

INSTRUCTIONS

(a) If Convention application insert "Convention"

AMENDED



(a) Convention

AUSTRALIA

Patents Act

(b) Delete one

APPLICATION FOR A (b) STANDARD/~~PETTY~~ PATENT

(c) Insert FULL name(s) of applicant(s)

+ We (c) Bristol-Myers Squibb Company

614068

(d) Insert FULL address(es) of applicant(s)

of (d) 345 Park Avenue,  
New York, New York, 10154,  
United States of America,

(e) Delete one

hereby apply for the grant of a (e) Standard/~~Petty~~ Patent for an invention entitled

(f) Insert TITLE of invention

(f) REBECCAMYCIN ANALOGS

(g) Insert "complete" or "provisional" or "petty patent"

which is described in the accompanying (g) complete specification.

(Note: The following applies only to Convention applications)

Details of basic application(s)

(h) Insert number, country and filing date for the/or each basic application

	Application No.	Country	Filing Date
(h)	933428	United States of America	21 November 1986

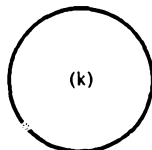
Address for Service:

PHILLIPS ORMONDE AND FITZPATRICK  
Patent and Trade Mark Attorneys  
367 Collins Street  
Melbourne, Australia 3000

(i) Insert date of signing

Dated (i) 19 January, 1990.

(j) Signature of applicant(s) (For body corporate see headnote\*)



(j) PHILLIPS ORMONDE & FITZPATRICK  
ATTORNEYS FOR:  
BRISTOL-MYERS SQUIBB COMPANY

(k) Corporate seal if any

*David Fitzpatrick*

Note: No legalization or other witness required

PHILLIPS ORMONDE AND FITZPATRICK  
Patent and Trade Mark Attorneys 81148/87  
367 Collins Street  
Melbourne, Australia

1

C

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DECLARATION FOR A PATENT APPLICATION

▼ INSTRUCTIONS

(a) Insert "Convention"  
if applicable

(b) Insert FULL name(s)  
of applicant(s)

(c) Insert "of addition"  
if applicable

(d) Insert TITLE of  
invention

(e) Insert FULL name(s)  
AND address(es) of  
declarant(s)  
(See headnote\*)

In support of the (a) Convention application made by

(b)

BRISTOL-MYERS COMPANY

(hereinafter called "applicant(s) for a patent (c)  
invention entitled (d)

for an

REBECCAMYCIN ANALOGS

I/~~XXX~~ (e) Isaac Jarkovsky, Assistant Secretary of Bristol-Myers Company,  
of 345 Park Avenue, New York, New York, United States of America

do solemnly and sincerely declare as follows:

1. I am/We are the applicant(s).

(or, in the case of an application by a body corporate)

1. I am/We are authorized to make this declaration on behalf of the applicant(s).

2. I am/We are the actual inventor(s) of the invention.

(or, where the applicant(s) is/are not the actual inventor(s))

2. (f) Takushi KANEKO: 398 Northwood Drive, Guilford, Connecticut 06437,  
Henry S. WONG: 38 Black Walnut Drive, Durham, Connecticut 06422 and  
Jacob J. UTZIG: 51 Ullman Street, Buffalo, New York 14207 all  
respectively in the United States of America

(f) Insert FULL name(s)  
AND address(es) of  
actual inventor(s)

(g) Recite how appli-  
cant(s) derive(s)  
title from actual  
inventor(s)  
(See headnote\*\*)

~~XX~~ are the actual inventor(s) of the invention and the facts upon which the applicant(s)  
is/~~XX~~ entitled to make the application are as follows:

(g)

Applicant is the assignee of the inventors.

(Note: Paragraphs 3 and 4 apply only to Convention applications)

3. The basic application(s) for patent or similar protection on which the application is based  
is/~~XX~~ identified by country, filing date, and basic applicant(s) as follows:

(h) UNITED STATES OF AMERICA

21st November, 1986

Takushi Kaneko, Henry S. Wong and Jacob J. Utzig

(h) Insert country,  
filing date, and  
basic applicant(s)  
for the/or EACH  
basic application

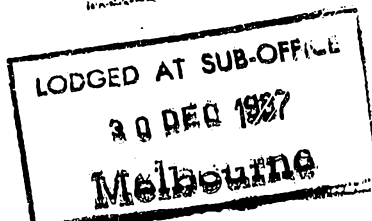
4. The basic application(s) referred to in paragraph 3 hereof was/~~XXX~~ the first application(s)  
made in a Convention country in respect of the invention the subject of the application.

(k) Insert PLACE of  
signing

(l) Insert DATE of  
signing

(m) Signature(s) of  
declarant(s)

Note: No legalization or  
other witness required



Declared at (k) New York, N.Y. 10154

Dated (l) December 10, 1987

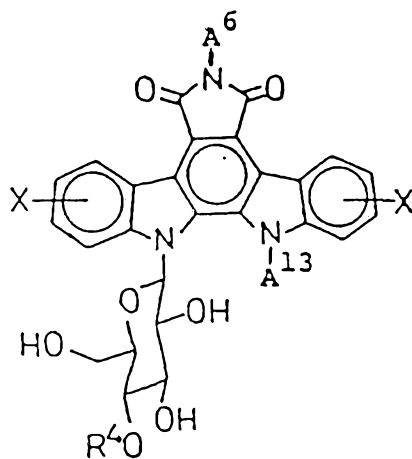
(m) Isaac Jarkovsky  
Assistant Secretary

To: The Commissioner of Patents

(12) PATENT ABRIDGMENT (11) Document No. AU-B-81148/87  
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 614068

- (54) Title  
REBECCAMYCIN ANALOGS
- (51)<sup>4</sup> International Patent Classification(s)  
C07H 019/23 A61K 031/71
- (21) Application No. : 81148/87 (22) Application Date : 12.11.87
- (30) Priority Data
- (31) Number (32) Date (33) Country  
933428 21.11.86 US UNITED STATES OF AMERICA
- (43) Publication Date : 26.05.88
- (44) Publication Date of Accepted Application : 22.08.91
- (71) Applicant(s)  
BRISTOL-MYERS SQUIBB COMPANY
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- (74) Attorney or Agent  
PHILLIPS ORMONDE & FITZPATRICK, 367 Collins Street, MELBOURNE VIC 3000
- (56) Prior Art Documents  
US 4487925  
US 4552842
- (57) Claim

1. A compound having the formula



wherein:

n is an integer from 1 to 6;

A<sup>6</sup> and A<sup>13</sup> are selected from H and  $-(CH_2)_nNR^1R^2$  and at least one of A<sup>6</sup> and A<sup>13</sup> is  $-(CH_2)_nNR^1R^2$ ;

R<sup