を30分間攪拌した後、4-クロロデアセチルコルヒチン(30 mg, 0.0766 mmol)を添加し、室温で一晩攪拌した。酢酸エチルと飽和重曹水を加えた後、有機層を取った。有機層を飽和重曹水次いでブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(淡黄色固体, 18 mg, 0.0321 mmol, 42%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.48 (1H, s), 7.28 (1H, d, $J=10.7$ Hz), 7.22 (1H, t, $J=8.2$ Hz), 7.05–7.01 (2H, br-m), 6.93–6.86 (1H, br-m), 6.81 (1H, d, $J=10.7$ Hz), 6.78–6.73 (1H, br-m), 4.77–4.71 (1H, m), 4.00 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.70 (3H, s), 3.32–3.27 (5H, m), 2.38–2.19 (2H, m), 2.01–1.97 (4H, m), 1.94–1.86 (1H, m).

WO 2011/021397

122

PCT/JP2010/005136

ESI-MS m/z : $[M+H]^+$.

[0194] 実施例 1 1 7

4-クロロ-N-[4-(4-モルホリニルメチル)ベンゾイル]デアセチルコルヒチンの合成

4-クロロ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(淡褐色固体, 41 mg, 0.0683 mmol, 89%)を得た。3-(ピロリジン-1-イル)安息香酸の代わりに4-(4-モルホリニルメチル)安息香酸を用いた。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.75 (2H, d, $J=7.8$ Hz), 7.50 (1H, s), 7.36 (2H, d, $J=7.8$ Hz), 7.30 (1H, d, $J=10.7$ Hz), 6.83 (1H, d, $J=10.7$ Hz), 4.79–4.73 (1H, m), 4.01 (3H, s), 4.00 (3H, s), 3.98 (3H, s), 3.74 (4H, t, $J=4.3$ Hz), 3.71 (3H, s), 3.56 (2H, s), 3.30 (1H, dd, $J=13.2$, 4.9 Hz), 2.49 (4H, s), 2.39–2.19 (2H, m), 2.00–1.92 (1H, m).

ESI-MS m/z : 595 $[M+H]^+$.

[0195] 実施例 1 1 8

4-クロロ-N-(ジエチルチオカルバモイル)デアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン(30 mg, 0.0766 mmol)のジクロロメタン(1.5 mL)溶液に、氷冷、アルゴンガス雰囲気下にて、チオホスゲン(6.12 μL , 0.0766 $\times$ 1.05 mmol)及びトリエチルアミン(25.6 μL , 0.0766 $\times$ 2.4 mmol)を添加して、同温度で2時間攪拌した。ジエチルアミン(15.8 μL , 0.0766 $\times$ 2 mmol)を添加した後、室温に徐々に戻しながら一晩攪拌した。反応液に酢酸エチル及び10%クエン酸を添加して、有機層を取った。有機層を飽和重曹水次いでブラインにて洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(乳白色固体, 33 mg, 0.0648mmol, 85%)を得た。

$^1\text{H-NMR}$ (400MHz, $\text{DMSO}-d_6$) δ [ppm]: 7.54 (1H, d, $J=6.8\text{Hz}$), 7.15 (1H, d, $J=11.0$ Hz), 7.08 (1H, s), 7.03 (1H, d, $J=11.0$ Hz), 5.02–4.95 (1H, m), 3

WO 2011/021397

123

PCT/JP2010/005136

. 91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.72–3.64 (4H, m), 3.62 (3H, s), 3.12 (1H, dd, J=13.5, 5.5 Hz), 2.33–2.24 (1H, m), 2.15–2.07 (1H, m), 2.02–1.95 (1H, m), 1.09 (6H, t, J=7.0 Hz).

ESI-MS m/z: 507 [M+H]⁺.

[0196] 実施例 1 1 9

4-クロロ-N-(ピロリジン-1-チオカルボニル) デアセチルコルヒチンの合成

4-クロロ-N-(ジエチルチオカルバモイル) デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(淡黄色固体, 28 mg, 0.0550 mmol, 72%)を得た。ジエチルアミンの代わりにピロリジンを用いた。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 7.55 (1H, d, J=7.3 Hz), 7.15 (1H, d, J=10.7 Hz), 7.12 (1H, s), 7.03 (1H, d, J=10.7 Hz), 4.94–4.87 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.61 (3H, s), 3.58–3.45 (4H, m), 3.12 (1H, dd, J=13.4, 5.1 Hz), 2.25–1.84 (7H, m).

ESI-MS m/z: 505 [M+H]⁺.

[0197] 実施例 1 2 0

4-クロロ-N-(ピペリジン-1-チオカルボニル) デアセチルコルヒチンの合成

4-クロロ-N-(ジエチルチオカルバモイル) デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 28 mg, 0.0536 mmol, 70%)を得た。ジエチルアミンの代わりにピペリジンを用いた。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 7.85 (1H, d, J=7.1 Hz), 7.15 (1H, d, J=10.7 Hz), 7.06 (1H, s), 7.03 (1H, d, J=10.7 Hz), 4.96–4.90 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.86–3.76 (4H, m), 3.63 (3H, s), 3.12 (1H, dd, J=13.1, 5.2 Hz), 2.23–1.96 (3H, m), 1.64–1.58 (2H, m), 1.50–1.44 (4H, m).

ESI-MS m/z: 519 [M+H]⁺.

[0198] 実施例 1 2 1

4-クロロ-N-(モルホリン-4-チオカルボニル) デアセチルコルヒチンの合成

4-クロロ-N-(ジエチルチオカルバモイル) デアセチルコルヒチンの合成と同

WO 2011/021397

124

PCT/JP2010/005136

様の方法にて、標記の化合物(乳白色固体, 28 mg, 0.0536 mmol, 70%)を得た。
。ジエチルアミンの代わりにモルホリンを用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.03 (1H, d, $J=7.1$ Hz), 7.16 (1H, d, $J=10.7$ Hz), 7.05 (1H, s), 7.03 (1H, d, $J=10.7$ Hz), 4.92–4.86 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.87–3.75 (4H, m), 3.62 (3H, s), 3.59 (4H, t, $J=4.8$ Hz), 3.12 (1H, dd, $J=12.2, 5.1$ Hz), 2.21–1.99 (3H, m).

ESI-MS m/z : 521 $[\text{M}+\text{H}]^+$.

[0199] 実施例 1 2 2

4-クロロ-N-(チオモルホリン-4-チオカルボニル)デアセチルコルヒチンの合成

4-クロロ-N-(ジエチルチオカルバモイル)デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 27 mg, 0.0504 mmol, 66%)を得た。
。ジエチルアミンの代わりにチオモルホリンを用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.97 (1H, d, $J=6.8$ Hz), 7.16 (1H, d, $J=10.7$ Hz), 7.04 (1H, s), 7.03 (1H, d, $J=10.7$ Hz), 4.95–4.89 (1H, m), 4.21–4.09 (4H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.63 (3H, s), 3.13 (1H, dd, $J=12.4, 4.9$ Hz), 2.60 (4H, t, $J=4.9$ Hz), 2.23–2.00 (3H, m).

ESI-MS m/z : 537 $[\text{M}+\text{H}]^+$.

[0200] 実施例 1 2 3

4-クロロ-N-(1,1-ジオキソチオモルホリン-4-チオカルボニル)デアセチルコルヒチンの合成

4-クロロ-N-(ジエチルチオカルバモイル)デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 30 mg, 0.0522 mmol, 68%)を得た。
。ジエチルアミンの代わりにチオモルホリン 1,1-ジオキシドを用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.38 (1H, d, $J=6.6$ Hz), 7.17 (1H, d, $J=10.7$ Hz), 7.04 (1H, s), 7.04 (1H, d, $J=10.7$ Hz), 4.83–4.77 (1H, m),

WO 2011/021397

125

PCT/JP2010/005136

4.38–4.31 (2H, m), 4.25–4.18 (2H, m), 3.90 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.64 (3H, s), 3.28–3.21 (2H, m), 3.15–3.08 (2H, m), 2.17–2.08 (3H, m).

ESI-MS m/z : 569 $[M+H]^+$.

[0201] 実施例 1 2 4

4-クロロ-N-(1-メチルピペラジン-4-チオカルボニル) デアセチルコルヒチンの合成

4-クロロ-N-(ジエチルチオカルバモイル) デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 27 mg, 0.0504 mmol, 66%)を得た。ジエチルアミンの代わりに1-メチルピペラジンを用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.98 (1H, d, $J=6.8$ Hz), 7.15 (1H, d, $J=10.7$ Hz), 7.05 (1H, s), 7.03 (1H, d, $J=10.7$ Hz), 4.93–4.87 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.88–3.77 (4H, m), 3.62 (3H, s), 3.12 (1H, dd, $J=12.6, 4.8$ Hz), 2.31 (4H, t, $J=4.8$ Hz), 2.21–1.97 (3H, m), 2.18 (3H, s).

ESI-MS m/z : 534 $[M+H]^+$.

[0202] 実施例 1 2 5

(S)-4-クロロ-N-(2-アセトキシプロピオニル) デアセチルコルヒチンの合成

4-クロロデアセチルコルヒチン(100 mg, 0.255 mmol)のジクロロメタン(5 mL)に、氷冷、アルゴンガス雰囲気下にて、(S)-2-アセトキシプロピオン酸クロリド(35.5 μL , 0.255×1.1 mmol)及びトリエチルアミン(39.1 μL , 0.255×1.1 mmol)を添加して、徐々に室温に戻しながら一晩攪拌した。反応液をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(乳白色固体, 123 mg, 0.243 mmol, 95%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.80 (1H, d, $J=7.3$ Hz), 7.14 (1H, d, $J=10.7$ Hz), 7.06 (1H, s), 7.03 (1H, d, $J=10.7$ Hz), 4.93 (1H, q, $J=6.7$ Hz), 4.27–4.21 (1H, m), 3.91 (3H, s), 3.89 (6H, s), 3.53 (3H, s), 3.1

WO 2011/021397

126

PCT/JP2010/005136

3 (1H, dd, $J=12.3, 3.8$ Hz), 2.16–2.08 (1H, m), 2.02 (3H, s), 1.99–1.90 (2H, m), 1.33 (3H, d, $J=6.7$ Hz).

ESI-MS m/z : 506 $[M+H]^+$.

[0203] 実施例 1 2 6

(S)-4-クロロ-N-(2-ヒドロキシプロピオニル)デアセチルコルヒチンの合成

(S)-4-クロロ-N-(2-アセトキシプロピオニル)デアセチルコルヒチン (123 mg, 0.250 mmol) のメタノール (2.5 mL) 溶液に室温で、ナトリウムメトキシド (3mg, 0.250×0.2 mmol) を加えて、室温で1時間攪拌した。反応液にクロロホルム及びブラインを加えて有機層を取った。有機層は無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法 (装置: Biotage社 Isolera One, クロロホルム/メタノール) にて精製し、標記の化合物 (乳白色固体, 107 mg, 0.231 mmol, 0.231 mmol) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.50 (1H, d, $J=7.6$ Hz), 7.16 (1H, s), 7.12 (1H, d, $J=10.7$ Hz), 7.02 (1H, d, $J=10.7$ Hz), 5.58 (1H, d, $J=5.4$ Hz), 4.29–4.22 (1H, m), 4.02 (1H, m), 3.91 (3H, s), 3.88 (6H, s), 3.53 (3H,), 3.10 (1H, dd, $J=12.4, 6.1$ Hz), 2.14–2.06 (2H, m), 1.93–1.84 (1H, m), 1.17 (3H, d, $J=6.8$ Hz).

ESI-MS m/z : 464 $[M+H]^+$.

[0204] 実施例 1 2 7

(R)-4-クロロ-N-(2-アセトキシプロピオニル)デアセチルコルヒチンの合成

(S)-4-クロロ-N-(2-ヒドロキシプロピオニル)デアセチルコルヒチン (100 mg, 0.216mmol) のトルエン (4.3 mL) 溶液に、氷冷、アルゴンガス雰囲気下にて酢酸 (14.8 μL , 0.216×1.2 mmol) 及びトリフェニルホスフィン (170 mg, 0.216×3 mmol) を添加し、次いでアゾジカルボン酸ジエチル (2.2 mol/L in toluene, 295 μL , 0.216×3 mmol) を滴下した後、徐々に室温に戻しながら一晩攪拌した。反応液をフラッシュクロマトグラフ法にて精製し、標記の化合物 (131 mg) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.76 (1H, d, $J=7.3$ Hz), 7.17 (1H, s),

WO 2011/021397

127

PCT/JP2010/005136

7.12 (1H, d, J=10.7 Hz), 7.02 (1H, d, J=10.7 Hz), 4.93 (1H, q, J=6.8 Hz), 4.22-4.15 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.14-3.08 (1H, m), 2.15-2.07 (1H, m), 2.03 (3H, s), 1.96-1.88 (2H, m), 1.29 (3H, d, J=6.8 Hz).

ESI-MS m/z: 506 [M+H]⁺.

[0205] 実施例 1 2 8

(R)-4-クロロ-N-(2-ヒドロキシプロピオニル)デアセチルコルヒチンの合成

(S)-4-クロロ-N-(2-ヒドロキシプロピオニル)デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 42 mg, 0.0905 mmol, 2工程64%)を得た。(S)-4-クロロ-N-(2-アセトキシプロピオニル)デアセチルコルヒチンの代わりに(R)-4-クロロ-N-(2-アセトキシプロピオニル)デアセチルコルヒチンを用いた。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 8.48 (1H, d, J=7.6 Hz), 7.15 (1H, s), 7.12 (1H, d, J=10.7 Hz), 7.02 (1H, d, J=10.7 Hz), 5.52 (1H, d, J=5.1 Hz), 4.27-4.20 (1H, m), 4.00-3.93 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.10 (1H, dd, J=12.7, 5.9 Hz), 2.15-2.04 (2H, m), 1.95-1.85 (1H, m), 1.17 (3H, d, J=6.6 Hz).

ESI-MS m/z: 464 [M+H]⁺.

[0206] 実施例 1 2 9

4-クロロ-N-(2-ヒドロキシ-2-メチルプロピオニル)デアセチルコルヒチンの合成

4-クロロ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 37 mg, 0.0766 mmol, 定量的)を得た。3-(ピロリジン-1-イル)安息香酸の代わりに2-ヒドロキシ-2-メチルプロピオン酸を用いた。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 8.37 (1H, d, J=7.3 Hz), 7.17 (1H, s), 7.12 (1H, d, J=10.7 Hz), 7.02 (1H, d, J=10.7 Hz), 5.49 (1H, s), 4.24-4.17 (1H, m), 3.92 (3H, s), 3.88 (6H, s), 3.54 (3H, s), 3.10 (1H, dd,

WO 2011/021397

128

PCT/JP2010/005136

$J=12.2, 5.4$ Hz), 2.18–2.06 (2H, m), 1.94–1.85 (1H, m), 1.21 (3H, s), 1.20 (3H, s).

ESI-MS m/z : 478 $[M+H]^+$.

[0207] 実施例 130

4-クロロ-N-(3-ヒドロキシ-2,2-ジメチルプロピオニル)デアセチルコルヒチンの合成

4-クロロ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 39 mg, 0.0766 mmol, 定量的)を得た。3-(ピロリジン-1-イル)安息香酸の代わりに3-ヒドロキシ-2,2-ジメチルプロピオン酸を用いた。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.65 (1H, br-s), 7.41 (1H, d, $J=11.0$ Hz), 7.32 (1H, br-s), 6.95 (1H, d, $J=11.0$ Hz), 4.58–4.51 (1H, m), 4.04 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.70 (1H, d, $J=11.0$ Hz), 3.64 (3H, s), 3.57 (1H, d, $J=11.0$ Hz), 2.31–2.09 (2H, m), 1.87–1.79 (1H, m), 1.22 (3H, s), 1.14 (3H, s).

ESI-MS m/z : 492 $[M+H]^+$.

[0208] 実施例 131

4-クロロ-N-[2,2-ビス(ヒドロキシメチル)プロピオニル]デアセチルコルヒチンの合成

4-クロロ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 30 mg, 0.0589 mmol, 77%)を得た。3-(ピロリジン-1-イル)安息香酸の代わりに2,2-ビス(ヒドロキシメチル)プロピオン酸を用いた。

$^1\text{H-NMR}$ (400MHz, $\text{DMSO}-d_6$) δ [ppm]: 8.03 (1H, d, $J=7.1$ Hz), 7.15 (1H, s), 7.11 (1H, d, $J=10.7$ Hz), 7.01 (1H, d, $J=10.7$ Hz), 4.72 (1H, t, $J=5.2$ Hz), 4.62 (1H, t, $J=5.2$ Hz), 4.28–4.21 (1H, m), 3.91 (3H, s), 3.88 (6H, s), 3.55 (3H, s), 3.48 (2H, d, $J=5.4$ Hz), 3.43 (2H, dd, $J=5.1, 2.2$ Hz), 3.10 (1H, dd, $J=13.4, 4.9$ Hz), 2.15–2.06 (1H, m), 2.02–1.91 (2

WO 2011/021397

129

PCT/JP2010/005136

H, m), 1.00 (3H, s).

ESI-MS m/z : 508 $[M+H]^+$.

[0209] 実施例 1 3 2

4-クロロ-N-(1-アセチルピペリジン-4-カルボニル) デアセチルコルヒチンの合成

4-クロロ-N-[3-(ピロリジン-1-イル) ベンゾイル] デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 27 mg, 0.0492 mmol, 64%)を得た。3-(ピロリジン-1-イル)安息香酸の代わりに1-アセチルピペリジン-4-カルボン酸を用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.61–8.58 (1H, m), 7.12 (1H, d, $J=10.7$ Hz), 7.10 (1H, d, $J=2.4$ Hz), 7.02 (1H, d, $J=10.7$ Hz), 4.35–4.29 (1H, m), 4.24–4.18 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.85–3.75 (1H, m), 3.53 (3H, s), 3.11 (1H, dd, $J=13.4, 5.4$ Hz), 3.07–2.98 (1H, m), 2.59–2.43 (2H, m), 2.15–2.07 (1H, m), 2.01–1.81 (2H, m), 1.96 (3H, d, $J=1.0$ Hz), 1.75–1.59 (2H, m), 1.48–1.27 (2H, m).

ESI-MS m/z : 545 $[M+H]^+$.

[0210] 実施例 1 3 3

4-クロロ-N-(1-ベンゾイルピペリジン-4-カルボニル) デアセチルコルヒチンの合成

4-クロロ-N-[3-(ピロリジン-1-イル) ベンゾイル] デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 42 mg, 0.0684 mmol, 89%)を得た。3-(ピロリジン-1-イル)安息香酸の代わりに1-ベンゾイルピペリジン-4-カルボン酸を用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.63 (1H, d, $J=7.1$ Hz), 7.45–7.32 (5H, m), 7.13 (1H, d, $J=10.7$ Hz), 7.10 (1H, s), 7.03 (1H, d, $J=10.7$ Hz), 4.50–4.37 (1H, m), 4.25–4.18 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.66–3.51 (1H, m), 3.54 (3H, s), 3.14–2.74 (2H, m), 3.11 (1H, dd, $J=13.2, 5.4$ Hz), 2.57–2.51 (1H, m), 2.15–2.07 (1H, m), 2.01–1

WO 2011/021397

130

PCT/JP2010/005136

.36 (6H, m).

ESI-MS m/z : 607 $[M+H]^+$.

[0211] 実施例 1 3 4

4-クロロ-N-[1-(tert-ブチルオキシカルボニル)ピペリジン-4-カルボニル]デアセチルコルヒチンの合成

4-クロロ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(淡黄色固体, 48 mg, 0.0798 mmol, 62%)を得た。3-(ピロリジン-1-イル)安息香酸の代わりに1-(tert-ブチルオキシカルボニル)ピペリジン-4-カルボン酸を用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.60 (1H, d, $J=7.3$ Hz), 7.13 (1H, d, $J=10.7$ Hz), 7.09 (1H, s), 7.03 (1H, d, $J=10.7$ Hz), 4.23-4.17 (1H, m), 3.96-3.86 (2H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.10 (1H, dd, $J=12.9, 5.4$ Hz), 2.79-2.67 (1H, m), 2.53-2.48 (1H, m), 2.43-2.35 (1H, m), 2.15-2.07 (1H, m), 2.00-1.80 (2H, m), 1.64 (2H, dd, $J=32.4, 11.2$), 1.40-1.24 (2H, m), 1.37 (9H, s).

ESI-MS m/z : 603 $[M+H]^+$.

[0212] 実施例 1 3 5

4-クロロ-N-(ピペリジン-4-カルボニル)デアセチルコルヒチン トリフルオロ酢酸塩の合成

4-クロロ-N-[1-(tert-ブチルオキシカルボニル)ピペリジン-4-カルボニル]デアセチルコルヒチン(30 mg, 0.0797 mmol)のジクロロメタン(1 mL)溶液に、氷冷下、トリフルオロ酢酸(400 μL)を添加して、氷冷下にて5分間、次いで室温で2時間攪拌した。反応液を濃縮乾固した後、残留物にエーテルとヘキサンを加えて超音波処理した。析出物をろ取、ヘキサンによる洗浄、次いで減圧乾燥し、標記の化合物(乳白色固体, 27 mg, 0.0433 mmol, 87%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.71 (1H, d, $J=7.3$ Hz), 8.47 (1H, br-s), 8.19 (1H, br-s), 7.14 (1H, d, $J=10.7$ Hz), 7.08 (1H, s), 7.04 (1H, d, $J=10.7$ Hz), 4.25-4.18 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3

WO 2011/021397

131

PCT/JP2010/005136

H, s), 3.55 (3H, s), 3.28 (2H, t, J=12.1 Hz), 3.11 (1H, dd, J=13.4, 4.9 Hz), 2.96–2.83 (2H, m), 2.56–2.48 (1H, m), 2.16–2.07 (1H, m), 2.01–1.77 (4H, m), 1.67–1.57 (2H, m).

ESI-MS m/z: 503 [M+H]⁺.

[0213] 実施例 136

4-クロロ-N-[3-(ジメチルアミノメチル)ベンゾイル]デアセチルコルヒチンの合成

4-クロロ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 35 mg, 0.0638 mmol, 83%)を得た。3-(ピロリジン-1-イル)安息香酸の代わりに3-(ジメチルアミノメチル)安息香酸塩酸塩を用いた。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 9.08 (1H, d, J=7.3 Hz), 7.81–7.78 (2H, m), 7.48–7.41 (2H, m), 7.21 (1H, s), 7.17 (1H, d, J=10.7 Hz), 7.05 (1H, d, J=10.7 Hz), 4.53–4.46 (1H, m), 3.94 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.60 (3H, s), 3.44 (2H, s), 3.17 (1H, dd, J=12.2, 5.6 Hz), 2.21–1.98 (3H, m), 2.15 (6H, s).

ESI-MS m/z: 553 [M+H]⁺.

[0214] 参考例 20

3-(トリフェニルメチルオキシ)プロパノールの合成

1,3-プロパンジオール(2.0 mL, 27.6 mmol)のジクロロメタン(140 mL)溶液に、氷冷、アルゴンガス雰囲気下にてトリエチルアミン(4.04 mL, 27.6×1.05 mmol)及びトリフェニルメチルクロリド(7.69 g, 27.6 mmol)を添加して、徐々に室温に戻しながら一晩攪拌した。反応液にクロロホルム及び水を添加して、有機層を取った。有機層は水次いでブラインにて洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, ヘキサン/酢酸エチル/クロロホルム)にて精製し、標記の化合物(白色固体, 4.59 g, 14.4 mmol, 52%)を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.45–7.41 (6H, m), 7.33–7.22 (9H, m), 3.

WO 2011/021397

132

PCT/JP2010/005136

77 (2H, t, J=5.9 Hz), 3.28 (2H, t, J=5.9 Hz), 1.89–1.84 (2H, m).

[0215] 参考例 2 1

3-(トリフェニルメチルオキシ)プロピオンアルデヒドの合成

塩化オキサリル(862 μ L, 6.28 $\times$ 1.6 mmol)のジクロロメタン(15 mL)溶液に、 -78°C 付近、アルゴンガス雰囲気下にて、ジメチルスルホキシド(1.56 mL, 6.28 $\times$ 3.5 mmol)のジクロロメタン(2.5 mL)溶液を滴下した。 -78°C 付近で10分間攪拌した後、3-(トリフェニルメチルオキシ)プロパノール(2.00 g, 6.28 mmol)のジクロロメタン(9 mL)溶液を滴下した。 -78°C 付近で10分間攪拌した後、トリエチルアミン(3.50 mL, 6.28 $\times$ 4 mmol)を滴下した。次いで、ゆっくりと室温に戻し、クロロホルムと水を添加した。有機層を取り、ブラインにて洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をヘキサンで再結晶して、標記の化合物(白色固体, 1.633 g, 5.16 mmol, 82%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 9.76 (1H, t, J=2.2 Hz), 7.44–7.40 (6H, m), 7.33–7.22 (9H, m), 3.47 (2H, t, J=6.1 Hz), 2.64 (2H, td, J=6.1, 2.2 Hz).

[0216] 参考例 2 2

3-(トリフェニルメチルオキシ)プロピオン酸の合成

3-(トリフェニルメチルオキシ)プロピオンアルデヒド(1.20 g, 3.79 mmol)のアセトン(19.5 mL)-水(3.8 mL)混液に氷冷下、過マンガンカリウム(0.599 g, 3.79 mmol)を数回に分けて添加した。反応液を室温で2時間攪拌した後、塩酸(1 mol/L)にてpH5付近に調製し、次いで10%チオ硫酸ナトリウムを添加して褐色を消去した。次いでクロロホルム、水及び塩酸(1 mol/L)を添加して、pH2付近に調製し、有機層を取った。水層は再びクロロホルムにて抽出した。有機層は合わせて、ブラインにて洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物に酢酸エチル(10 mL)を加えて超音波処理した後、ヘキサン(10 mL)を加えて室温で一晩静置した。析出物をろ取、ヘキサンで洗浄、減圧乾燥して、標記の化合物(白色固体, 1.15 g, 3.47 mmol, 92%)

WO 2011/021397

133

PCT/JP2010/005136

を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.45–7.41 (6H, m), 7.32–7.21 (9H, m), 3.42 (2H, t, $J=6.3$ Hz), 2.62 (2H, t, $J=6.3$ Hz).

[0217] 実施例 1 3 7

4-クロロ-N-[3-(トリフェニルメチルオキシ)プロピオニル]デアセチルコルヒチンの合成

4-クロロ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(乳白色固体, 118 mg, 0.168 mmol, 94 %)を得た。3-(ピロリジン-1-イル)安息香酸の代わりに3-(トリフェニルメチルオキシ)プロピオン酸を用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.78 (1H, d, $J=7.3$ Hz), 7.34–7.20 (16H, m), 7.13 (1H, d, $J=10.7$ Hz), 7.03 (1H, d, $J=10.7$ Hz), 4.35–4.28 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.54 (3H, s), 3.18–3.09 (2H, m), 3.05–2.99 (1H, m), 2.53–2.39 (2H, m), 2.18–2.09 (1H, m), 2.02–1.83 (2H, m).

ESI-MS m/z : 706 $[\text{M}+\text{H}]^+$.

[0218] 実施例 1 3 8

4-クロロ-N-(3-ヒドロキシプロピオニル)デアセチルコルヒチンの合成

4-クロロ-N-[3-(トリフェニルメチルオキシ)プロピオニル]デアセチルコルヒチン(95 mg, 0.135 mmol)のメタノール(0.5 mL)溶液に、室温でp-トルエンスルホン酸一水和物(5 mg, 0.135×0.2 mmol)を添加して、5時間攪拌した。反応液を濃縮乾固して、フラッシュクロマトグラフ法(装置:Biotage社Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(淡黄色固体, 54 mg, 0.116 mmol, 86%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.59 (1H, d, $J=7.1$ Hz), 7.13 (2H, s), 7.12 (2H, d, $J=10.7$ Hz), 7.02 (1H, d, $J=10.7$ Hz), 4.54 (1H, t, $J=5.2$ Hz), 4.28–4.22 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.57–3.52 (2H, m), 3.53 (3H, s), 3.10 (1H, dd, $J=13.7, 5.1$ Hz), 2.35–2.2

WO 2011/021397

134

PCT/JP2010/005136

3 (2H, m), 2.15–2.06 (1H, m), 1.99–1.80 (2H, m).

ESI-MS m/z : 464 $[M+H]^+$.

[0219] 実施例 139

4-クロロ-N-(メタンシルホニルアセチル)デアセチルコルヒチンの合成

アルゴン雰囲気下、メタンシルホニル酢酸 (21 mg, 0.128×1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (21 mg, 0.128×1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 17 mg, 0.033 mmol, 29%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.67 (1H, br. s), 7.73 (1H, s), 7.36 (1H, d, $J = 10.7$ Hz), 6.92 (1H, d, $J = 10.7$ Hz), 4.63–4.56 (1H, m), 4.15 (1H, d, $J = 14.6$ Hz), 4.06 (1H, d, $J = 14.6$ Hz), 4.03 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.63 (3H, s), 3.25 (1H, dd, $J = 13.4, 5.1$ Hz), 3.08 (3H, s), 2.34–2.25 (1H, m), 2.16–2.13 (1H, m), 1.93–1.84 (1H, m).

ESI-MS m/z : 512 ($[M+H]^+$)

[0220] 実施例 140

4-クロロ-N-[3-(ジメチルアミノ)プロピオニル]デアセチルコルヒチンの合成

アルゴン雰囲気下、3-ジメチルアミノプロピオン酸塩酸塩 (39 mg, 0.128×2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (49 mg, 0.128×2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (35 mg

WO 2011/021397

135

PCT/JP2010/005136

, 0.128 × 2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (乳白色固体, 27 mg, 0.055 mmol, 43%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.64 (1H, d, 7.1 Hz), 7.13 (1H, s), 7.12 (1H, d, J = 11.0 Hz), 7.02 (1H, d, J = 11.0 Hz), 4.26-4.20 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.11 (1H, d, J = 13.4, 5.1 Hz), 2.43-2.37 (2H, m), 2.31-2.27 (2H, m), 2.11 (3H, s), 2.10-2.08 (1H, m), 1.99-1.90 (1H, m), 1.86-1.78 (1H, m).

ESI-MS m/z: 491 ([M+H]⁺)

[0221] 実施例 1 4 1

4-クロロ-N-[3-(ジエチルアミノ)プロピオニル]デアセチルコルヒチンの合成
アルゴン雰囲気下、3-ジエチルアミノプロピオン酸塩酸塩 (47 mg, 0.128 × 2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (49 mg, 0.128 × 2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物 (35 mg, 0.128 × 2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (乳白色固体, 34 mg, 0.066 mmol, 51%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.70 (1H, d, J = 6.6 Hz), 7.13 (1H, s), 7.12 (1H, d, J = 11.0 Hz), 7.02 (1H, d, J = 11.0 Hz), 4.26-4.20 (1H,

WO 2011/021397

136

PCT/JP2010/005136

m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.11 (1H, dd, $J = 13.5, 5.2$ Hz), 2.64–2.55 (2H, m), 2.51–2.40 (4H, m), 2.27–2.22 (2H, m), 2.13–2.10 (1H, m), 1.99–1.92 (1H, m), 1.85–1.78 (1H, m), 0.93 (6H, t, $J = 6.7$ Hz).

ESI-MS m/z : 519 ($[M+H]^+$)

[0222] 実施例 1 4 2

4-クロロ-N-(3-ピペリジノプロピオニル)デアセチルコルヒチンの合成

アルゴン雰囲気下、1-ピペリジンプロピオン酸 (40 mg, 0.128×2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (49 mg, 0.128×2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (35 mg, 0.128×2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 20 mg, 0.038 mmol, 29%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.71 (1H, d, $J = 7.3$ Hz), 7.12 (1H, s), 7.12 (1H, d, $J = 10.5$ Hz), 7.02 (1H, d, $J = 11.0$ Hz), 4.26–4.20 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.11 (1H, dd, $J = 13.4, 5.1$ Hz), 2.43 (2H, t, $J = 7.0$ Hz), 2.31–2.26 (6H, m), 2.13–2.10 (1H, m), 2.00–1.91 (1H, m), 1.83–1.80 (1H, m), 1.48–1.47 (4H, m), 1.37–1.35 (2H, m).

ESI-MS m/z : 531 ($[M+H]^+$)

[0223] 実施例 1 4 3

N-[N'-(*t*-ブチルオキシカルボニル)-N'-メチルアミノアセチル]-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、*t*-ブチルオキシカルボニルサルコシン (96 mg, 0.255 × 2 mmol) を N,N -ジメチルホルムアミド (1.5 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (98 mg, 0.255 × 2 mmol)、1-ヒドロキシベンゾトリアゾール-水和物 (69 mg, 0.255 × 2 mmol) を加え、0°Cで30分攪拌した。そこへ4-クロロデアセチルコルヒチン (100 mg, 0.255 mmol) を加え、室温に戻して90分攪拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 71 mg, 0.126 mmol, 50%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.67, 8.64 (total 1H, each d, $J = 7.8$ Hz), 7.13 (1H, d, $J = 10.5$ Hz), 7.11 (1H, s), 7.02 (1H, d, $J = 10.5$ Hz), 4.29–4.23 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.32–3.30 (2H, m), 3.11 (1H, dd, $J = 13.4, 5.1$ Hz), 2.74, 2.72 (total 3H, each s), 2.13–2.10 (1H, m), 1.99–1.93 (1H, m), 1.91–1.82 (1H, m), 1.36, 1.29 (total 6H, each s).

ESI-MS m/z : 563 ($[\text{M}+\text{H}]^+$)

[0224] 実施例 1 4 4

N -[N' -ベンジル- N' -(*t*-ブチルオキシカルボニル)アミノアセチル]-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、 N -ベンジル- N -*t*-ブチルオキシカルボニルグリシン (135 mg, 0.255 × 2 mmol) を N,N -ジメチルホルムアミド (1.5 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (98 mg, 0.255 × 2 mmol)、1-ヒドロキシベンゾトリアゾール-水和物 (69 mg, 0.255 × 2 mmol) を加え、0°Cで30分攪拌した。そこへ4-クロロデアセチルコルヒチン (100 mg, 0.255 mmol) を加え、室温に戻して90分攪拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水

WO 2011/021397

138

PCT/JP2010/005136

、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 87 mg, 0.136 mmol, 57%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.64, 8.60 (total 1H, each d, $J = 6.8$ Hz), 7.36–7.31 (2H, m), 7.28–7.20 (3H, m), 7.15–7.12 (2H, m), 7.03 (1H, d, $J = 11.0$ Hz), 4.37–4.19 (3H, m), 3.92 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.85–3.66 (2H, m), 3.56 (3H, s), 3.10 (1H, dd, $J = 13.4$, 5.1 Hz), 2.16–2.07 (1H, m), 1.99–1.91 (1H, m), 1.87–1.79 (1H, m), 1.33 (9H, s).

ESI-MS m/z : 639 ($[\text{M}+\text{H}]^+$)

[0225] 実施例 145

4-クロロ-N-(1-メチルピペリジン-4-イルカルボニル)デアセチルコルヒチンの合成

アルゴン雰囲気下、1-メチルピペリジン-4-カルボン酸塩酸塩 (46 mg, 0.128 $\times$ 2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (49 mg, 0.128 $\times$ 2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物 (35 mg, 0.128 $\times$ 2 mmol) を加え、0°Cで30分攪拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して150分攪拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 17 mg, 0.033 mmol, 26%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.54 (1H, d, $J = 7.3$ Hz), 7.12 (1H, d, $J = 10.7$ Hz), 7.10 (1H, s), 7.02 (1H, d, $J = 10.7$ Hz), 4.24–4.18 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.10 (1

WO 2011/021397

139

PCT/JP2010/005136

H, dd, $J = 13.8, 5.5$ Hz), 2.76–2.70 (2H, m), 2.17–2.06 (2H, m), 2.11 (3H, s), 1.97–1.92 (1H, m), 1.88–1.77 (3H, m), 1.67–1.64 (1H, m), 1.53–1.48 (3H, m).

ESI-MS m/z : 517 ($[M+H]^+$)

[0226] 実施例 1 4 6

4-クロロ-N-[1-(ベンジルオキシカルボニル)ピペリジン-4-イルカルボニル]
デアセチルコルヒチンの合成

アルゴン雰囲気下、1-(ベンジルオキシカルボニル)ピペリジン-4-カルボン酸 (134 mg, 0.255×2 mmol) をN,N-ジメチルホルムアミド (1.5 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (98 mg, 0.255×2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物 (69 mg, 0.255×2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (100 mg, 0.255 mmol) を加え、室温に戻して150分撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 110 mg, 0.173 mmol, 68%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.61 (1H, d, $J = 7.1$ Hz), 7.36–7.31 (5H, m), 7.13 (1H, d, $J = 10.7$ Hz), 7.10 (1H, s), 7.03 (1H, d, $J = 11.0$ Hz), 5.05 (2H, s), 4.24–4.17 (1H, m), 4.01–3.98 (2H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.10 (1H, dd, $J = 13.2, 5.1$ Hz), 2.88–2.75 (2H, m), 2.44–2.41 (1H, m), 2.12–2.09 (1H, m), 2.00–1.91 (1H, m), 1.89–1.81 (1H, m), 1.71–1.64 (2H, m), 1.39–1.34 (2H, m).

ESI-MS m/z : 637 ($[M+H]^+$)

[0227] 実施例 1 4 7

4-クロロ-N-(N'-メチルアミノアセチル)デアセチルコルヒチンの合成

WO 2011/021397

140

PCT/JP2010/005136

アルゴン雰囲気下、4-クロロ-N-(N'-*t*-ブチルオキシカルボニル-N'-メチルアミノアセチル)デアセチルコルヒチン (30 mg, 0.053 mmol) をジクロロメタン (426 μ l) に溶解し、0°Cに冷却した。そこへトリフルオロ酢酸 (171 μ l) を加え、0°Cで150分撹拌した。反応液を濃縮乾固し、クロロホルムに再溶解して飽和重曹水、飽和食塩水で洗浄した。クロロホルム層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 16 mg, 0.035 mmol, 65%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.66 (1H, d, $J = 7.3$ Hz), 7.13 (1H, d, $J = 10.5$ Hz), 7.12 (1H, s), 7.03 (1H, d, $J = 10.7$ Hz), 4.31–4.25 (1H, m), 3.92 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.17 (2H, s), 3.11 (1H, dd, $J = 13.7, 3.4$ Hz), 2.27 (3H, s), 2.16–2.08 (1H, m), 1.97–1.92 (2H, m).

ESI-MS m/z : 463 ($[\text{M}+\text{H}]^+$)

[0228] 実施例 148

N-(N'-ベンジルアミノアセチル)-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロ-N-(N'-ベンジル-N'-*t*-ブトキシカルボニルアミノアセチル)デアセチルコルヒチン (23 mg, 0.036 mmol) をジクロロメタン (288 μ l) に溶解し、0°Cに冷却した。そこへトリフルオロ酢酸 (115 μ l) を加え、0°Cで150分撹拌した。反応液を濃縮乾固し、クロロホルムに再溶解して飽和重曹水、飽和食塩水で洗浄した。クロロホルム層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 21 mg, 定量的) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.66 (1H, d, $J = 7.1$ Hz), 7.34–7.32 (4H, m), 7.26–7.24 (1H, m), 7.14 (1H, s), 7.14 (1H, d, $J = 10.5$ Hz), 7.04 (1H, d, $J = 10.7$ Hz), 4.29–4.23 (1H, m), 3.92 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.71 (2H, s), 3.54 (3H, s), 3.19 (2H, s), 3.11 (1H,

WO 2011/021397

141

PCT/JP2010/005136

dd, $J = 13.3, 4.3$ Hz), 2.13–2.10 (1H, m), 1.97–1.89 (2H, m).

ESI-MS m/z : 539 ($[M+H]^+$)

[0229] 実施例 149

4-クロロ-N-[4-(ジメチルアミノ)ブチリル]デアセチルコルヒチンの合成

アルゴン雰囲気下、4-ジメチルアミノ酪酸塩酸塩 (43 mg, 0.128×2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (49 mg, 0.128×2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (35 mg, 0.128×2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して1時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 35 mg, 0.069 mmol, 54%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.62 (1H, d, $J = 7.1$ Hz), 7.12 (1H, d, $J = 10.5$ Hz), 7.11 (1H, s), 7.03 (1H, d, $J = 11.0$ Hz), 4.26–4.20 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.11 (1H, dd, $J = 13.5, 5.0$ Hz), 2.36–2.34 (2H, m), 2.24 (6H, s), 2.19–2.16 (2H, m), 2.13–2.07 (1H, m), 2.00–1.91 (1H, m), 1.87–1.79 (1H, m), 1.66–1.59 (2H, m).

ESI-MS m/z : 505 ($[M+H]^+$)

[0230] 実施例 150

4-クロロ-N-[2-(ジメチルアミノ)ベンゾイル]デアセチルコルヒチンの合成

アルゴン雰囲気下、2-(ジメチルアミノ)安息香酸 (25 mg, 0.128×1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (21 mg, 0.

WO 2011/021397

142

PCT/JP2010/005136

128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチル
コルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して終夜撹拌した。反応
液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗
浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した
。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SN
AP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 2
4 mg, 0.045 mmol, 35%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 9.54 (1H, d, J = 7.3 Hz), 7.42–7.38 (2
H, m), 7.33 (1H, s), 7.17–7.15 (2H, m), 7.06–6.99 (2H, m), 4.52–4.46
(1H, m), 3.94 (3H, s), 3.90 (3H, s), 3.89 (3H, s), 3.60 (3H, s), 3.15
(1H, dd, J = 12.9, 3.7 Hz), 2.70 (6H, s), 2.17–2.14 (1H, m), 2.10–1.
98 (2H, m).

ESI-MS m/z: 539 ([M+H]⁺)

[0231] 実施例 151

4-クロロ-N- (イソブチルチオカルバモイル) デアセチルコルヒチンの合成
アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol)
をジクロロメタン (1 mL) に溶解し、0°Cに冷却した。そこへイソチオシアン
酸イソブチル (31 μl, 0.128 × 2 mmol) を加え、室温まで昇温させながら
4時間撹拌した。反応液に10%クエン酸水溶液を加え、クロロホルムで抽出し
た。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥さ
せ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biota
ge Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の
化合物 (淡黄色固体, 26 mg, 0.051 mmol, 40%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.00 (1H, br. s), 7.57 (1H, br. s), 7.14
(1H, d, J = 10.5 Hz), 7.06 (1H, s), 7.03 (1H, d, J = 10.7 Hz), 4.79–
4.70 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.60 (3H, s),
3.21–3.11 (3H, m), 2.15–2.07 (2H, m), 1.84–1.76 (1H, m), 0.84 (6H, d
, J = 6.3 Hz).

WO 2011/021397

143

PCT/JP2010/005136

ESI-MS m/z : 507 ($[M+H]^+$)

[0232] 実施例 1 5 2

N-(ベンジルチオカルバモイル)-4-クロロデアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (1 mL) に溶解し、0°Cに冷却した。そこへイソチオシアン酸ベンジル (34 μ l, 0.128 $\times$ 2 mmol) を加え、室温まで昇温させながら4時間攪拌した。反応液に10% クエン酸水溶液を加え、クロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 43 mg, 0.079 mmol, 62%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.19 (1H, br. s), 7.97 (1H, br. s), 7.31–7.30 (2H, m), 7.25–7.24 (3H, m), 7.15 (1H, d, $J = 10.7$ Hz), 7.11 (1H, s), 7.04 (1H, d, $J = 11.0$ Hz), 4.79–4.74 (1H, m), 4.65–4.60 (2H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.60 (3H, s), 3.12 (1H, d, $J = 12.4, 4.6$ Hz), 2.18–2.06 (2H, m), 1.93–1.86 (1H, m).

ESI-MS m/z : 541 ($[M+H]^+$)

[0233] 実施例 1 5 3

4-クロロ-N-(シクロヘキシルチオカルバモイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (1 mL) に溶解し、0°Cに冷却した。そこへイソチオシアン酸シクロヘキシル (35 μ l, 0.128 $\times$ 2 mmol) を加え、室温に戻して6時間攪拌した。反応液に10% クエン酸水溶液を加え、クロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 26 mg, 0.049 mmol, 38%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.86 (1H, d, $J = 6.6$ Hz), 7.48 (1H, br

WO 2011/021397

144

PCT/JP2010/005136

. s), 7.14 (1H, d, J = 10.7 Hz), 7.04 (1H, s), 7.03 (1H, d, J = 11.0 Hz), 4.78–4.71 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.60 (3H, s), 3.11 (1H, dd, J = 12.8, 4.5 Hz), 2.12–2.07 (2H, m), 1.88–1.77 (3H, m), 1.66–1.61 (2H, m), 1.53–1.50 (1H, m), 1.25–1.14 (6H, m).
ESI-MS m/z: 533 ([M+H]⁺)

[0234] 実施例 1 5 4

4-クロロ-N-[(2-ピペリジノエチル)チオカルバモイル]デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (1 mL) に溶解し、0°Cに冷却した。そこへイソチオシアン酸2-ピペリジノエチル(42 μ l, 0.128 $\times$ 2 mmol) を加え、室温まで昇温させながら終夜撹拌した。反応液に10% クエン酸水溶液を加え、クロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 33 mg, 0.059 mmol, 46%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.59 (1H, br. s), 7.94 (1H, br. s), 7.15 (1H, d, J = 10.7 Hz), 7.06 (1H, s), 7.03 (1H, d, J = 11.0 Hz), 4.75–4.70 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.80–3.74 (2H, m), 3.61 (3H, s), 3.20–3.06 (2H, m), 3.12 (1H, dd, J = 12.7, 4.4 Hz), 2.96–2.81 (2H, m), 2.19–2.02 (2H, m), 1.97–1.88 (1H, m), 1.73–1.61 (4H, m), 1.42–1.33 (1H, m), 1.28–1.25 (3H, m).

ESI-MS m/z: 562 ([M+H]⁺)

[0235] 実施例 1 5 5

4-クロロ-N-[[2-(4-モルフォリノ)エチル]チオカルバモイル]デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (1 mL) に溶解し、0°Cに冷却した。そこへイソチオシアン

WO 2011/021397

145

PCT/JP2010/005136

酸2-(4-モルフォリノ)エチル (39 μ l, 0.128 $\times$ 2 mmol) を加え、室温まで昇温させながら終夜攪拌した。反応液に10% クエン酸水溶液を加え、クロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (褐色固体, 35 mg, 0.062 mmol, 49%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.29 (1H, d, $J = 6.1$ Hz), 7.45 (1H, br. s), 7.14 (1H, d, $J = 10.7$ Hz), 7.05 (1H, s), 7.03 (1H, d, $J = 11.0$ Hz), 4.78-4.71 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.60 (3H, s), 3.59-3.57 (4H, m), 3.51-3.42 (2H, m), 3.12 (1H, dd, $J = 12.7, 4.6$ Hz), 2.39-2.35 (6H, m), 2.17-2.02 (2H, m), 1.91-1.78 (1H, m).

ESI-MS m/z : 564 ($[\text{M}+\text{H}]^+$)

[0236] 実施例 156

4-クロロ-N-(3-ピリジルチオカルバモイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (1 mL) に溶解し、0°Cに冷却した。そこへイソチオシアン酸3-ピリジル (29 μ l, 0.128 $\times$ 2 mmol) を加え、室温に戻して4時間攪拌した。反応液に10%クエン酸水溶液を加え、クロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄褐色固体, 51 mg, 0.097 mmol, 76%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.79 (1H, s), 8.67 (1H, d, $J = 6.8$ Hz), 8.57 (1H, d, $J = 2.2$ Hz), 8.28 (1H, d, $J = 3.7$ Hz), 7.94 (1H, d, $J = 8.1$ Hz), 7.32 (1H, dd, $J = 8.2, 4.8$ Hz), 7.17 (1H, d, $J = 10.7$ Hz), 7.14 (1H, s), 7.05 (1H, d, $J = 11.0$ Hz), 4.81-4.75 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.61 (3H, s), 3.17-3.12 (1H, m), 2.19-2.13 (2H, m), 2.06-1.99 (1H, m).

WO 2011/021397

146

PCT/JP2010/005136

ESI-MS m/z : 528 ($[M+H]^+$)

[0237] 実施例 1 5 7

4-クロロ-N-[p-(ジメチルアミノ)フェニルチオカルバモイル]デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (1 mL) に溶解し、0°Cに冷却した。そこへイソチオシアン酸p-(ジメチルアミノ)フェニル (43 mg, 0.128 × 2 mmol) を加え、室温に戻して4時間撹拌した。反応液に10% クエン酸水溶液を加え、クロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 46 mg, 0.081 mmol, 63%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.40 (1H, s), 8.02 (1H, d, $J = 6.6$ Hz), 7.15 (1H, d, $J = 10.7$ Hz), 7.14 (2H, d, $J = 8.3$ Hz), 7.10 (1H, s), 7.03 (1H, d, $J = 11.0$ Hz), 6.68 (2H, d, $J = 8.8$ Hz), 4.84–4.79 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.61 (3H, s), 3.11 (1H, dd, $J = 12.8, 5.5$ Hz), 2.87 (6H, s), 2.13–2.09 (2H, m), 2.03–1.98 (1H, m).

ESI-MS m/z : 570 ($[M+H]^+$)

[0238] 実施例 1 5 8

4-クロロ-N-(4-ニトロベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μl , 0.128 × 1.1 mmol)、4-ニトロベンゾイルクロリド (24 mg, 0.128 × 1 mmol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g,

WO 2011/021397

147

PCT/JP2010/005136

メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 36 mg, 0.098 mmol, 77%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.31 (1H, d, $J = 8.3$ Hz), 8.07–8.02 (2H, m), 7.95 (2H, d, $J = 8.5$ Hz), 7.64 (1H, s), 7.41 (1H, d, $J = 10.7$ Hz), 6.95 (1H, d, $J = 10.7$ Hz), 4.80–4.74 (1H, m), 4.05 (3H, s), 4.02 (3H, s), 3.99 (3H, s), 3.72 (3H, s), 3.32 (1H, dd, $J = 13.5, 5.5$ Hz), 2.45–2.36 (1H, m), 2.22–2.19 (1H, m), 2.11–2.04 (1H, m).

ESI-MS m/z : 541 ($[\text{M}+\text{H}]^+$)

[0239] 実施例 159

4-クロロ-N-(2-ナフトイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μl , 0.128×1.1 mmol)、2-ナフトイルクロリド (24 mg, 0.128×1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 42 mg, 0.077 mmol, 60%) を得た。

$^1\text{H-NMR}$ (400MHz, $\text{DMSO}-d_6$) δ [ppm]: 9.26 (1H, d, $J = 7.1$ Hz), 8.53 (1H, s), 8.02–7.97 (4H, m), 7.65–7.59 (2H, m), 7.26 (1H, s), 7.19 (1H, d, $J = 10.5$ Hz), 7.06 (1H, d, $J = 10.7$ Hz), 4.58–4.52 (1H, m), 3.95 (3H, s), 3.90 (3H, s), 3.89 (3H, s), 3.63 (3H, s), 3.19 (1H, dd, $J = 11.5, 4.6$ Hz), 2.19–2.12 (3H, m).

ESI-MS m/z : 546 ($[\text{M}+\text{H}]^+$)

[0240] 実施例 160

4-クロロ-N-(2-ニトロベンゾイル) デアセチルコルヒチンの合成

アルゴン雰囲気下、2-ニトロ安息香酸 (26 mg, 0.128×1.2 mmol) をN,N

WO 2011/021397

148

PCT/JP2010/005136

-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水和物 (21 mg, 0.128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して終夜撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 23 mg, 0.043 mmol, 33%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 8.04 (1H, d, J = 7.8 Hz), 7.71-7.63 (3H, m), 7.58-7.56 (1H, m), 7.35 (1H, d, J = 10.2 Hz), 6.88 (1H, d, J = 10.7 Hz), 6.86 (1H, br. s), 4.85-4.79 (1H, m), 4.02 (3H, s), 4.02 (3H, s), 4.02 (3H, s), 3.66 (3H, s), 3.31 (1H, dd, J = 13.7, 5.4 Hz), 2.44-2.34 (1H, m), 2.22-2.18 (1H, m), 1.95-1.81 (1H, m).

ESI-MS m/z: 541 ([M+H]⁺)

[0241] 実施例 161

4-クロロ-N-(4-メチルベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、p-トルイル酸 (21 mg, 0.128 × 1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水和物 (21 mg, 0.128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して終夜撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 28 mg, 0.055 mmol, 33%) を得た。

WO 2011/021397

149

PCT/JP2010/005136

mol, 43%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.70 (2H, d, $J = 8.1$ Hz), 7.62 (1H, s), 7.35 (1H, d, $J = 10.7$ Hz), 7.19 (2H, d, $J = 8.1$ Hz), 6.90 (1H, br. s), 6.87 (1H, d, $J = 10.7$ Hz), 4.80–4.74 (1H, m), 4.01 (3H, s), 4.00 (3H, s), 3.98 (3H, s), 3.70 (3H, s), 3.31 (1H, dd, $J = 13.4, 5.1$ Hz), 2.41–2.30 (1H, m), 2.37 (3H, s), 2.24–2.20 (1H, m), 1.96–1.93 (1H, m).

ESI-MS m/z : 510 ($[\text{M}+\text{H}]^+$)

[0242] 実施例 1 6 2

4-クロロ-N-(4-フェニルベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、 0°C に冷却した。そこへトリエチルアミン (19 μl , 0.128×1.1 mmol)、4-フェニルベンゾイルクロリド (28 mg, 0.128×1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 40 mg, 0.070 mmol, 55%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.12 (1H, d, $J = 7.1$ Hz), 7.99 (2H, d, $J = 8.3$ Hz), 7.80 (2H, d, $J = 8.3$ Hz), 7.74 (2H, d, $J = 7.3$ Hz), 7.50 (2H, t, $J = 7.6$ Hz), 7.41 (1H, t, $J = 7.2$ Hz), 7.23 (1H, s), 7.18 (1H, d, $J = 10.5$ Hz), 7.06 (1H, d, $J = 11.0$ Hz), 4.55–4.49 (1H, m), 3.94 (3H, s), 3.90 (3H, s), 3.89 (3H, s), 3.62 (3H, s), 3.18 (1H, dd, $J = 12.0, 4.9$ Hz), 2.23–2.06 (3H, m).

ESI-MS m/z : 572 ($[\text{M}+\text{H}]^+$)

[0243] 実施例 1 6 3

4-クロロ-N-(3,5-ジニトロベンゾイル)デアセチルコルヒチンの合成

WO 2011/021397

150

PCT/JP2010/005136

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、3,5-ジニトロベンゾイルクロリド (30 mg, 0.128 $\times$ 1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 47 mg, 0.080 mmol, 63%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 9.91 (1H, d, J = 5.9 Hz), 8.95 (1H, t, J = 1.8 Hz), 8.63 (2H, s), 7.94 (1H, s), 7.54 (1H, d, J = 10.7 Hz), 7.14 (1H, d, J = 11.0 Hz), 4.78-4.72 (1H, m), 4.12 (3H, s), 4.03 (3H, s), 3.98 (3H, s), 3.67 (3H, s), 3.37 (1H, dd, J = 12.2, 2.9 Hz), 2.49-2.42 (2H, m), 2.28-2.19 (1H, m).

ESI-MS m/z: 586 ([M+H]⁺)

[0244] 実施例 164

4-クロロ-N-(3,5-ジメチルベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、3,5-ジメチルベンゾイルクロリド (19 μ l, 0.128 $\times$ 1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 35mg, 0.067 mmol, 52%) を得た。

¹H-NMR (400MHz, CDCl₃) δ [ppm]: 7.64 (1H, s), 7.39 (2H, s), 7.36 (1H, d, J = 11.0 Hz), 7.12 (1H, s), 6.88 (1H, d, J = 11.0 Hz), 6.78 (1H, d,

WO 2011/021397

151

PCT/JP2010/005136

$J = 6.8 \text{ Hz}$), 4.80–4.74 (1H, m), 4.01 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.69 (3H, s), 3.32 (1H, dd, $J = 13.2, 5.1 \text{ Hz}$), 2.37–2.32 (1H, m), 2.32 (6H, s), 2.26–2.18 (1H, m), 1.98–1.93 (1H, m).

ESI-MS m/z : 524 ($[M+H]^+$)

[0245] 実施例 165

4-クロロ-N-(3-ニトロベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, $0.128 \times 1.1 \text{ mmol}$)、3-ニトロベンゾイルクロリド (27 mg, $0.128 \times 1 \text{ mmol}$) を加え、室温に戻して1時間撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 59 mg, 0.109 mmol, 85%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.90 (1H, d, $J = 6.1 \text{ Hz}$), 8.57 (1H, s), 8.16 (1H, d, $J = 8.1 \text{ Hz}$), 7.96 (1H, d, $J = 7.6 \text{ Hz}$), 7.89 (1H, s), 7.47 (1H, d, $J = 10.7 \text{ Hz}$), 7.34 (1H, t, $J = 7.9 \text{ Hz}$), 7.03 (1H, d, $J = 11.0 \text{ Hz}$), 4.83–4.77 (1H, m), 4.07 (3H, s), 4.02 (3H, s), 3.98 (3H, s), 3.70 (3H, s), 3.33 (1H, dd, $J = 13.4, 5.6 \text{ Hz}$), 2.47–2.38 (1H, m), 2.34–2.29 (1H, m), 2.27–2.17 (1H, m).

ESI-MS m/z : 541 ($[M+H]^+$)

[0246] 実施例 166

4-クロロ-N-(3,5-ジメトキシベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, $0.128 \times 1.1 \text{ mmol}$)、3,5-ジメトキシベンゾイルクロリド (26 mg, $0.128 \times 1 \text{ mmol}$) を加え、室温に戻して1時間撹拌した。反応液に水を

WO 2011/021397

152

PCT/JP2010/005136

加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 47 mg, 0.085mmol, 66%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.65 (1H, s), 7.38 (1H, d, $J = 10.7$ Hz), 7.01 (1H, d, $J = 7.1$ Hz), 6.93 (2H, s), 6.90 (1H, d, $J = 10.7$ Hz), 6.56 (1H, s), 4.77–4.71 (1H, m), 4.02 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.79 (6H, s), 3.69 (3H, s), 3.31 (1H, dd, $J = 13.3, 5.2$ Hz), 2.40–2.30 (1H, m), 2.25–2.17 (1H, m), 2.02–1.95 (1H, m).

ESI-MS m/z : 556 ($[\text{M}+\text{H}]^+$)

[0247] 実施例 167

4-クロロ-N-(2,4-ジメトキシベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μl , 0.128×1.1 mmol)、2,4-ジメトキシベンゾイルクロリド (26 mg, 0.128×1 mmol) を加え、室温に戻して1時間攪拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 33 mg, 0.059mmol, 46%) を得た。

$^1\text{H-NMR}$ (400MHz, $\text{DMSO}-d_6$) δ [ppm]: 8.29 (1H, d, $J = 6.3$ Hz), 7.98 (1H, d, $J = 8.8$ Hz), 7.64 (1H, s), 7.34 (1H, d, $J = 10.7$ Hz), 6.85 (1H, d, $J = 10.7$ Hz), 6.54 (1H, dd, $J = 8.7, 2.1$ Hz), 6.51 (1H, d, $J = 2.0$ Hz), 4.78–4.71 (1H, m), 4.02 (3H, s), 4.00 (3H, s), 4.00 (3H, s), 3.97 (3H, s), 3.85 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, $J = 13.2, 5.1$ Hz), 2.40–2.31 (1H, m), 2.26–2.18 (1H, m), 1.93–1.84 (1H, m).

WO 2011/021397

153

PCT/JP2010/005136

ESI-MS m/z : 556 ($[M+H]^+$)

[0248] 実施例 168

4-クロロ-N-(2,4,6-トリメチルベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、2,4,6-トリメチルベンゾイルクロリド (21 μ l, 0.128 $\times$ 1 mmol) を加え、室温に戻して90分撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 28 mg, 0.052 mmol, 41%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.50 (1H, s), 7.29 (1H, d, J = 10.7 Hz), 6.86–6.81 (3H, m), 6.09 (1H, d, J = 7.8 Hz), 4.84–4.78 (1H, m), 4.02 (3H, s), 4.01 (3H, s), 4.01 (3H, s), 3.69 (3H, s), 3.29 (1H, dd, J = 13.2, 4.4 Hz), 2.33–2.16 (2H, m), 2.26 (9H, s), 1.80–1.73 (1H, m).

ESI-MS m/z : 538 ($[M+H]^+$)

[0249] 実施例 169

4-クロロ-N-(4-シアノベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、0°Cに冷却した。そこへトリエチルアミン (19 μ l, 0.128 $\times$ 1.1 mmol)、4-シアノベンゾイルクロリド (21 mg, 0.128 $\times$ 1 mmol) を加え、室温に戻して1時間撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 51 mg, 0.098 mmol, 77%) を得た。

WO 2011/021397

154

PCT/JP2010/005136

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.45 (1H, d, $J = 5.9$ Hz), 7.93 (2H, d, $J = 8.3$ Hz), 7.71 (1H, s), 7.52 (2H, d, $J = 8.1$ Hz), 7.44 (1H, d, $J = 11.0$ Hz), 6.98 (1H, d, $J = 11.0$ Hz), 4.81–4.75 (1H, m), 4.06 (3H, s), 4.02 (3H, s), 3.99 (3H, s), 3.72 (3H, s), 3.31 (1H, dd, $J = 13.2, 5.6$ Hz), 2.44–2.35 (1H, m), 2.24–2.17 (1H, m), 2.16–2.06 (1H, m).

ESI-MS m/z : 521 ($[\text{M}+\text{H}]^+$)

[0250] 実施例 170

4-クロロ-N-(3-ヒドロキシ-3-メチルブチリル)デアセチルコルヒチンの合成
アルゴン雰囲気下、 β -ヒドロキシイソ吉草酸 (16 μl , 0.128×1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、 0°C に冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128×1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水和物 (21 mg, 0.128×1.2 mmol) を加え、 0°C で30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 33 mg, 0.067 mmol, 52%) を得た。

$^1\text{H-NMR}$ (400MHz, $\text{DMSO}-d_6$) δ [ppm]: 8.60 (1H, d, $J = 7.3$ Hz), 7.17 (1H, s), 7.13 (1H, d, $J = 10.5$ Hz), 7.03 (1H, d, $J = 10.7$ Hz), 4.64 (1H, s), 4.29–4.23 (1H, m), 3.92 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.55 (3H, s), 3.11 (1H, dd, $J = 13.2, 4.9$ Hz), 2.26 (2H, s), 2.15–2.07 (1H, m), 2.01–1.92 (1H, m), 1.89–1.81 (1H, m), 1.12 (3H, s), 1.11 (3H, s).

ESI-MS m/z : 492 ($[\text{M}+\text{H}]^+$)

[0251] 実施例 171

4-クロロ-N-[4-(ベンジルオキシ)ブチリル]デアセチルコルヒチンの合成

アルゴン雰囲気下、4-ベンジルオキシ酪酸 (27 μl , 0.128×1.2 mmol)

WO 2011/021397

155

PCT/JP2010/005136

をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (21 mg, 0.128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 36 mg, 0.063 mmol, 50%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.61 (1H, d, J = 7.1 Hz), 7.34–7.30 (4 H, m), 7.28–7.24 (1H, m), 7.13 (1H, d, J = 10.5 Hz), 7.12 (1H, s), 7.03 (1H, d, J = 11.0 Hz), 4.46 (1H, d, J = 12.0 Hz), 4.41 (1H, d, J = 12.0 Hz), 4.26–4.20 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.37 (2H, t, J = 6.5 Hz), 3.10 (1H, dd, J = 13.4, 5.1 Hz), 2.23 (2H, t, J = 6.8 Hz), 2.14–2.06 (1H, m), 1.98–1.88 (1H, m), 1.86–1.78 (1H, m), 1.75–1.68 (2H, m).

ESI-MS m/z: 568 ([M+H]⁺)

[0252] 実施例 172

4-クロロ-N-(2-シアノベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、2-シアノ安息香酸 (23 mg, 0.128 × 1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (21 mg, 0.128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた

WO 2011/021397

156

PCT/JP2010/005136

残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄褐色固体, 41 mg, 0.079 mmol, 62%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.96 (1H, d, $J = 7.8$ Hz), 7.72 (1H, d, $J = 6.6$ Hz), 7.64–7.53 (3H, m), 7.46 (1H, br. s), 7.35 (1H, d, $J = 10.5$ Hz), 6.89 (1H, d, $J = 11.0$ Hz), 4.86–4.80 (1H, m), 4.02 (3H, s), 4.01 (3H, s), 4.00 (3H, s), 3.70 (3H, s), 3.32 (1H, dd, $J = 13.5, 5.5$ Hz), 2.47–2.38 (1H, m), 2.26–2.18 (1H, m), 2.03–1.95 (1H, m).

ESI-MS m/z : 521 ($[\text{M}+\text{H}]^+$)

[0253] 実施例 173

4-クロロ-N-(3-シアノベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をジクロロメタン (2 mL) に溶解し、 0°C に冷却した。そこへトリエチルアミン (19 μl , 0.128×1.1 mmol)、3-シアノベンゾイルクロリド (21 mg, 0.128×1 mmol) を加え、室温に戻して2時間撹拌した。反応液に水を加えクエンチし、飽和重曹水を加え、クロロホルムで抽出した。有機層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 44 mg, 0.084 mmol, 66%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.33 (1H, br. s), 8.14 (1H, s), 7.98 (1H, d, $J = 8.1$ Hz), 7.67 (2H, s), 7.66–7.65 (2H, m), 7.42–7.40 (1H, m), 7.41 (1H, d, $J = 11.0$ Hz), 6.95 (1H, d, $J = 10.7$ Hz), 4.81–4.75 (1H, m), 4.03 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.71 (3H, s), 3.33 (1H, dd, $J = 12.7, 5.4$ Hz), 2.41–2.35 (1H, m), 2.26–2.15 (2H, m).

ESI-MS m/z : 521 ($[\text{M}+\text{H}]^+$)

[0254] 実施例 174

4-クロロ-N-(2-エチル-2-ヒドロキシブチリル)デアセチルコルヒチンの合成

WO 2011/021397

157

PCT/JP2010/005136

アルゴン雰囲気下、2-エチル-2-ヒドロキシ酪酸 (20 mg, 0.128 × 1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール水合物 (21 mg, 0.128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SN AP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 38 mg, 0.075 mmol, 59%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.32 (1H, d, J = 7.8 Hz), 7.22 (1H, s), 7.11 (1H, d, J = 10.5 Hz), 7.01 (1H, d, J = 11.0 Hz), 4.97 (1H, s), 4.33-4.27 (1H, m), 3.92 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.55 (3H, s), 3.10 (1H, dd, J = 12.9, 5.4 Hz), 2.20-2.06 (2H, m), 1.90-1.82 (1H, m), 1.67-1.54 (2H, m), 1.47-1.38 (2H, m), 0.76-0.69 (6H, m).

ESI-MS m/z: 506 ([M+H]⁺)

[0255] 実施例 175

4-クロロ-N-(1-ヒドロキシシクロプロパンカルボニル)デアセチルコルヒチンの合成

アルゴン雰囲気下、1-ヒドロキシ-1-シクロプロパンカルボン酸 (16 mg, 0.128 × 1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール水合物 (21 mg, 0.128 × 1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、

WO 2011/021397

158

PCT/JP2010/005136

溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 28 mg, 0.059 mmol, 50%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.66 (1H, d, $J = 7.8$ Hz), 7.26 (1H, s), 7.12 (1H, d, $J = 10.5$ Hz), 7.02 (1H, d, $J = 10.7$ Hz), 6.43 (1H, s), 4.35–4.28 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.51 (3H, s), 3.11 (1H, dd, $J = 13.5, 5.5$ Hz), 2.25–2.17 (1H, m), 2.14–2.06 (1H, m), 1.94–1.85 (1H, m), 0.96–0.79 (4H, m).

ESI-MS m/z : 476 ($[\text{M}+\text{H}]^+$)

[0256] 実施例 176

4-クロロ-N-(3-フェニルベンゾイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、3-ビフェニルカルボン酸 (30 mg, 0.128×1.2 mmol) をN,N-ジメチルホルムアミド (1 mL) に溶解し、0°Cに冷却した。そこへ1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (29 mg, 0.128×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水合物 (21 mg, 0.128×1.2 mmol) を加え、0°Cで30分撹拌した。そこへ4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) を加え、室温に戻して3時間撹拌した。反応液に水を加えクエンチし、酢酸エチルを加え、飽和重曹水、飽和食塩水で洗浄した。酢酸エチル層を無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 26 mg, 0.045 mmol, 36%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.18 (1H, d, $J = 7.3$ Hz), 8.18 (1H, t, $J = 1.8$ Hz), 7.86 (1H, dd, $J = 7.8, 1.7$ Hz), 7.75–7.73 (1H, m), 7.58 (1H, t, $J = 7.7$ Hz), 7.53–7.49 (1H, m), 7.44–7.39 (1H, m), 7.24 (1H, s), 7.19 (1H, d, $J = 10.5$ Hz), 7.06 (1H, d, $J = 11.2$ Hz), 4.56–4.50 (1H, m), 3.94 (3H, s), 3.90 (3H, s), 3.89 (3H, s), 3.62 (3H, s), 3.18 (1H, dd, $J = 12.6, 4.0$ Hz), 2.23–2.08 (3H, m).

WO 2011/021397

159

PCT/JP2010/005136

ESI-MS m/z : 572 ($[M+H]^+$)

[0257] 実施例 177

4-クロロ-N-(4-ヒドロキシブチル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロ-N-(4-ベンジルオキシブチル)デアセチルコルヒチン (41 mg, 0.072 mmol) をジクロロメタン (500 μ l) に溶解し、 -78°C に冷却した。そこへボロントリブロミド (1M ジクロロメタン溶液, 72 μ l, 0.072 $\times$ 1 mmol) を加え、 -78°C で30分攪拌した。反応液に飽和重曹水を加えクエンチし、クロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (白色固体, 14 mg, 0.029 mmol, 41%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.59 (1H, d, $J = 7.1$ Hz), 7.13 (1H, d, $J = 10.7$ Hz), 7.11 (1H, s), 7.03 (1H, d, $J = 10.7$ Hz), 4.46 (1H, t, $J = 5.1$ Hz), 4.26–4.20 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.34–3.30 (2H, m), 3.10 (1H, dd, $J = 13.5, 5.5$ Hz), 2.17 (2H, t, $J = 7.6$ Hz), 2.13–2.06 (1H, m), 1.99–1.90 (1H, m), 1.87–1.79 (1H, m), 1.62–1.54 (2H, m).

ESI-MS m/z : 478 ($[M+H]^+$)

[0258] 実施例 178

4-ブロモ-N-(*t*-ブチルオキシカルボニル)コルヒチンの合成

4-ブロモコルヒチン (363 mg, 0.76 mmol) をアセトニトリル (18 mL) に溶解した。そこへジメチルアミノピリジン (93 mg, 0.76 $\times$ 1 mmol)、トリエチルアミン (0.318 mL, 0.76 $\times$ 3 mmol)、二炭酸-*t*-ブチル (0.873 mL, 0.76 $\times$ 5 mmol) を加えて7時間加熱還流した。反応後、クエン酸水溶液を加えた。クロロホルム抽出した。飽和食塩水で洗った。無水硫酸マグネシウムで乾燥した。溶媒を留去し、残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 25 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄土

WO 2011/021397

160

PCT/JP2010/005136

色固体, 338 mg, 76.9%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.21 (1H, s), 7.12 (1H, d, $J = 10.7$ Hz), 7.03 (1H, d, $J = 10.7$ Hz), 4.81 (1H, dd, $J = 12.2, 5.9$ Hz), 3.90 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.56 (3H, s), 3.20 (1H, dd, $J = 13.4, 5.1$ Hz), 2.33–2.27 (1H, m), 2.25 (3H, s), 1.91–1.81 (1H, m), 1.49 (9H, s), 1.44–1.38 (1H, m).

ESI-MS m/z : 578 $[\text{M}+\text{H}]^+$, 580 $[\text{M}+2+\text{H}]^+$.

[0259] 実施例 179

4-ブロモ-N-(*t*-ブチルオキシカルボニル)デアセチルコルヒチンの合成

4-ブロモ-N-(*t*-ブチルオキシカルボニル)コルヒチン(324 mg, 0.56 mmol)をメタノール(6.5 mL)に溶解した。そこへ1 mol/L水酸化ナトリウム水溶液(1.12 mL, 0.56×2 mmol)を加えて室温で5時間攪拌した。飽和食塩水を加えてクロロホルム抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 25 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 280 mg, 93.2%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.77 (1H, d, $J = 7.8$ Hz), 7.20 (1H, s), 7.11 (1H, d, $J = 10.7$ Hz), 7.03 (1H, d, $J = 10.7$ Hz), 4.00–3.94 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.55 (3H, s), 3.08 (1H, dd, $J = 13.2, 5.1$ Hz), 2.18–2.10 (1H, m), 1.97–1.87 (1H, m), 1.83–1.74 (1H, m), 1.33 (9H, s).

ESI-MS m/z : 536 $[\text{M}+\text{H}]^+$, 538 $[\text{M}+2+\text{H}]^+$.

[0260] 実施例 180

4-ブロモデアセチルコルヒチンの合成

4-ブロモ-N-(*t*-ブチルオキシカルボニル)デアセチルコルヒチン(251 mg, 0.47 mmol)にトリフルオロ酢酸(0.663 mL, 0.47×1.9 mmol)を加え、室温で1時間攪拌した。1 mol/L水酸化ナトリウム水溶液でpH=14にし、クロロホルム:メタノール=5:1で抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した

WO 2011/021397

161

PCT/JP2010/005136

。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 185 mg, 90.2%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.65 (1H, s), 7.06 (1H, d, $J = 10.7$ Hz), 6.99 (1H, d, $J = 10.7$ Hz), 3.88 (3H, s), 3.87 (3H, s), 3.85 (3H, s), 3.56 (3H, s), 3.37–3.34 (1H, m), 3.01 (1H, dd, $J = 13.1, 4.8$ Hz), 2.19–2.11 (1H, m), 2.09–1.93 (3H, m), 1.49–1.42 (1H, m).

ESI-MS m/z : 436 $[\text{M}+\text{H}]^+$, 438 $[\text{M}+2+\text{H}]^+$.

[0261] 実施例 1 8 1

4-ブromo-N-プロピオニルデアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン(37 mg, 0.084 mmol)をジクロロメタン(1.0 mL)に溶解した。そこへプロピオニルクロリド(0.009 mL, 0.084×1.2 mmol)、トリエチルアミン(0.018 mL, 0.084×1.5 mmol)を加えて室温で2時間攪拌した。反応後、溶媒を留去し、残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(白色固体, 40 mg, 96.7%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.55 (1H, d, $J = 7.3$ Hz), 7.14–7.11 (2H, m), 7.03 (1H, d, $J = 11.0$ Hz), 4.24–4.18 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.53 (3H, s), 3.11 (1H, dd, $J = 13.8, 5.0$ Hz), 2.23–2.12 (3H, m), 1.99–1.89 (1H, m), 1.84–1.77 (1H, m), 0.95 (3H, t, $J = 7.6$ Hz).

ESI-MS m/z : 492 $[\text{M}+\text{H}]^+$, 494 $[\text{M}+2+\text{H}]^+$.

[0262] 実施例 1 8 2

4-ブromo-N-(シクロヘキサンカルボニル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン(39 mg, 0.089 mmol)をジクロロメタン(1.0 mL)に溶解した。そこへシクロヘキサンカルボニルクロリド(0.014 mL, 0.089×1.2 mmol)、トリエチルアミン(0.019 mL, 0.089×1.5 mmol)を加えて室温で2時間攪拌した。反応後、溶媒を留去し、残渣をシリカゲルカラムクロマト

WO 2011/021397

162

PCT/JP2010/005136

グラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(白色固体, 44 mg, 90.5%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.49 (1H, d, $J = 7.6$ Hz), 7.13–7.10 (2H, m), 7.02 (1H, d, $J = 11.0$ Hz), 4.21–4.14 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.53 (3H, s), 3.11 (1H, dd, $J = 13.4, 5.4$ Hz), 2.23–2.14 (2H, m), 1.97–1.89 (1H, m), 1.87–1.78 (1H, m), 1.72–1.60 (5H, m), 1.30–1.19 (5H, m).

ESI-MS m/z : 546 $[\text{M}+\text{H}]^+$, 548 $[\text{M}+2+\text{H}]^+$.

[0263] 実施例 183

4-ブロモ-N-ベンゾイルデアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(34 mg, 0.078 mmol)をジクロロメタン(1.0 mL)に溶解した。そこへベンゾイルクロリド(0.011 mL, 0.078×1.2 mmol)、トリエチルアミン(0.018 mL, 0.078×1.5 mmol)を加えて室温で1時間攪拌した。反応後、溶媒を留去し、残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 41 mg, 97.3%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.08 (1H, d, $J = 7.3$ Hz), 7.89–7.87 (2H, m), 7.58–7.55 (1H, m), 7.51–7.47 (2H, m), 7.20 (1H, s), 7.17 (1H, d, $J = 10.7$ Hz), 7.05 (1H, d, $J = 10.7$ Hz), 4.50–4.44 (1H, m), 3.93 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.60 (3H, s), 3.17 (1H, dd, $J = 13.3, 5.0$ Hz), 2.30–2.21 (1H, m), 2.15–1.99 (2H, m).

ESI-MS m/z : 540 $[\text{M}+\text{H}]^+$, 542 $[\text{M}+2+\text{H}]^+$.

[0264] 実施例 184

4-ブロモ-N-(フェニルアセチル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(51 mg, 0.117 mmol)をジクロロメタン(1.3 mL)に溶解した。そこへフェニルアセチルクロリド(0.019 mL, 0.117×1.2 mmol)、トリエチルアミン(0.024 mL, 0.117×1.5 mmol)を加えて室温で1時間攪拌した。反応後、溶媒を留去し、残渣をシリカゲルカラムクロマトグラフ

WO 2011/021397

163

PCT/JP2010/005136

イー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 66 mg, quant.)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.89 (1H, d, $J = 7.3$ Hz), 7.28–7.18 (5H, m), 7.16 (1H, s), 7.11 (1H, d, $J = 10.5$ Hz), 7.02 (1H, d, $J = 10.5$ Hz), 4.23–4.16 (1H, m), 3.89 (3H, s), 3.88 (3H, s), 3.85 (3H, s), 3.49 (3H, s), 3.48 (2H, s), 3.12 (1H, dd, $J = 13.4, 5.4$ Hz), 2.25–2.16 (1H, m), 2.01–1.82 (2H, m).

ESI-MS m/z : 554 $[\text{M}+\text{H}]^+$, 556 $[\text{M}+2+\text{H}]^+$.

[0265] 実施例 185

N-アリル-4-ブromo-N-(*t*-ブチルオキシカルボニル)デアセチルコルヒチンの合成

4-ブromo-N-(*t*-ブチルオキシカルボニル)デアセチルコルヒチン(158 mg, 0.29 mmol)をN,N-ジメチルホルムアミド (3.2 mL)に溶解した。そこへ50%水素化ナトリウム(28 mg, 0.29×2 mmol)、アリルブロミド(0.074 mL, 0.29×3 mmol)を加えて、室温で一晩攪拌した。反応後、溶媒を留去し、水を加えてクロロホルム抽出した。飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 25 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 75 mg, 44.9%)を得た。

ESI-MS m/z : 576 $[\text{M}+\text{H}]^+$, 578 $[\text{M}+2+\text{H}]^+$.

[0266] 実施例 186

4-ブromo-N-(トリフルオロアセチル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン(40 mg, 0.09 mmol)をジクロロメタン(1.0 mL)に溶解した。そこへトリフルオロ無水酢酸(0.015 mL, 0.09×1.2 mmol)、トリエチルアミン(0.019mL, 0.09×1.5 mmol)を加えて室温で2時間攪拌した。反応後、溶媒を留去し、残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 41 mg, 85.6%)を得た。

WO 2011/021397

164

PCT/JP2010/005136

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 10.17 (1H, d, $J = 7.1$ Hz), 7.17 (1H, d, $J = 10.7$ Hz), 7.07 (1H, d, $J = 11.0$ Hz), 7.03 (1H, s), 4.31–4.25 (1H, m), 3.91 (3H, s), 3.90 (3H, s), 3.86 (3H, s), 3.54 (3H, s), 3.18–3.13 (1H, m), 2.27–2.19 (1H, m), 2.09–2.03 (2H, m).

ESI-MS m/z : 532 $[\text{M}+\text{H}]^+$, 534 $[\text{M}+2+\text{H}]^+$.

[0267] 実施例 187

N-(アセトキシアセチル)-4-ブロモデアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン (100 mg, 0.23 mmol) をジクロロメタン (2.5 mL) に溶解した。そこへアセトキシアセチルクロリド (0.030 mL, 0.23×1.2 mmol)、トリエチルアミン (0.048 mL, 0.23×1.5 mmol) を加えて室温で2時間攪拌した。反応後、溶媒を留去し、残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 145 mg, quant.) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.80 (1H, d, $J = 7.3$ Hz), 7.14–7.10 (2H, m), 7.03 (1H, d, $J = 10.7$ Hz), 4.50 (2H, s), 4.26–4.20 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.53 (3H, s), 3.12 (1H, dd, $J = 13.7, 4.4$ Hz), 2.24–2.16 (1H, m), 2.05 (3H, s), 1.99–1.83 (2H, m).

ESI-MS m/z : 536 $[\text{M}+\text{H}]^+$, 538 $[\text{M}+2+\text{H}]^+$.

[0268] 実施例 188

4-ブロモ-N-(2-ピリジンカルボニル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン (46 mg, 0.105 mmol) をジクロロメタン (1.2 mL) に溶解した。そこへ2-ピリジンカルボニルクロリド塩酸塩 (22 mg, 0.105×1.2 mmol)、トリエチルアミン (0.022 mL, 0.105×1.5 mmol) を加えて室温で3時間攪拌した。反応後、溶媒を留去し、残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 42 mg, 73.9%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.56 (1H, d, $J = 7.8$ Hz), 8.74 (1H,

WO 2011/021397

165

PCT/JP2010/005136

dq, $J = 4.8, 0.9$ Hz), 7.99 (1H, td, $J = 7.7, 1.4$ Hz), 7.93 (1H, d, $J = 7.7$ Hz), 7.65 (1H, ddd, $J = 7.7, 4.8, 1.4$ Hz), 7.21 (1H, s), 7.16 (1H, d, $J = 10.7$ Hz), 7.05 (1H, d, $J = 10.7$ Hz), 4.53–4.47 (1H, m), 3.93 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.60 (3H, s), 3.16 (1H, dd, $J = 13.5, 6.0$ Hz), 2.37–2.21 (2H, m), 2.03–1.94 (1H, m).

ESI-MS m/z : 542 $[M+H]^+$, 544 $[M+2+H]^+$.

[0269] 実施例 189

N-アリル-4-ブロモデアセチルコルヒチンの合成

N-アリル-4-ブロモ-N-(*t*-ブチルオキシカルボニル)デアセチルコルヒチン(90 mg, 0.156 mmol)にトリフルオロ酢酸(0.22 mL, 0.156×19 mmol)を加えて室温で1時間攪拌した。クロロホルムを加えて、1 mol/L水酸化ナトリウム水溶液を加えてpH=14にした。クロロホルム:メタノール=5:1で抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 39 mg, 52.5%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO- d_6) δ [ppm]: 7.56 (1H, s), 7.11 (1H, d, $J = 10.5$ Hz), 7.01 (1H, d, $J = 10.5$ Hz), 5.71 (1H, ddd, $J = 22.0, 10.6, 6.2$ Hz), 4.98 (1H, dd, $J = 17.3, 1.7$ Hz), 4.92 (1H, dd, $J = 10.6, 1.7$ Hz), 3.89 (6H, s), 3.85 (3H, s), 3.48 (3H, s), 3.14–3.08 (1H, m), 3.06–3.00 (2H, m), 2.86–2.79 (1H, m), 2.61–2.56 (1H, m), 2.18–2.00 (2H, m), 1.56–1.49 (1H, m).

ESI-MS m/z : 477 $[M+H]^+$, 479 $[M+2+H]^+$.

[0270] 実施例 190

N-(ベンジルオキシカルボニル)-4-ブロモデアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(63 mg, 0.144 mmol)をジクロロメタン(1.3 mL)に溶解した。ベンジルオキシカルボニルクロリド(0.025 mL, 0.144×1.2 mmol)、トリエチルアミン(0.030 mL, 0.144×1.5 mmol)を加えて室温で1晩攪拌した。反応後、溶媒を留去し、残渣をシリカゲルカラムクロマトグラフ

WO 2011/021397

166

PCT/JP2010/005136

イー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 18 mg, 21.9%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.23 (1H, d, $J = 7.3$ Hz), 7.37–7.30 (5H, m), 7.21 (1H, s), 7.13 (1H, d, $J = 11.0$ Hz), 7.04 (1H, d, $J = 11.0$ Hz), 4.98 (2H, d, $J = 5.9$ Hz), 4.07–4.01 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.52 (3H, s), 3.10 (1H, dd, $J = 13.3, 5.2$ Hz), 2.21–2.12 (1H, m), 2.03–1.93 (1H, m), 1.84–1.77 (1H, m).

ESI-MS m/z : 571 $[\text{M}+\text{H}]^+$, 573 $[\text{M}+2+\text{H}]^+$.

[0271] 実施例 191

4-ブロモ-N-[(メチルチオ)アセチル]デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(126 mg, 0.29 mmol)をN,N-ジメチルホルムアミド(2.5 mL)に溶解し、氷冷した。そこへメチルチオ酢酸(0.030 mL, 0.29×1.2 mmol)、1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(67 mg, 0.29×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物(47 mg, 0.29×1.2 mmol)を加えて室温で2時間攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、1 mol/L塩酸、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 112 mg, 73.6%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.77 (1H, d, $J = 7.3$ Hz), 7.16 (1H, s), 7.12 (1H, d, $J = 11.0$ Hz), 7.03 (1H, d, $J = 11.0$ Hz), 4.24–4.18 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.54 (3H, s), 3.15–3.13 (1H, m), 3.11 (2H, d, $J = 6.8$ Hz), 2.25–2.16 (1H, m), 2.06 (3H, s), 2.01–1.91 (1H, m), 1.88–1.80 (1H, m).

ESI-MS m/z : 525 $[\text{M}+\text{H}]^+$, 527 $[\text{M}+2+\text{H}]^+$.

[0272] 実施例 192

4-ブロモ-N-[(N',N'-ジメチルアミノ)アセチル]デアセチルコルヒチンの合成

WO 2011/021397

167

PCT/JP2010/005136

4-ブロモデアセチルコルヒチン(124 mg, 0.28 mmol)をN,N-ジメチルホルムアミド(2.5 mL)に溶解し、氷冷した。そこへN,N-ジメチルアミノ酢酸(35 mg, 0.28×1.2 mmol)、1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(65 mg, 0.28×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水合物(46 mg, 0.28×1.2 mmol)を加えて室温で2.5時間攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、1 mol/L塩酸、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 109 mg, 74.7%)を得た。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 8.58 (1H, d, J = 7.6 Hz), 7.15 (1H, s), 7.12 (1H, d, J = 11.0 Hz), 7.02 (1H, d, J = 11.0 Hz), 4.30-4.24 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.54 (3H, s), 3.11 (1H, dd, J = 13.4, 5.1 Hz), 2.94-2.84 (2H, m), 2.21 (6H, s), 2.18-2.14 (1H, m), 2.06-1.98 (1H, m), 1.94-1.85 (1H, m).

ESI-MS m/z: 522 [M+H]⁺, 524 [M+2+H]⁺.

[0273] 実施例 193

4-ブromo-N-プロピルデアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(96 mg, 0.22 mmol)をメタノール(2.0 mL)に溶解し、プロピオンアルデヒド(0.017 mL, 0.22×1.1 mmol)、オルトギ酸トリメチル(0.041 mL, 0.22×1.5 mmol)を加えて室温で1時間攪拌した。氷冷し、水素化ホウ素ナトリウム(12 mg, 0.22×1.5 mmol)を加えて室温で1時間攪拌した。水を加えて、クロロホルム抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 43 mg, 40.9%)を得た。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 7.56 (1H, s), 7.10 (1H, d, J = 10.5 Hz), 7.01 (1H, d, J = 10.5 Hz), 3.90 (3H, s), 3.89 (3H, s), 3.86 (3H,

WO 2011/021397

168

PCT/JP2010/005136

s), 3.50 (3H, s), 3.07–3.01 (2H, m), 2.34–2.20 (2H, m), 2.18–2.01 (3H, m), 1.54–1.47 (1H, m), 1.39–1.30 (2H, m), 0.81 (3H, t, $J = 7.3$ Hz).

ESI-MS m/z : 479 $[M+H]^+$, 481 $[M+2+H]^+$.

[0274] 実施例 194

4-ブromo-N-(ヘキシルカルバモイル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン (68 mg, 0.16 mmol) をジクロロメタン (1.4 mL) に溶解し、ヘキシルイソシアネート (0.047 mL, 0.16×2 mmol) を加えて室温で3時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 80 mg, 88.7%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.18 (1H, s), 7.10 (1H, d, $J = 10.5$ Hz), 7.02 (1H, d, $J = 10.5$ Hz), 6.66 (1H, d, $J = 7.6$ Hz), 5.92 (1H, t, $J = 5.7$ Hz), 4.13–4.07 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.52 (3H, s), 3.09 (1H, dd, $J = 13.5, 5.5$ Hz), 2.97–2.83 (2H, m), 2.21–2.13 (1H, m), 2.00–1.91 (1H, m), 1.71–1.64 (1H, m), 1.31–1.17 (8H, m), 0.83 (3H, t, $J = 6.7$ Hz).

ESI-MS m/z : 564 $[M+H]^+$, 566 $[M+2+H]^+$.

[0275] 実施例 195

4-ブromo-N-(フェニルカルバモイル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン (71 mg, 0.16 mmol) をジクロロメタン (1.4 mL) に溶解し、フェニルイソシアネート (0.035 mL, 0.16×2 mmol) を加えて室温で4時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 74 mg, 83.3%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.55 (1H, s), 7.32–7.29 (2H, m), 7.25 (1H, s), 7.20–7.13 (3H, m), 7.04 (1H, d, $J = 11.2$ Hz), 7.00 (1H, d, $J = 7.3$ Hz), 6.88 (1H, t, $J = 7.3$ Hz), 4.21–4.15 (1H, m), 3.92 (3H,

WO 2011/021397

169

PCT/JP2010/005136

s), 3.89 (3H, s), 3.87 (3H, s), 3.56 (3H, s), 3.13 (1H, dd, $J = 13.8$, 5.2 Hz), 2.26–2.18 (1H, m), 2.11–2.01 (1H, m), 1.81–1.74 (1H, m).

ESI-MS m/z : 556 $[M+H]^+$, 558 $[M+2+H]^+$.

[0276] 実施例 196

4-ブromo-N-(ヒドロキシアセチル)デアセチルコルヒチンの合成

N-(アセトキシアセチル)-4-ブromoデアセチルコルヒチン (58 mg, 0.11 mmol) をメタノール (1.2 mL)、ジクロロメタン (1.2 mL) に溶解した。そこへナトリウムメトキシド (12 mg, 0.11×2 mmol) を加えて室温で2時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 31 mg, 57.0%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.54 (1H, d, $J = 7.8$ Hz), 7.16 (1H, s), 7.12 (1H, d, $J = 10.5$ Hz), 7.03 (1H, d, $J = 10.5$ Hz), 5.54 (1H, t, $J = 5.9$ Hz), 4.33–4.26 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.85 (3H, s), 3.85–3.82 (2H, m), 3.83–3.80 (2H, m), 3.53 (3H, s), 3.11 (1H, dd, $J = 13.9$, 5.6 Hz), 2.23–2.15 (1H, m), 2.11–2.04 (1H, m), 1.93–1.85 (1H, m).

ESI-MS m/z : 495 $[M+H]^+$, 497 $[M+2+H]^+$.

[0277] 実施例 197

4-ブromo-N-(イソブタンシルホニル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン (57 mg, 0.13 mmol) をジクロロメタン (1.0 mL) に溶解した。そこへイソブタンシルホニルクロリド (0.02 mL, 0.13×1.2 mmol)、トリエチルアミン (0.027 mL, 0.13×1.5 mmol) を加えて室温で2時間攪拌した。反応後、1 mol/L塩酸を加えてクロロホルム抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 39 mg, 53.9%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.11 (1H, d, $J = 8.1$ Hz), 7.43 (1H,

WO 2011/021397

170

PCT/JP2010/005136

s), 7.12 (1H, d, $J = 10.7$ Hz), 7.04 (1H, d, $J = 10.7$ Hz), 3.91 (3H, s), 3.91–3.90 (1H, m), 3.90 (3H, s), 3.87 (3H, s), 3.48 (3H, s), 3.10 (1H, dd, $J = 13.9, 4.6$ Hz), 2.73 (2H, dd, $J = 6.3, 2.2$ Hz), 2.19–2.11 (1H, m), 2.07–1.94 (2H, m), 1.85–1.78 (1H, m), 0.90 (6H, dd, $J = 6.8, 2.2$ Hz).

ESI-MS m/z : 557 $[M+H]^+$, 559 $[M+2+H]^+$.

[0278] 実施例 198

4-ブロモ-N-トシルデアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン (60 mg, 0.14 mmol) をジクロロメタン (1.2 mL) に溶解した。そこへトシルクロリド (32 mg, 0.14×1.2 mmol)、トリエチルアミン (0.029 mL, 0.14×1.5 mmol) を加えて室温で一晩攪拌した。反応後、1 mol/L 塩酸を加えてクロロホルム抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 75 mg, 90.7%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO- d_6) δ [ppm]: 8.50 (1H, d, $J = 7.6$ Hz), 7.39 (1H, s), 7.37 (1H, s), 7.23 (1H, s), 7.22 (1H, s), 7.21 (1H, s), 7.08 (1H, d, $J = 10.5$ Hz), 6.98 (1H, d, $J = 10.5$ Hz), 3.92 (3H, s), 3.88 (3H, s), 3.85 (3H, s), 3.53 (3H, s), 3.51–3.48 (1H, m), 2.93 (1H, dd, $J = 13.9, 5.6$ Hz), 2.31 (3H, s), 2.09–2.00 (1H, m), 1.71–1.63 (1H, m), 1.55–1.48 (1H, m).

ESI-MS m/z : 591 $[M+H]^+$, 593 $[M+2+H]^+$.

[0279] 実施例 199

4-ブロモ-N-(2-チオフエンスルホニル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン (66 mg, 0.15 mmol) をジクロロメタン (1.3 mL) に溶解した。そこへ2-チオフエンスルホニルクロリド (33 mg, 0.15×1.2 mmol)、トリエチルアミン (0.031 mL, 0.15×1.5 mmol) を加えて室温で一晩攪拌した。反応後、1 mol/L 塩酸を加えてクロロホルム抽出した。無水硫酸マグ

WO 2011/021397

171

PCT/JP2010/005136

ネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 76 mg, 87.0%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.83 (1H, d, $J = 7.6$ Hz), 7.84 (1H, dd, $J = 4.9, 1.5$ Hz), 7.30 (1H, dd, $J = 3.8, 1.5$ Hz), 7.27 (1H, s), 7.11 (1H, d, $J = 10.7$ Hz), 7.05–7.00 (2H, m), 3.92 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.71–3.64 (1H, m), 3.52 (3H, s), 2.99 (1H, dd, $J = 13.5, 5.5$ Hz), 2.14–2.05 (1H, m), 1.78–1.58 (2H, m).

ESI-MS m/z : 583 $[\text{M}+\text{H}]^+$, 585 $[\text{M}+2+\text{H}]^+$.

[0280] 実施例 200

4-ブromo-N-(2,4-ジフルオロベンゾイル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン(62 mg, 0.14 mmol)をN,N-ジメチルホルムアミド(1.3 mL)に溶解し、氷冷した。そこへ2,4-ジフルオロ安息香酸(27 mg, 0.14×1.2 mmol)、1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(33 mg, 0.14×1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水合物(23 mg, 0.14×1.2 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、水、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 41 mg, 50.8%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.12 (1H, d, $J = 7.1$ Hz), 7.62 (1H, dd, $J = 15.1, 8.3$ Hz), 7.39 (1H, td, $J = 10.0, 2.1$ Hz), 7.24 (1H, s), 7.20 (1H, dd, $J = 8.3, 2.4$ Hz), 7.16 (1H, d, $J = 10.7$ Hz), 7.05 (1H, d, $J = 10.7$ Hz), 4.44–4.38 (1H, m), 3.93 (3H, s), 3.90 (3H, s), 3.88 (3H, s), 3.58 (3H, s), 3.15 (1H, dd, $J = 13.3, 4.8$ Hz), 2.28–2.20 (1H, m), 2.08–1.94 (2H, m).

ESI-MS m/z : 577 $[\text{M}+\text{H}]^+$, 578 $[\text{M}+2+\text{H}]^+$.

WO 2011/021397

172

PCT/JP2010/005136

[0281] 実施例 201

4-ブロモ-N-[3-(N', N'-ジメチルアミノ)ベンゾイル]デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(63 mg, 0.144 mmol)をN,N-ジメチルホルムアミド(1.3 mL)に溶解し、氷冷した。そこへ3-(N,N-ジメチルアミノ)安息香酸(29 mg, 0.144×1.2 mmol)、1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(33 mg, 0.144×1.2 mmol)、1-ヒドロキシベンゾトリアゾール-水和物(23 mg, 0.144×1.2 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、水、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 75 mg, 89.3%)を得た。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 8.94 (1H, d, J = 7.3 Hz), 7.28 (1H, t, J = 7.8 Hz), 7.20-7.13 (4H, m), 7.05 (1H, d, J = 11.0 Hz), 6.89 (1H, dd, J = 7.9, 2.3 Hz), 4.49-4.43 (1H, m), 3.93 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.60 (3H, s), 3.17 (1H, dd, J = 13.1, 4.8 Hz), 2.92 (6H, s), 2.29-2.21 (1H, m), 2.16-1.98 (2H, m).

ESI-MS m/z: 584 [M+H]⁺, 586 [M+2+H]⁺.

[0282] 実施例 202

4-ブロモ-N-(2-フルオロベンゾイル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(46 mg, 0.11 mmol)をジクロロメタン(1.0 mL)に溶解した。そこへ2-フルオロベンゾイルクロリド(0.016 mL, 0.11×1.2 mmol)、トリエチルアミン(0.023 mL, 0.11×1.5 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 56 mg, 91.2%)を得た。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 9.13 (1H, d, J = 7.1 Hz), 7.58-7.52

WO 2011/021397

173

PCT/JP2010/005136

(2H, m), 7.34–7.29 (2H, m), 7.27 (1H, s), 7.16 (1H, d, $J = 10.7$ Hz), 7.06 (1H, d, $J = 10.7$ Hz), 4.45–4.39 (1H, m), 3.93 (3H, s), 3.90 (3H, s), 3.88 (3H, s), 3.59 (3H, s), 3.17–3.13 (1H, m), 2.28–2.20 (1H, m), 2.07–1.95 (2H, m).

ESI-MS m/z : 559 $[M+H]^+$, 561 $[M+2+H]^+$.

[0283] 実施例 203

4-ブロモ-N-(3-メトキシベンゾイル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(48 mg, 0.11 mmol)をジクロロメタン(1.0 mL)に溶解した。そこへ3-メトキシベンゾイルクロリド(0.018 mL, 0.11×1.2 mmol)、トリエチルアミン(0.023 mL, 0.11×1.5 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 63 mg, quant.)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.05 (1H, d, $J = 7.3$ Hz), 7.49 (1H, d, $J = 7.8$ Hz), 7.43–7.39 (2H, m), 7.19 (1H, s), 7.17 (1H, d, $J = 10.7$ Hz), 7.13 (1H, dd, $J = 8.3, 2.4$ Hz), 7.05 (1H, d, $J = 10.7$ Hz), 4.49–4.43 (1H, m), 3.93 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.80 (3H, s), 3.60 (3H, s), 3.17 (1H, dd, $J = 13.9, 4.9$ Hz), 2.30–2.21 (1H, m), 2.15–2.01 (2H, m).

ESI-MS m/z : 571 $[M+H]^+$, 573 $[M+2+H]^+$.

[0284] 実施例 204

4-ブロモ-N-(エチルチオカルバモイル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(68 mg, 0.16 mmol)をジクロロメタン(1.4 mL)に溶解した。そこへエチルイソチオシアネート(0.028 mL, 0.16×2 mmol)を加えて室温で4時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 33 mg, 39.4%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.97 (1H, br s), 7.52 (1H, br s), 7.

WO 2011/021397

174

PCT/JP2010/005136

14 (1H, d, $J = 10.5$ Hz), 7.06 (1H, s), 7.03 (1H, d, $J = 10.5$ Hz), 4.79–4.70 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.60 (3H, s), 3.41–3.27 (2H, m), 3.12 (1H, dd, $J = 13.3, 5.7$ Hz), 2.25–2.17 (1H, m), 2.09–1.81 (2H, m), 1.05 (3H, t, $J = 7.1$ Hz).

ESI-MS m/z : 524 $[M+H]^+$, 526 $[M+2+H]^+$.

[0285] 実施例 205

4-ブロモ-N-(フェニルチオカルバモイル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン (68 mg, 0.16 mmol) をジクロロメタン (1.4 mL) に溶解した。そこへフェニルイソチオシアネート (0.038 mL, 0.16×2 mmol) を加えて室温で5時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 97 mg, quant.) を得た。
 $^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.70 (1H, br s), 8.43 (1H, d, $J = 7.1$ Hz), 7.46 (1H, s), 7.44 (1H, s), 7.32–7.28 (2H, m), 7.17–7.03 (4H, m), 4.80–4.74 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.62 (3H, s), 3.15 (1H, dd, $J = 13.1, 4.8$ Hz), 2.28–2.09 (2H, m), 2.02–1.95 (1H, m).

ESI-MS m/z : 572 $[M+H]^+$, 574 $[M+2+H]^+$.

[0286] 実施例 206

4-ブロモ-N-(N', N'-ジエチルカルバモイル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン (113 mg, 0.26 mmol) を1,2-ジクロロエタン (2.2 mL) に溶解した。そこへジエチルカルバモイルクロリド (0.049 mL, 0.26×1.5 mmol)、トリエチルアミン (0.072 mL, 0.26×2 mmol) を加えて3時間加熱還流した。反応後、1 mol/L塩酸を加えてクロロホルム抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 51 mg, 36.6%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.21 (1H, s), 7.10 (1H, d, $J = 10.7$

WO 2011/021397

175

PCT/JP2010/005136

Hz), 7.01 (1H, d, $J = 10.7$ Hz), 6.76 (1H, d, $J = 7.6$ Hz), 4.25–4.18 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.53 (3H, s), 3.29–3.15 (4H, m), 3.11 (1H, dd, $J = 13.7, 5.1$ Hz), 2.21–2.12 (1H, m), 2.04–1.96 (1H, m), 1.93–1.85 (1H, m), 1.00 (6H, t, $J = 7.0$ Hz).

ESI-MS m/z : 536 $[M+H]^+$, 538 $[M+2+H]^+$.

[0287] 実施例 207

4-ブロモ-N-(ピペリジン-1-イルカルボニル) デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(107 mg, 0.25 mmol)を1,2-ジクロロエタン(2.1 mL)に溶解した。そこへ1-ピペリジんカルボニルクロリド(0.047 mL, 0.25×1.5 mmol)、トリエチルアミン(0.070 mL, 0.25×2 mmol)を加えて3時間加熱還流した。反応後、1 mol/L塩酸を加えてクロロホルム抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 105 mg, 76.7%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO- d_6) δ [ppm]: 7.18 (1H, s), 7.11 (1H, d, $J = 10.5$ Hz), 7.03–6.98 (2H, m), 4.20–4.13 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.53 (3H, s), 3.30–3.24 (4H, m), 3.10 (1H, dd, $J = 13.9, 3.7$ Hz), 2.21–2.12 (1H, m), 1.98–1.85 (2H, m), 1.55–1.50 (2H, m), 1.42–1.36 (4H, m).

ESI-MS m/z : 548 $[M+H]^+$, 550 $[M+2+H]^+$.

[0288] 実施例 208

4-ブロモ-N-(3-ヒドロキシ-2,2-ジメチルプロピオニル) デアセチルコルヒチンの合成

ヒドロキシピバル酸(20 mg, 0.14×1.2 mmol)をN,N-ジメチルホルムアミド(0.4 mL)に溶解した。1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(33 mg, 0.14×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水合物(23 mg, 0.14×1.2 mmol)を加えて室温で30分攪拌した。そこへ4-ブロモデアセチルコルヒチン(62 mg, 0.14 mmol)を加えて室温で一晩攪拌した。反

WO 2011/021397

176

PCT/JP2010/005136

応後、溶媒を留去した。クロロホルムに溶解し、1mol/L塩酸、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 79 mg, quant.)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.02 (1H, d, $J = 7.3$ Hz), 7.13–7.10 (2H, m), 7.02 (1H, d, $J = 11.0$ Hz), 4.80 (1H, t, $J = 5.4$ Hz), 4.25–4.19 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.55 (3H, s), 3.42–3.34 (2H, m), 3.11 (1H, dd, $J = 13.5, 5.0$ Hz), 1.07 (3H, s), 1.00 (3H, s).

ESI-MS m/z : 537 $[\text{M}+\text{H}]^+$, 539 $[\text{M}+2+\text{H}]^+$.

[0289] 実施例 209

4-ブromo-N-(2-ヒドロキシ-2-メチルプロピオニル)デアセチルコルヒチンの合成

2-ヒドロキシイソ酪酸(18 mg, 0.14×1.2 mmol)をN,N-ジメチルホルムアミド(0.4 mL)に溶解した。1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(33 mg, 0.14×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物(23 mg, 0.14×1.2 mmol)を加えて室温で30分攪拌した。そこへ4-ブromoデアセチルコルヒチン(62 mg, 0.14 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、1mol/L塩酸、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 79 mg, quant.)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.38 (1H, d, $J = 7.6$ Hz), 7.17 (1H, s), 7.11 (1H, d, $J = 10.7$ Hz), 7.02 (1H, d, $J = 10.7$ Hz), 5.49 (1H, s), 4.22–4.15 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.54 (3H, s), 3.11 (1H, dd, $J = 12.8, 5.7$ Hz), 2.23–2.08 (2H, m), 1.94–1.8

WO 2011/021397

177

PCT/JP2010/005136

3 (1H, m), 1.21 (3H, s), 1.20 (3H, s).

ESI-MS m/z : 523 $[M+H]^+$, 525 $[M+2+H]^+$.

[0290] 実施例 2 1 0

4-ブロモ-N-(2-エチル-2-ヒドロキシブチリル)デアセチルコルヒチンの合成

2-エチル-2-ヒドロキシ酪酸 (23 mg, 0.14×1.2 mmol) を N,N-ジメチルホルムアミド (0.4 mL) に溶解した。1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (33 mg, 0.14×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (23 mg, 0.14×1.2 mmol) を加えて室温で 30 分攪拌した。そこへ 4-ブロモデアセチルコルヒチン (62 mg, 0.14 mmol) を加えて室温で一晩攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、1 mol/L 塩酸、1 mol/L 水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage IsoleraOne, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 68 mg, 88.2%) を得た。

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ [ppm]: 8.32 (1H, d, $J = 6.8$ Hz), 7.22 (1H, s), 7.10 (1H, d, $J = 10.7$ Hz), 7.01 (1H, d, $J = 10.7$ Hz), 4.97 (1H, s), 4.32-4.25 (1H, m), 3.92 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.55 (3H, s), 3.11 (1H, dd, $J = 12.7, 5.6$ Hz), 2.23-2.11 (2H, m), 1.89-1.79 (1H, m), 1.65-1.56 (2H, m), 1.47-1.38 (2H, m), 0.76-0.69 (6H, m).

ESI-MS m/z : 551 $[M+H]^+$, 553 $[M+2+H]^+$.

[0291] 実施例 2 1 1

4-ブロモ-N-(1-ヒドロキシシクロプロパンカルボニル)デアセチルコルヒチンの合成

1-ヒドロキシ-1-シクロプロパンカルボン酸 (17 mg, 0.14×1.2 mmol) を N,N-ジメチルホルムアミド (0.4 mL) に溶解した。1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (33 mg, 0.14×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (23 mg, 0.14×1.2 mmol) を加えて室温で 1 時間攪拌した。そこへ 4-ブロモデアセチルコルヒチン (62 mg, 0.14 mmol) を加えて室

WO 2011/021397

178

PCT/JP2010/005136

温で一晩攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、1mol/L塩酸、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 67 mg, 92.0%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.66 (1H, d, $J = 7.8$ Hz), 7.25 (1H, s), 7.11 (1H, d, $J = 10.5$ Hz), 7.02 (1H, d, $J = 10.5$ Hz), 6.43 (1H, s), 4.32–4.26 (1H, m), 3.90 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.51 (3H, s), 3.11 (1H, dd, $J = 12.6, 6.0$ Hz), 2.23–2.15 (2H, m), 1.94–1.84 (1H, m), 0.95–0.79 (4H, m).

ESI-MS m/z : 521 $[\text{M}+\text{H}]^+$, 523 $[\text{M}+2+\text{H}]^+$.

[0292] 実施例 2 1 2

4-ブromo-N-[2,2-ビス(ヒドロキシメチル)プロピオニル]デアセチルコルヒチンの合成

2,2-ビス(ヒドロキシメチル)プロピオン酸 (23 mg, 0.14×1.2 mmol) をN,N-ジメチルホルムアミド(0.4 mL)に溶解した。1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(33 mg, 0.14×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物(23 mg, 0.14×1.2 mmol)を加えて室温で1時間攪拌した。そこへ4-ブromoデアセチルコルヒチン(62mg, 0.14 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、1mol/L塩酸、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 74 mg, 95.6%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.02 (1H, d, $J = 7.1$ Hz), 7.15 (1H, s), 7.11 (1H, d, $J = 10.5$ Hz), 7.01 (1H, d, $J = 10.5$ Hz), 4.73 (1H, t, $J = 5.2$ Hz), 4.62 (1H, t, $J = 5.4$ Hz), 4.26–4.20 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.55 (3H, s), 3.48 (2H, d, $J = 5.4$ Hz).

WO 2011/021397

179

PCT/JP2010/005136

z), 3.43 (2H, dd, $J = 5.2, 2.6$ Hz), 3.14–3.08 (1H, m), 2.23–2.15 (1H, m), 1.98–1.90 (2H, m), 1.00 (3H, s).

ESI-MS m/z : 553 $[M+H]^+$, 555 $[M+2+H]^+$.

[0293] 実施例 2 1 3

4-ブロモ-N-(3-ヒドロキシ-3-メチルブチリル)デアセチルコルヒチンの合成
 β -ヒドロキシイソ吉草酸(41 mg, 0.29×1.2 mmol)をN,N-ジメチルホルムアミド(0.8 mL)に溶解した。1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(67 mg, 0.29×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物(47 mg, 0.29×1.2 mmol)を加えて室温で30分攪拌した。そこへ4-ブロモデアセチルコルヒチン(127 mg, 0.29 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、1mol/L塩酸、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage IsoleraOne, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 153 mg, 98.4%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.59 (1H, d, $J = 7.3$ Hz), 7.17 (1H, s), 7.12 (1H, d, $J = 10.7$ Hz), 7.03 (1H, d, $J = 10.7$ Hz), 4.63 (1H, s), 4.27–4.21 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.55 (3H, s), 3.11 (1H, dd, $J = 13.7, 4.9$ Hz), 2.26 (2H, s), 2.24–2.15 (1H, m), 2.00–1.90 (1H, m), 1.87–1.79 (1H, m), 1.12 (6H, d, $J = 3.7$ Hz).

ESI-MS m/z : 537 $[M+H]^+$, 539 $[M+2+H]^+$.

[0294] 実施例 2 1 4

4-ブロモ-N-[4-(ベンジルオキシ)ブチリル]デアセチルコルヒチンの合成

4-ベンジルオキシ酪酸(0.060 mL, 0.28×1.2 mmol)をN,N-ジメチルホルムアミド(1.2 mL)に溶解した。1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(65 mg, 0.28×1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物(46 mg, 0.28×1.2 mmol)を加えて室温で30分攪拌した。そこへ4-ブロモデアセチルコルヒチン(124 mg, 0.28 mmol)を加えて室温で一晩攪拌し

WO 2011/021397

180

PCT/JP2010/005136

た。反応後、溶媒を留去した。クロロホルムに溶解し、1mol/L塩酸、1 mol/L水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 155 mg, 90.4%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.60 (1H, d, $J = 7.3$ Hz), 7.34–7.25 (5H, m), 7.13–7.11 (2H, m), 7.03 (1H, d, $J = 11.0$ Hz), 4.44 (2H, dd, $J = 17.4, 12.1$ Hz), 4.24–4.18 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.53 (3H, s), 3.37 (2H, t, $J = 6.3$ Hz), 3.11 (1H, dd, $J = 14.1, 4.9$ Hz), 2.25–2.21 (2H, m), 2.19–2.14 (1H, m), 1.97–1.88 (1H, m), 1.84–1.78 (1H, m), 1.76–1.68 (2H, m).

ESI-MS m/z : 613 $[\text{M}+\text{H}]^+$, 615 $[\text{M}+2+\text{H}]^+$.

[0295] 実施例 2 1 5

4-ブロモ-N-(3-ニトロベンゾイル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(67 mg, 0.15 mmol)をジクロロメタン(0.7 mL)に溶解した。そこへ3-ニトロベンゾイルクロリド(34 mg, 0.15×1.2 mmol)、トリエチルアミン(0.031 mL, 0.15×1.5 mmol)を加えて室温で3時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 77 mg, 87.7%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.46 (1H, d, $J = 7.1$ Hz), 8.77 (1H, t, $J = 2.0$ Hz), 8.42 (1H, dq, $J = 8.2, 1.0$ Hz), 8.32 (1H, d, $J = 7.9$ Hz), 7.81 (1H, t, $J = 7.9$ Hz), 7.19–7.17 (2H, m), 7.06 (1H, d, $J = 11.0$ Hz), 4.53–4.46 (1H, m), 3.93 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.61 (3H, s), 3.21–3.17 (1H, m), 2.33–2.24 (1H, m), 2.15–2.08 (2H, m).

ESI-MS m/z : 586 $[\text{M}+\text{H}]^+$, 588 $[\text{M}+2+\text{H}]^+$.

[0296] 実施例 2 1 6

WO 2011/021397

181

PCT/JP2010/005136

4-ブromo-N-(4-シアノベンゾイル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン(62 mg, 0.14 mmol)をジクロロメタン(0.6 mL)に溶解した。そこへ4-シアノベンゾイルクロリド(28 mg, 0.14×1.2 mmol)、トリエチルアミン(0.029 mL, 0.14×1.5 mmol)を加えて室温で3時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 72 mg, 90.9%)を得た。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 9.32 (1H, d, J = 7.1 Hz), 8.05–7.98 (4H, m), 7.19–7.16 (2H, m), 7.06 (1H, d, J = 11.0 Hz), 4.49–4.43 (1H, m), 3.93 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.60 (3H, s), 3.20–3.15 (1H, m), 2.30–2.22 (1H, m), 2.12–2.05 (2H, m).

ESI-MS m/z: 566 [M+H]⁺, 568 [M+2+H]⁺

[0297] 実施例 2 1 7

4-ブromo-N-(3,5-ジメチルベンゾイル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン(65 mg, 0.15 mmol)をジクロロメタン(0.7 mL)に溶解した。そこへ3,5-ジメチルベンゾイルクロリド(30 mg, 0.15×1.2 mmol)、トリエチルアミン(0.031 mL, 0.15×1.5 mmol)を加えて室温で3時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 56 mg, 65.7%)を得た。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 8.99 (1H, d, J = 7.1 Hz), 7.50 (2H, s), 7.19–7.15 (3H, m), 7.05 (1H, d, J = 11.2 Hz), 4.48–4.42 (1H, m), 3.93 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.60 (3H, s), 3.17 (1H, dd, J = 13.5, 5.2 Hz), 2.32 (6H, s), 2.29–2.21 (1H, m), 2.16–1.97 (2H, m).

ESI-MS m/z: 569 [M+H]⁺, 571 [M+2+H]⁺

[0298] 実施例 2 1 8

4-ブromo-N-(4-メチルベンゾイル)デアセチルコルヒチンの合成

WO 2011/021397

182

PCT/JP2010/005136

p-トルイル酸 (23 mg, 0.14 × 1.2 mmol) を N,N-ジメチルホルムアミド (0.4 mL) に溶解した。そこへ 1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (33 mg, 0.14 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (23 mg, 0.14 × 1.2 mmol) を加えて室温で 30 分攪拌した。そこへ 4-ブロモデアセチルコルヒチン (62 mg, 0.14 mmol) を加えて室温で一晩攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、1 mol/L 塩酸、1 mol/L 水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 61 mg, 78.6%) を得た。

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 8.99 (1H, d, J = 7.3 Hz), 7.80 (2H, d, J = 8.1 Hz), 7.29 (2H, d, J = 8.1 Hz), 7.19 (1H, s), 7.16 (1H, d, J = 10.7 Hz), 7.05 (1H, d, J = 10.7 Hz), 4.49-4.43 (1H, m), 3.93 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.60 (3H, s), 3.17 (1H, dd, J = 13.4, 5.4 Hz), 2.36 (3H, s), 2.29-2.21 (1H, m), 2.15-1.97 (2H, m).

ESI-MS m/z: 555 [M+H]⁺, 557 [M+2+H]⁺

[0299] 実施例 219

4-ブロモ-N-[3-(ピロリジン-1-イル)ベンゾイル]デアセチルコルヒチンの合成

3-(ピロリジン-1-イル)安息香酸 (33 mg, 0.14 × 1.2 mmol) を N,N-ジメチルホルムアミド (0.6 mL) に溶解した。そこへ 1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩 (33 mg, 0.14 × 1.2 mmol)、1-ヒドロキシベンゾトリアゾール水和物 (23 mg, 0.14 × 1.2 mmol) を加えて室温で 30 分攪拌した。そこへ 4-ブロモデアセチルコルヒチン (62 mg, 0.14 mmol) を加えて室温で一晩攪拌した。反応後、溶媒を留去した。クロロホルムに溶解し、1 mol/L 塩酸、1 mol/L 水酸化ナトリウム水溶液、飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し

WO 2011/021397

183

PCT/JP2010/005136

、標記の化合物(黄色固体, 10 mg, 11.7%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.93 (1H, d, $J = 7.3$ Hz), 7.27–7.12 (4H, m), 7.05 (1H, d, $J = 11.0$ Hz), 6.95 (1H, s), 6.69 (1H, dd, $J = 8.2, 2.1$ Hz), 4.49–4.42 (1H, m), 3.93 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.60 (3H, s), 3.24 (4H, t, $J = 6.6$ Hz), 3.17 (1H, dd, $J = 13.7, 5.1$ Hz), 2.29–2.21 (1H, m), 2.16–2.08 (1H, m), 2.07–1.98 (1H, m), 1.97–1.94 (4H, m).

ESI-MS m/z : 610 $[\text{M}+\text{H}]^+$, 612 $[\text{M}+2+\text{H}]^+$

[0300] 実施例 2 2 0

4-ブロモ-N-(イソブチルチオカルバモイル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(62 mg, 0.14 mmol)をジクロロメタン(1.2 mL)に溶解した。そこへイソブチルイソチオシアネート(32 mg, 0.14×2 mmol)を加えて室温で6時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotagel Solera One, SANP 25 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 33 mg, 42.7%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.01 (1H, br s), 7.58 (1H, br s), 7.14 (1H, d, $J = 10.7$ Hz), 7.05–7.02 (2H, m), 4.77–4.66 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.60 (3H, s), 3.25–3.10 (3H, m), 2.26–2.17 (1H, m), 2.12–2.01 (1H, m), 1.88–1.74 (2H, m), 0.84 (6H, d, $J = 6.6$ Hz).

ESI-MS m/z : 552 $[\text{M}+\text{H}]^+$, 554 $[\text{M}+2+\text{H}]^+$

[0301] 実施例 2 2 1

4-ブロモ-N-(ベンジルチオカルバモイル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(64 mg, 0.15 mmol)をジクロロメタン(1.2 mL)に溶解した。そこへベンジルイソチオシアネート(45 mg, 0.15×2 mmol)を加えて室温で6時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 25 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 77 mg, 87.7%)を得た。

WO 2011/021397

184

PCT/JP2010/005136

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.21 (1H, br s), 7.97 (1H, br s), 7.33–7.22 (5H, m), 7.15 (1H, d, $J = 10.7$ Hz), 7.11 (1H, s), 7.04 (1H, d, $J = 10.7$ Hz), 4.77–4.71 (1H, m), 4.68–4.58 (2H, m), 3.91 (3H, s), 3.90 (3H, s), 3.87 (3H, s), 3.60 (3H, s), 3.13 (1H, dd, $J = 13.1, 5.0$ Hz), 2.26–2.18 (1H, m), 2.13–2.02 (1H, m), 1.93–1.83 (1H, m).

ESI-MS m/z : 586 $[\text{M}+\text{H}]^+$, 588 $[\text{M}+2+\text{H}]^+$

[0302] 実施例 2 2 2

4-ブromo-N-[4-(N', N' -ジメチルアミノ)フェニルチオカルバモイル]デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン (65 mg, 0.15 mmol) をジクロロメタン (1.3 mL) に溶解した。そこへ 4- N', N' -ジメチルアミノフェニルイソチオシアネート (53 mg, 0.15×2 mmol) を加えて室温で 7 時間攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 25 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 86 mg, 93.3%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.41 (1H, s), 8.03 (1H, d, $J = 7.3$ Hz), 7.15–7.13 (3H, m), 7.09 (1H, s), 7.03 (1H, d, $J = 11.0$ Hz), 6.68 (2H, d, $J = 9.0$ Hz), 4.82–4.76 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.61 (3H, s), 3.12 (1H, dd, $J = 12.7, 4.9$ Hz), 2.87 (6H, s), 2.24–2.16 (1H, m), 2.11–1.96 (2H, m).

ESI-MS m/z : 615 $[\text{M}+\text{H}]^+$, 617 $[\text{M}+2+\text{H}]^+$

[0303] 実施例 2 2 3

4-ブromo-N-(ピリジン-3-イルチオカルバモイル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン (61 mg, 0.14 mmol) をジクロロメタン (1.2 mL) に溶解した。そこへピリジン-3-イルイソチオシアネート (0.053 mL, 0.14×2 mmol) を加えて室温で一晩攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 25 g, クロ

WO 2011/021397

185

PCT/JP2010/005136

クロホルム/メタノール)で精製し、標記の化合物(黄色固体, 82 mg, quant.)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.80 (1H, s), 8.67 (1H, d, $J = 7.1$ Hz), 8.56 (1H, d, $J = 2.4$ Hz), 8.28 (1H, dd, $J = 4.8, 1.3$ Hz), 7.94 (1H, d, $J = 8.2$ Hz), 7.32 (1H, dd, $J = 8.2, 4.8$ Hz), 7.16 (1H, d, $J = 10.7$ Hz), 7.13 (1H, s), 7.05 (1H, d, $J = 10.7$ Hz), 4.79–4.73 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.61 (3H, s), 3.15 (1H, dd, $J = 12.6, 5.0$ Hz), 2.28–2.10 (2H, m), 2.03–1.95 (1H, m).

ESI-MS m/z : 573 $[\text{M}+\text{H}]^+$, 575 $[\text{M}+2+\text{H}]^+$

[0304] 実施例 2 2 4

4-ブロモ-N-(N', N'-ジエチルチオカルバモイル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(59 mg, 0.14 mmol)をジクロロメタン(1.2 mL)に溶解した。そこへチオホスゲン(0.011 mL, 0.14×1.05 mmol)、トリエチルアミン(0.047 mL, 0.14×2.4 mmol)を加えて室温で2時間攪拌した。そこへジエチルアミン(0.020 mL, 0.14×2 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 39 mg, 50.5%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.54 (1H, d, $J = 7.1$ Hz), 7.14 (1H, d, $J = 10.7$ Hz), 7.08 (1H, s), 7.03 (1H, d, $J = 10.7$ Hz), 4.99–4.93 (1H, m), 3.90 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.70–3.64 (4H, m), 3.62 (3H, s), 3.13 (1H, dd, $J = 13.2, 5.4$ Hz), 2.30–2.16 (2H, m), 2.01–1.94 (1H, m), 1.09 (6H, t, $J = 7.0$ Hz).

ESI-MS m/z : 552 $[\text{M}+\text{H}]^+$, 554 $[\text{M}+2+\text{H}]^+$

[0305] 実施例 2 2 5

4-ブロモ-N-(ピロリジン-1-イルチオカルボニル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(58 mg, 0.13 mmol)をジクロロメタン(1.2

WO 2011/021397

186

PCT/JP2010/005136

mL)に溶解した。そこへチオホスゲン(0.011 mL, 0.13×1.05 mmol)、トリエチルアミン(0.043 mL, 0.13×2.4 mmol)を加えて室温で2時間攪拌した。そこへピロリジン(0.022 mL, 0.13×2 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 25 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 27 mg, 37.8%)を得た。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 7.55 (1H, d, J = 7.1 Hz), 7.14 (1H, d, J = 10.7 Hz), 7.11 (1H, s), 7.03 (1H, d, J = 10.7 Hz), 4.92–4.85 (1H, m), 3.90 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.61 (3H, s), 3.58–3.44 (4H, m), 3.12 (1H, dd, J = 12.4, 5.9 Hz), 2.24–2.15 (2H, m), 2.01–1.96 (1H, m), 1.93–1.83 (4H, m).

ESI-MS m/z: 550 [M+H]⁺, 552 [M+2+H]⁺

[0306] 実施例 2 2 6

4-ブロモ-N-(ピペリジン-1-イルチオカルボニル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン(60 mg, 0.14 mmol)をジクロロメタン(1.2 mL)に溶解した。そこへチオホスゲン(0.011 mL, 0.14×1.05 mmol)、トリエチルアミン(0.047 mL, 0.14×2.4 mmol)を加えて室温で2時間攪拌した。そこへピペリジン(0.029 mL, 0.14×2 mmol)を加えて室温で一晩攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 49 mg, 62.1%)を得た。

¹H-NMR(400MHz, DMSO-d₆) δ [ppm]: 7.85 (1H, d, J = 7.1 Hz), 7.14 (1H, d, J = 10.7 Hz), 7.06 (1H, s), 7.03 (1H, d, J = 10.7 Hz), 4.94–4.87 (1H, m), 3.90 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.84–3.76 (4H, m), 3.63 (3H, s), 3.12 (1H, dd, J = 12.7, 5.9 Hz), 2.24–2.14 (2H, m), 2.03–1.93 (1H, m), 1.64–1.58 (2H, m), 1.51–1.44 (4H, m).

ESI-MS m/z: 564 [M+H]⁺, 566 [M+2+H]⁺

WO 2011/021397

187

PCT/JP2010/005136

[0307] 実施例 2 2 7

4-ブromo-N-(4-ヒドロキシブチリル)デアセチルコルヒチンの合成

4-ブromo-N-(4-ベンジルオキシブチロイル)デアセチルコルヒチン (86 mg, 0.14 mmol) をジクロロメタン (1.7 mL) に溶解し、 -78°C で攪拌した。そこへ 1 mol/L 三臭化ホウ素ジクロロメタン溶液 (0.14 mL, 0.14 mmol) を加えて -78°C で 20 分攪拌した。水、メタノールを加えてクロロホルム抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 13 mg, 17.8%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.58 (1H, d, $J = 7.3$ Hz), 7.13–7.11 (2H, m), 7.03 (1H, d, $J = 10.7$ Hz), 4.45 (1H, t, $J = 5.1$ Hz), 4.24–4.18 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.53 (3H, s), 3.36–3.31 (2H, m), 3.11 (1H, dd, $J = 13.4, 5.4$ Hz), 2.23–2.15 (3H, m), 1.98–1.88 (1H, m), 1.85–1.77 (1H, m), 1.62–1.54 (2H, m).

ESI-MS m/z : 523 $[\text{M}+\text{H}]^+$, 525 $[\text{M}+2+\text{H}]^+$

[0308] 実施例 2 2 8

4-ブromo-N-(チオモルホリン-4-イルチオカルボニル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン (60 mg, 0.14 mmol) をジクロロメタン (1.2 mL) に溶解した。そこへチオホスゲン (0.011 mL, 0.14×1.05 mmol)、トリエチルアミン (0.047 mL, 0.14×2.4 mmol) を加えて室温で 2 時間攪拌した。そこへチオモルホリン (0.028 mL, 0.14×2 mmol) を加えて室温で一晩攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 23 mg, 28.3%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.97 (1H, d, $J = 6.8$ Hz), 7.15 (1H, d, $J = 10.5$ Hz), 7.05–7.02 (2H, m), 4.92–4.86 (1H, m), 4.21–4.09 (4H, m), 3.90 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.63 (3H, s), 3.13 (1H

WO 2011/021397

188

PCT/JP2010/005136

, dd, $J = 12.1, 5.2$ Hz), 2.60 (4H, t, $J = 4.9$ Hz), 2.25–2.14 (2H, m), 2.07–1.98 (1H, m).

ESI-MS m/z : 582 $[M+H]^+$, 584 $[M+2+H]^+$

[0309] 実施例 2 2 9

4-ブロモ-N-(1,1-ジオキソチオモルホリン-4-イルチオカルボニル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン (59 mg, 0.14 mmol) をジクロロメタン (1.2 mL) に溶解した。そこへチオホスゲン (0.011 mL, 0.14×1.05 mmol)、トリエチルアミン (0.047 mL, 0.14×2.4 mmol) を加えて室温で2時間攪拌した。そこへチオモルホリン-1,1-ジオキシド (38mg, 0.14×2 mmol) を加えて室温で一晩攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標記の化合物 (黄色固体, 78 mg, 90.8%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ 8.38 (1H, d, $J = 7.1$ Hz), 7.16 (1H, d, $J = 10.5$ Hz), 7.05–7.02 (2H, m), 4.78 (1H, dd, $J = 16.0, 8.4$ Hz), 4.37–4.31 (2H, m), 4.23–4.18 (2H, m), 3.90 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.63 (3H, s), 3.28–3.22 (2H, m), 3.16–3.08 (3H, m), 2.25–2.09 (3H, m).

ESI-MS m/z : 614 $[M+H]^+$, 616 $[M+2+H]^+$

[0310] 実施例 2 3 0

4-ブロモ-N-(4-メチルピペラジン-1-イルチオカルボニル)デアセチルコルヒチンの合成

4-ブロモデアセチルコルヒチン (60 mg, 0.14 mmol) をジクロロメタン (1.2 mL) に溶解した。そこへチオホスゲン (0.011 mL, 0.14×1.05 mmol)、トリエチルアミン (0.047 mL, 0.14×2.4 mmol) を加えて室温で2時間攪拌した。そこへ4-メチルピペラジン (0.031 mL, 0.14×2 mmol) を加えて室温で一晩攪拌した。反応後、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー (Biotage Isolera One, SANP 10 g, クロロホルム/メタノール) で精製し、標

WO 2011/021397

189

PCT/JP2010/005136

記の化合物(黄色固体, 37 mg, 45.7%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.01 (1H, d, $J = 6.3$ Hz), 7.15 (1H, d, $J = 10.7$ Hz), 7.04–7.01 (2H, m), 4.90–4.84 (1H, m), 3.90 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.84–3.80 (2H, m), 3.62 (3H, s), 3.15–3.03 (4H, m), 2.39–2.30 (3H, m), 2.24–2.14 (5H, m), 2.04–1.94 (1H, m).

ESI-MS m/z : 579 $[\text{M}+\text{H}]^+$, 581 $[\text{M}+2+\text{H}]^+$

[0311] 実施例 2 3 1

4-ブromo-N-(モルホリン-4-イルチオカルボニル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン(60 mg, 0.14 mmol)をジクロロメタン(1.2 mL)に溶解し、氷冷した。そこへチオホスゲン(0.011 mL, 0.14×1.05 mmol)、トリエチルアミン(0.047 mL, 0.14×2.4 mmol)を加えて氷冷下2時間攪拌した。そこへモルホリン(0.024 mL, 0.14×2 mmol)を加えて室温で一晩攪拌した。クロロホルムを加えて0.1 mol/L塩酸で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 69 mg, 87.2%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.04 (1H, d, $J = 6.8$ Hz), 7.15 (1H, d, $J = 10.5$ Hz), 7.05–7.02 (2H, m), 4.90–4.83 (1H, m), 3.90 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.84–3.78 (4H, m), 3.62 (3H, s), 3.59 (4H, t, $J = 4.8$ Hz), 3.13 (1H, dd, $J = 12.9, 5.9$ Hz), 2.25–2.11 (2H, m), 2.06–1.97 (1H, m).

ESI-MS m/z : 566 $[\text{M}+\text{H}]^+$, 568 $[\text{M}+2+\text{H}]^+$

[0312] 実施例 2 3 2

4-ブromo-N-(エタンスルホニル)デアセチルコルヒチンの合成

4-ブromoデアセチルコルヒチン(240 mg, 0.55 mmol)をジクロロメタン(5.0 mL)に溶解し、氷冷した。そこへエタンスルホニルクロリド(0.78 mL, 0.55×1.5 mmol)、トリエチルアミン(0.153 mL, 0.55×2 mmol)を加えて室温で3

WO 2011/021397

190

PCT/JP2010/005136

日間攪拌した。反応後、1 mol/L塩酸を加えてクロロホルム抽出した。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 25 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 267 mg, 91.9%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.09 (1H, d, $J = 7.8$ Hz), 7.45 (1H, s), 7.11 (1H, d, $J = 10.5$ Hz), 7.04 (1H, d, $J = 10.5$ Hz), 3.91 (3H, s), 3.90 (3H, s), 3.89–3.88 (1H, m), 3.87 (3H, s), 3.48 (3H, s), 3.10 (1H, dd, $J = 12.9, 4.6$ Hz), 2.90–2.77 (2H, m), 2.18–1.98 (2H, m), 1.86–1.78 (1H, m), 1.10 (3H, t, $J = 7.3$ Hz).

ESI-MS m/z : 529 $[\text{M}+\text{H}]^+$, 531 $[\text{M}+2+\text{H}]^+$.

[0313] 実施例 2 3 3

4-クロロ-2,3-ジデメチルコルヒチンの合成

4-クロロコルヒチン(1.148 g, 2.65 mmol)を濃硫酸(3.8 mL)に溶解し、55℃で3時間攪拌し、さらに87℃で2時間攪拌した。反応液を氷水に注ぎ、1 mol/L水酸化ナトリウム水溶液(100 mL)を加えた。クロロホルム:メタノール=5:1で抽出した。飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 25 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 162 mg, 15.1%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.49 (1H, s), 9.28 (1H, s), 8.60 (1H, d, $J = 7.6$ Hz), 7.13–7.10 (2H, m), 7.02 (1H, d, $J = 11.0$ Hz), 4.30–4.24 (1H, m), 3.88 (3H, s), 3.40 (3H, s), 3.03 (1H, dd, $J = 13.3, 5.0$ Hz), 2.07–1.98 (1H, m), 1.96–1.88 (1H, m), 1.85 (3H, s), 1.81–1.74 (1H, m).

ESI-MS m/z : 406 $[\text{M}+\text{H}]^+$.

[0314] 実施例 2 3 4

4-クロロ-2,3-(メチレンジオキシ)-2,3-ジデメトキシコルヒチンの合成

4-クロロ-2,3-ジデメチルコルヒチン(51 mg, 0.126 mmol)をアセトニトリ

WO 2011/021397

191

PCT/JP2010/005136

ル(1.5 mL)に溶解した。そこへ炭酸カリウム(35 mg, 0.126×2 mmol)、ブロモクロロメタン(0.016 mL, 0.126×2 mmol)を加えて7時間加熱還流した。さらに炭酸カリウム(35 mg, 0.126×2 mmol)、ブロモクロロメタン(0.016 mL, 0.126×2 mmol)、N,N-ジメチルホルムアミド(1.5 mL)を加えて90°Cで一晩撹拌した。反応後、溶媒を留去した。水を加えてクロロホルム:メタノール=5:1で抽出した。飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 36 mg, 68.4%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.57 (1H, d, $J = 7.6$ Hz), 7.12 (1H, s), 7.10 (1H, d, $J = 10.7$ Hz), 7.01 (1H, d, $J = 10.7$ Hz), 6.20 (1H, d, $J = 1.0$ Hz), 6.19 (1H, d, $J = 1.0$ Hz), 4.30–4.24 (1H, m), 3.88 (3H, s), 3.74 (3H, s), 2.99 (1H, dd, $J = 13.2, 4.9$ Hz), 2.09–2.01 (1H, m), 1.97–1.89 (1H, m), 1.86 (3H, s), 1.82–1.75 (1H, m).

ESI-MS m/z : 418 $[\text{M}+\text{H}]^+$.

[0315] 実施例 235

4-クロロ-2,3-(エチレンジオキシ)-2,3-ジデメトキシコルヒチンの合成

4-クロロ-2,3-ジデメチルコルヒチン(51 mg, 0.126 mmol)をアセトニトリル(1.5 mL)、N,N-ジメチルホルムアミド(1.5 mL)に溶解した。そこへ1,2-ジブromoエタン(0.012 mL, 0.126×1.1 mmol)、炭酸カリウム(44 mg, 0.126×2.5 mmol)、ヨウ化ナトリウム(1 mg, 0.126×0.05 mmol)を加えて90°Cで一晩撹拌した。反応後、溶媒を留去した。水を加えてクロロホルム:メタノール=5:1で抽出した。飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 9 mg, 16.5%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.60 (1H, d, $J = 7.3$ Hz), 7.11 (1H, s), 7.10 (1H, d, $J = 11.0$ Hz), 7.01 (1H, d, $J = 11.0$ Hz), 4.47–4.34 (

WO 2011/021397

192

PCT/JP2010/005136

4H, m), 4.28–4.22 (1H, m), 3.88 (3H, s), 3.51 (3H, s), 3.06 (1H, dd, $J = 13.7, 5.6$ Hz), 2.10–2.01 (1H, m), 1.96–1.88 (1H, m), 1.85 (3H, s), 1.83–1.75 (1H, m).

ESI-MS m/z : 432 $[M+H]^+$.

[0316] 実施例 2 3 6

1,2-ジデメチルコルヒチンの合成

コルヒチン(1.214 g, 3.04 mmol)を濃硫酸(4.2 mL)に溶解し、55°Cで3時間攪拌し、さらに87°Cで2時間攪拌した。反応液を氷水に注ぎ、1 mol/L水酸化ナトリウム水溶液(100 mL)を加えた。クロロホルム:メタノール=5:1で抽出した。飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage Isolera One, SANP 25 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 44 mg, 4.1%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.64 (1H, s), 8.55 (1H, s), 8.44 (1H, d, $J = 7.8$ Hz), 7.21 (1H, d, $J = 10.7$ Hz), 7.14 (1H, s), 7.00 (1H, d, $J = 10.7$ Hz), 6.42 (1H, s), 4.42–4.35 (1H, m), 3.86 (3H, s), 3.81 (3H, s), 2.48–2.43 (1H, m), 2.22–2.14 (1H, m), 2.04–1.95 (1H, m), 1.85 (3H, s), 1.80–1.72 (1H, m).

ESI-MS m/z : 372 $[M+H]^+$.

[0317] 実施例 2 3 7

1,2-(メチレンジオキシ)-1,2-ジデメトキシコルヒチンの合成

1,2-ジメトキシコルヒチン(37 mg, 0.10 mmol)をN,N-ジメチルホルムアミド(1.1 mL)に溶解した。そこへ炭酸カリウム(28 mg, 0.10×2 mmol)、ブROMOKROKLOMETAN(0.013 mL, 0.10×2 mmol)を加えて90°Cで一晩攪拌した。反応後、溶媒を留去した。水を加えてクロロホルム:メタノール=5:1で抽出した。飽和食塩水で洗った。無水硫酸マグネシウムで乾燥し、溶媒を留去した。残渣をシリカゲルカラムクロマトグラフィー(Biotage IsoleraOne, SANP 10 g, クロロホルム/メタノール)で精製し、標記の化合物(黄色固体, 38 mg, 99.

WO 2011/021397

193

PCT/JP2010/005136

1%)を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.52 (1H, d, J = 7.8 Hz), 7.29 (1H, d, J = 10.5 Hz), 7.13 (1H, s), 7.00 (1H, d, J = 10.5 Hz), 6.62 (1H, s), 6.07 (2H, dd, J = 19.4, 0.9 Hz), 4.43-4.36 (1H, m), 3.86 (6H, s), 3.32 (3H, s), 2.60 (1H, dd, J = 13.3, 6.2 Hz), 2.30-2.22 (1H, m), 2.12-2.01 (1H, m), 1.90-1.87 (1H, m), 1.85 (3H, s).

ESI-MS m/z: 384 [M+H]⁺.

[0318] 実施例 238

4-クロロ-1,2-(メチレンジオキシ)-1,2-ジデメトキシコルヒチンの合成

1,2-(メチレンジオキシ)-1,2-ジデメトキシコルヒチン (23 mg, 0.0599 mmol) をアセトニトリル (0.5 mL) に溶解した。そこへN-クロロスクシンイミド (10 mg, 0.0749 mmol) を加えて室温で2日間攪拌した。Waters社製MSトリガー付き分取LCシステムを用い、標記の化合物 (黄色固体, 11 mg, 43.9%) を得た。

¹H-NMR (400MHz, DMSO-d₆) δ [ppm]: 8.57 (1H, d, J = 7.6 Hz), 7.31 (1H, d, J = 10.7 Hz), 7.12 (1H, s), 7.01 (1H, d, J = 10.7 Hz), 6.16 (1H, d, J = 0.7 Hz), 6.13 (1H, d, J = 0.7 Hz), 4.35-4.28 (1H, m), 4.01 (3H, s), 3.88 (3H, s), 3.08 (1H, dd, J = 13.3, 5.2 Hz), 2.18-2.10 (1H, m), 2.04-1.95 (1H, m), 1.89-1.81 (4H, m).

ESI-MS m/z: 418 [M+H]⁺.

MSトリガー付き分取システム

装置 ; Mass Detector 3100

Binary Gradient Module 2545

Sample Manager 2767

System Fluidics Organizer

Photodiode Array Detector 2998

Mass Lynx

カラム ; waters Sun Fire Prep C18 OBD (5 μm 30 × 150 mm)

条件 ; 水:アセトニトリル=9:1を2分間、その後20分間かけて水:アセトニトリル

WO 2011/021397

194

PCT/JP2010/005136

ル=9:1から水:アセトニトリル=1:9にグラジエントした。

流量 ; 20 mL/分

[0319] 実施例 2 3 9

4-クロロ-N-(エチルチオカルバモイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をメタノール-水 (2:1, 1.5 mL) に溶解し、0°Cに冷却した。そこへイソチオシアン酸エチル (22 μ l, 0.128 $\times$ 2 mmol) を加え、0°Cで75分撹拌した。反応液をクロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (黄色固体, 30 mg, 0.063 mmol, 49%) を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.44 (1H, br. s), 7.84 (1H, s), 7.50 (1H, dd, $J = 10.7$ Hz), 7.04 (1H, d, $J = 11.0$ Hz), 5.07–5.01 (1H, m), 4.07 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.74 (3H, s), 3.63–3.56 (1H, m), 3.38–3.33 (1H, m), 3.25 (1H, dd, $J = 13.7, 5.4$ Hz), 2.49–2.40 (1H, m), 2.13–2.10 (1H, m), 1.96–1.89 (1H, m), 1.11 (3H, t, $J = 7.2$ Hz).
ESI-MS m/z : 479 ($[\text{M}+\text{H}]^+$)

[0320] 実施例 2 4 0

4-クロロ-N-(フェニルチオカルバモイル)デアセチルコルヒチンの合成

アルゴン雰囲気下、4-クロロデアセチルコルヒチン (50 mg, 0.128 mmol) をメタノール-水 (2:1, 1.5 mL) に溶解し、0°Cに冷却した。そこへイソチオシアン酸フェニル (30 μ l, 0.128 $\times$ 2 mmol) を加え、0°Cで75分撹拌した。反応液をクロロホルムで抽出した。クロロホルム層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥させ、溶媒を留去した。得られた残渣をシリカゲルクロマトグラフィー (Biotage Isolera One, SNAP 10 g, メタノール/クロロホルム) で精製し、標記の化合物 (淡黄色固体, 50 mg, 0.095 mmol, 74%) を得た。

WO 2011/021397

195

PCT/JP2010/005136

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 8.85 (1H, br. s), 8.01 (1H, br. s), 7.86 (1H, d, $J = 7.3$ Hz), 7.50 (1H, d, $J = 10.2$ Hz), 7.36 (1H, d, $J = 7.6$ Hz), 7.29–7.27 (2H, m), 7.15 (1H, t, $J = 7.2$ Hz), 7.02 (1H, d, $J = 11.2$ Hz), 5.13–5.07 (1H, m), 4.04 (3H, s), 3.99 (3H, s), 3.99 (3H, s), 3.72 (3H, s), 3.20 (1H, dd, $J = 13.8, 5.7$ Hz), 2.45–2.35 (1H, m), 2.04–1.98 (1H, m), 1.94–1.86 (1H, m).

ESI-MS m/z : 527 ($[\text{M}+\text{H}]^+$)

[0321] 実施例 2 4 1

4-クロロ-N-(4-ピペリジノピペリジン-1-カルボキシアセチル)デアセチルコルヒチンの合成

4-クロロ-N-(ヒドロキシアセチル)デアセチルコルヒチン (0.107 g, 0.238 mmol) のジクロロメタン (有機合成用, 5 mL) 溶液に、氷冷、アルゴンガス雰囲気下にて4-ジメチルアミノピリジン (12 mg, 0.238 $\times$ 0.4 mmol)、トリエチルアミン (82.9 μL , 0.238 $\times$ 2.5 mmol) 及び、4-ピペリジノピペリジン-1-カルボニルクロリド (0.121 g, 0.238 $\times$ 2.2 mmol) を添加した。本溶液を徐々に室温に戻しながら一晩攪拌した後、クロロホルムと飽和重曹水を加えた。有機層を取り、水層は再びクロロホルムで抽出した。有機層は合わせてブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法 (装置: Biotage社 Isolera One, クロロホルム/メタノール) にて精製し、標記化合物 (乳白色固体, 0.112 g, 0.174 mmol, 73 %) を得た。

$^1\text{H-NMR}$ (400MHz, $\text{DMSO}-d_6$) δ [ppm]: 8.74 (1H, d, $J=7.6$ Hz), 7.13 (1H, d, $J=10.7$ Hz), 7.11 (1H, s), 7.03 (1H, d, $J=10.7$ Hz), 4.48 (2H, s), 4.27–4.21 (1H, m), 4.07–3.94 (2H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.11 (1H, dd, $J=14.0, 4.5$ Hz), 2.83–2.68 (2H, m), 2.56–2.33 (5H, m), 2.16–2.07 (1H, m), 2.00–1.83 (2H, m), 1.75–1.24 (10H, m).

ESI-MS m/z : 644 $[\text{M}+\text{H}]^+$.

[0322] 実施例 2 4 2

4-クロロ-N-(4-ピペリジノピペリジン-1-カルボキシアセチル)デアセチルコ
ルヒチン塩酸塩の合成

4-クロロ-N-(4-ピペリジノピペリジン-1-カルボキシアセチル)デアセチル
コルヒチン(69 mg, 0.107 mmol)のアセトン(2 mL)溶液に、室温にて10%HCl-
メタノール(65 μ L, 0.107 $\times$ 1.5 mmol)を添加した。本溶液を減圧下、濃縮乾
固して標記化合物(乳白色固体, 72 mg, 0.106 mmol, 99%, HPLC純度97.8%)を
得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 9.89 (1H, s), 8.78 (1H, d, $J=7.3$ Hz),
7.14 (1H, d, $J=10.7$ Hz), 7.12 (1H, s), 7.05 (1H, d, $J=10.7$ Hz), 4.50
(2H, s), 4.28-4.21 (1H, m), 4.12-3.99 (2H, m), 3.91 (3H, s), 3.89 (3H,
s), 3.88 (3H, s), 3.54 (3H, s), 3.29-2.68 (6H, m), 3.12 (1H, dd, $J=$
13.4, 5.4 Hz), 2.16-2.07 (1H, m), 2.01-1.34 (12H, m).

[0323] 実施例 2 4 3

4-クロロ-N-(1-メチルピペラジン-4-カルボキシアセチル)デアセチルコルヒ
チンの合成

4-クロロ-N-(4-ピペリジノピペリジン-1-カルボキシアセチル)デアセチル
コルヒチンの合成と同様の方法にて、標記化合物(乳白色固体, 25 mg, 0.043
mmol, 61%)を得た。4-ピペリジノピペリジン-1-カルボニルクロリドの代わ
りに1-メチルピペラジン-4-カルボニルクロリド塩酸塩(8.8 eq.)、さらには
トリエチルアミン(19 eq.)を用いた。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.33 (1H, s), 7.25 (1H, d, $J=10.7$ Hz), 7
.09 (1H, s), 6.81 (1H, d, $J=10.7$ Hz), 4.65 (1H, d, $J=14.9$ Hz), 4.61-4.
54 (1H, m), 4.54 (1H, d, $J=14.9$ Hz), 4.00 (3H, s), 3.99 (3H, s), 3.97
(3H, s), 3.68-3.59 (4H, m), 3.62 (3H, s), 3.26 (1H, dd, $J=13.1$, 4.5
Hz), 2.62-2.40 (4H, m), 2.42 (3H, s), 2.28-2.14 (2H, m), 1.85-1.78 (1
H, m).

ESI-MS m/z : 576 $[\text{M}+\text{H}]^+$.

[0324] 実施例 2 4 4

4-クロロ-N-(ジメチルアミノアセトキシアセチル)デアセチルコルヒチンの合成

4-クロロ-N-(4-ピペリジノピペリジン-1-カルボキシアセチル)デアセチルコルヒチンの合成と同様の方法にて、標記化合物(乳白色固体, 35 mg)を得た。4-ピペリジノピペリジン-1-カルボニルクロリドの代わりにN,N-ジメチルグリシンを用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.86 (1H, d, $J=6.3$ Hz), 7.42 (1H, s), 7.27 (1H, d, $J=10.7$ Hz), 6.82 (1H, d, $J=10.7$ Hz), 4.67 (1H, d, $J=15.4$ Hz), 4.65–4.57 (1H, m), 4.60 (1H, d, $J=15.4$ Hz), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.41–3.24 (3H, m), 2.43 (3H, s), 2.42 (3H, s), 2.28–2.14 (2H, m), 1.97–1.90 (1H, m).

ESI-MS m/z : 535 $[\text{M}+\text{H}]^+$.

[0325] 実施例 2 4 5

4-クロロ-N-[3-(ジメチルアミノ)プロピオニルオキシアセチル]デアセチルコルヒチンの合成

4-クロロ-N-(4-ピペリジノピペリジン-1-カルボキシアセチル)デアセチルコルヒチンの合成と同様の方法にて、標記化合物(乳白色固体, 33 mg)を得た。4-ピペリジノピペリジン-1-カルボニルクロリドの代わりにN,N-ジメチルホモグリシンを用いた。

ESI-MS m/z : 549 $[\text{M}+\text{H}]^+$.

[0326] 実施例 2 4 6

4-クロロ-N-[1-(ベンジルオキシカルボニル)ピペリジン-4-カルボキシアセチル]デアセチルコルヒチンの合成

4-クロロ-N-(ヒドロキシアセチル)デアセチルコルヒチン (50 mg, 0.111 mmol) のジクロロメタン(有機合成用, 2.2 mL) 溶液に、室温、アルゴンガス雰囲気下にて1-(ベンジルオキシカルボニル)ピペリジン-4-カルボン酸 (58 mg, 0.111 $\times$ 2 mmol)、1-エチル-3-(3-ジメチルアミノプロピル)カルボジイミド塩酸

WO 2011/021397

198

PCT/JP2010/005136

塩(47 mg, 0.4111×2.2 mmol)、及び4-(ジメチルアミノ)ピリジン(5 mg, 0.11×0.4 mmol)を添加して一晩攪拌した。反応液をクロロホルム及びブラインで希釈して、有機層を取った。有機層は飽和重曹水、次いでブラインで洗浄後、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残留物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記化合物(乳白色固体, 72 mg, 0.104 mmol, 94%)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.41 (1H, s), 7.39–7.31 (5H, m), 7.30 (1H, d, $J=10.7$ Hz), 7.01 (1H, s), 6.85 (1H, d, $J=10.7$ Hz), 5.13 (2H, s), 4.65–4.55 (1H, m), 4.63 (1H, d, $J=15.6$ Hz), 4.56 (1H, d, $J=15.6$ Hz), 4.21–4.09 (2H, m), 4.02 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.61 (3H, s), 3.28 (1H, dd, $J=13.1, 4.5$ Hz), 2.97–2.86 (2H, m), 2.65–2.56 (1H, m), 2.32–2.14 (2H, m), 2.00–1.64 (5H, m).

ESI-MS m/z : 695 $[\text{M}+\text{H}]^+$.

[0327] 実施例 247

4-クロロ-N-(チオアセトキシアセチル)デアセチルコルヒチンの合成

4-クロロ-N-(クロロアセチル)デアセチルコルヒチン(50 mg, 0.12 mmol)をアセトン(12mL)に溶解し、チオ酢酸カリウム(82 mg, 0.72 mmol, 6 eq)を加え65 °Cで3時間攪拌した。反応液を減圧留去し、残渣をクロロホルムに溶解し、水で洗浄した。有機層をブライン洗浄、無水硫酸マグネシウムにて乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルフラッシュカラムクロマトグラフィー (5%メタノール/酢酸エチル→ 10%メタノール/酢酸エチル)にて精製し、目的物の4-クロロ-N-(チオアセトキシアセチル)デアセチルコルヒチン(55.8 mg, 98 %)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.29 (1H, br-d), 7.28 (1H, s), 7.24 (1H, d, $J=10.6$ Hz), 6.80 (1H, d, $J=10.8$ Hz), 4.49 (1H, ddd, $J=12.2, 6.1, 6.1$ Hz), 4.00 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.62 (1H, dd, $J=14.5, 1.8$ Hz), 3.60 (3H, s), 3.54 (1H, dd, $J=14.4, 2.5$ Hz), 3.25 (1H, m

WO 2011/021397

199

PCT/JP2010/005136

), 2.45 (3H, s), 2.25–2.12 (2H, overlapped), 1.78 (1H, ddd, $J=11.0, 11.0, 4.9$ Hz).

FAB-MS (NBA) m/z : 508 $[M+H]^+$

[0328] 実施例 248

4-クロロ-N-(メルカプトアセチル)デアセチルコルヒチンの合成

4-クロロ-N-(チオアセトキシアセチル)デアセチルコルヒチン(10 mg, 0.020 mmol)をメタノール(4.5 mL)に溶解し、1M ナトリウムメトキシド(30 μ L, 0.030 mmol, 1.5 eq)を加え室温で40分間撹拌した。反応液に酢酸(3滴)を加え、弱酸性とした後、溶媒を減圧留去した。残渣をクロロホルムに溶解し、水で洗浄、有機層をブライン洗浄、無水硫酸マグネシウム乾燥、ろ過、減圧留去、真空乾燥した。得られた残渣をシリカゲルフラッシュカラムクロマトグラフィー (2%メタノール/クロロホルム→5%メタノール/クロロホルム)にて精製し、目的物の4-クロロ-N-(チオアセチル)デアセチルコルヒチン(9.5 mg, 定量的)を得た。

$^1\text{H-NMR}$ (400MHz, CDCl_3) δ [ppm]: 7.67 (1H, br-d, $J=7.0$ Hz), 7.40 (1H, s), 7.28 (1H, d, $J=10.4$ Hz), 6.83 (1H, d, $J=10.8$ Hz), 4.53 (1H, ddd, $J=12.1, 6.2, 6.2$ Hz), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.26 (3H, m), 2.33 (2H, m), 1.84 (1H, ddd, $J=11.5, 11.5, 4.9$ Hz).

FAB-MS (NBA) m/z : 466 $[M+H]^+$

[0329] 実施例 249

4-クロロ-N-[1-(エトキシカルボニル)ピペリジン-4-イルカルボニル]デアセチルコルヒチンの合成

1-(エトキシカルボニル)ピペリジン-4-カルボン酸(17 mg, 0.0766×1.1 mmol)のジクロロメタン(2 mL)溶液に、氷冷、アルゴンガス雰囲気下にて1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(16 mg, 0.0766×1.1 mmol)及び1-ヒドロキシベンゾトリアゾール-水合物(13 mg, 0.0766×1.1 mmol)を添加して、30分間撹拌した。次いで、4-クロロデアセチルコルヒチ

WO 2011/021397

200

PCT/JP2010/005136

ン(30 mg, 0.0766 mmol)を添加して、徐々に室温に戻しながら一晩撹拌した。反応液をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(乳白色固体, 41 mg, 0.0714 mmol, 93%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.59 (1H, d, $J=7.3$ Hz), 7.12 (1H, d, $J=10.7$ Hz), 7.09 (1H, s), 7.02 (1H, d, $J=10.7$ Hz), 4.24–4.17 (1H, m), 4.01–3.87 (2H, m), 4.01 (2H, q, $J=7.1$ Hz), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.11 (1H, dd, $J=13.2, 5.4$ Hz), 2.86–2.72 (2H, m), 2.45–2.37 (1H, m), 2.15–2.06 (1H, m), 2.00–1.81 (2H, m), 1.66 (2H, dd, $J=28.8, 11.2$ Hz), 1.40–1.27 (2H, m), 1.16 (3H, t, $J=7.1$ Hz).

ESI-MS m/z : 575 $[\text{M}+\text{H}]^+$.

[0330] 参考例 2 3

4-([1, 3] ジチオラン-2-イル) コルヒチンの合成

4-ホルミルコルヒチン(50 mg, 0.117 mmol)のクロロホルム(1 mL)溶液に、室温、アルゴンガス雰囲気下にて1,2-エタンジチオール(14.6 μL , 0.117×1.5 mmol)及びヨウ素(3 mg, 0.117×0.1 mmol)を加えて、一晩撹拌した。反応液に10%亜硫酸ナトリウムを加えて、10分間撹拌した後、クロロホルムを加えて有機層を取った。有機層はブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残渣物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(乳白色固体, 32 mg, 0.0634 mmol, 54%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.56 (1H, d, $J=7.1$ Hz), 7.08 (1H, s), 7.05 (1H, d, $J=10.7$ Hz), 7.00 (1H, d, $J=10.7$ Hz), 6.38 (1H, s), 4.28–4.21 (1H, m), 3.88 (3H, s), 3.87 (3H, s), 3.84 (3H, s), 3.71 (1H, dd, $J=13.5, 5.5$ Hz), 3.58–3.51 (2H, m), 3.50 (3H, s), 3.42–3.35 (2H, m), 2.25–2.17 (1H, m), 2.03–1.95 (1H, m), 1.86 (3H, s), 1.80–1.72 (1H, m).

WO 2011/021397

201

PCT/JP2010/005136

ESI-MS m/z : 504 $[M+H]^+$.

[0331] 参考例 2 4

4-([1, 3] ジチアン-2-イル) コルヒチンの合成

4-([1, 3] ジチオラン-2-イル) コルヒチンの合成と同様の方法にて、標記化合物(淡褐色固体, 0.224 g, 0.432 mmol, 74%)を得た。1, 2-エタンジチオールに代わり、1, 3-プロパンジチオールを用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.58 (1H, d, $J=7.3$ Hz), 7.08 (1H, s), 7.05 (1H, d, $J=10.7$ Hz), 6.99 (1H, d, $J=10.7$ Hz), 4.26-4.20 (1H, m), 3.94-3.89 (1H, m), 3.88 (3H, m), 3.88 (3H, s), 3.87 (3H, s), 3.50 (3H, s), 3.18-3.09 (2H, m), 2.94-2.85 (2H, m), 2.19-2.01 (3H, m), 1.88-1.80 (1H, m), 1.86 (3H, s), 1.73-1.63 (1H, m).

ESI-MS m/z : 518 $[M+H]^+$.

[0332] 参考例 2 5

4-(ヒドロキシメチル) コルヒチンの合成

4-ホルミルコルヒチン(100 mg, 0.234 mmol)のメタノール(2.5 mL)溶液に、室温、アルゴンガス雰囲気下にて水素化ホウ素ナトリウム(13 mg, 0.234 $\times$ 1.5 mmol)を添加して、室温で3時間撹拌した。反応液にアセトンを加えて30分間撹拌した後、クロロホルムと水を加えた。有機層を取り、水層はさらにクロロホルムにて抽出した。有機層は合わせてブラインにて洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残渣物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(乳白色固体, 74 mg, 0.173 mmol, 74%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.55 (1H, d, $J=7.3$ Hz), 7.11 (1H, s), 7.09 (1H, d, $J=10.7$ Hz), 7.02 (1H, d, $J=10.7$ Hz), 4.87 (1H, t, $J=5.1$ Hz), 4.54 (1H, dd, $J=11.3, 4.4$ Hz), 4.47 (1H, dd, $J=11.3, 5.9$ Hz), 4.31-4.24 (1H, m), 3.88 (3H, s), 3.87 (3H, s), 3.83 (3H, s), 3.50 (3H, s), 3.00 (1H, dd, $J=12.6, 4.5$ Hz), 2.10-1.95 (2H, m), 1.85 (3H, s), 1.81-1.73 (1H, m).

WO 2011/021397

202

PCT/JP2010/005136

ESI-MS m/z : 430 $[M+H]^+$.

[0333] 実施例 250

4-(フルオロメチル)コルヒチンの合成

4-(ヒドロキシメチル)コルヒチン (30 mg, 0.0699 mmol) のジクロロメタン (1 mL) 溶液に、氷冷、アルゴンガス雰囲気下にて (ジエチルアミノ) サルファートリフルオリド (11.0 μ L, 0.0699 $\times$ 1.2 mmol) を添加して、氷冷下2時間攪拌した。反応液をフラッシュクロマトグラフ法 (装置: Biotage社 Isolera One, クロロホルム/メタノール) にて精製し、標記の化合物 (乳白色固体, 19 mg, 0.0442 mmol, 63%) を得た。

$^1\text{H-NMR}$ (400MHz, DMSO- d_6) δ [ppm]: 8.60 (1H, d, $J=7.3$ Hz), 7.11 (1H, d, $J=10.7$ Hz), 7.11 (1H, s), 7.03 (1H, d, $J=10.7$ Hz), 5.49 (2H, d, $J=48.5$ Hz), 4.28–4.21 (1H, m), 3.89 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.54 (3H, s), 2.93 (1H, dd, $J=13.9, 4.6$ Hz), 2.13–1.94 (2H, m), 1.86–1.77 (1H, m), 1.85 (3H, s).

ESI-MS m/z : 432 $[M+H]^+$.

[0334] 実施例 251

4-(ジフルオロメチル)コルヒチンの合成

N-ヨードコハク酸イミド (87 mg, 0.0966 $\times$ 4 mmol) のジクロロメタン (5 mL) 溶液に、 -78°C 付近、アルゴンガス雰囲気下にて、フッ化水素・ピリジン (HF: 65%, 119 mg, 0.0966 $\times$ 40 mmol) のジクロロメタン (1 mL) 溶液を添加した。 -78°C 付近で20分間攪拌した後、4-([1,3]ジチアン-2-イル)コルヒチン (50 mg, 0.0966 mmol) のジクロロメタン (1 mL) 溶液を添加して、徐々に室温に戻しながら一晩攪拌した。反応液に10%亜硫酸ナトリウムを加えて10分間攪拌した後、クロロホルムと飽和重曹水を加えた。有機層を取り、飽和重曹水、0.1mol/L塩酸、飽和重曹水次いでブラインで洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残渣物 [41 mg, HPLC純度: 60% (4-CHO体: 23%)] をメタノール (2 mL) に溶解し、室温、アルゴンガス雰囲気下にて、水素化ホウ素ナトリウム (2 mg, 0.0966 $\times$ 0.5 mmol) を添加して、30分間攪拌した後、アセ

WO 2011/021397

203

PCT/JP2010/005136

トンを加えて、さらに30分間攪拌した。反応液にクロロホルムと水を加えて有機層を取った。有機層はブラインにて洗浄、無水硫酸ナトリウムにて乾燥、ろ過、減圧下濃縮乾固した。残渣物をフラッシュクロマトグラフ法(装置:Biotage社 Isolera One, クロロホルム/メタノール)にて精製し、標記の化合物(白色固体, 30 mg, 0.0674 mmol, 70%)を得た。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 8.62 (1H, d, $J=7.3$ Hz), 7.25 (1H, t, $J=54.0$ Hz), 7.11 (1H, d, $J=10.7$ Hz), 7.10 (1H, s), 7.03 (1H, d, $J=10.7$ Hz), 4.28–4.21 (1H, m), 3.90 (3H, s), 3.90 (3H, s), 3.89 (3H, s), 3.56 (3H, s), 3.11 (1H, dd, $J=13.7, 4.9$ Hz), 2.17–2.08 (1H, m), 1.99–1.91 (1H, m), 1.87–1.80 (1H, m), 1.86 (3H, s).

ESI-MS m/z : 450 $[\text{M}+\text{H}]^+$.

[0335] 実施例 2 5 2

4-クロロ-N-(N', N'-ジメチルチオカルバモイル)デアセチルコルヒチンの合成

4-クロロ-N-(N', N'-ジエチルチオカルバモイル)デアセチルコルヒチンの合成と同様の方法にて、標記の化合物(淡黄色固体, 37 mg, 0.0766 mmol, 定量的)を得た。ジエチルアミンの代わりに、ジメチルアミン塩酸塩及びトリエチルアミンを用いた。

$^1\text{H-NMR}$ (400MHz, DMSO-d_6) δ [ppm]: 7.63 (1H, d, $J=7.1$ Hz), 7.15 (1H, d, $J=11.0$ Hz), 7.09 (1H, s), 7.02 (1H, d, $J=11.0$ Hz), 4.91–4.85 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.62 (3H, s), 3.20 (6H, s), 3.12 (1H, dd, $J=13.2, 5.1$ Hz), 2.26–2.18 (1H, m), 2.16–2.08 (1H, m), 2.04–1.95 (1H, m).

ESI-MS m/z : 479 $[\text{M}+\text{H}]^+$.

[0336] 実施例 2 5 3

4-クロロ-N-(N', N'-ジプロピルチオカルバモイル)デアセチルコルヒチンの合成

4-クロロ-N-(N', N'-ジエチルチオカルバモイル)デアセチルコルヒチンの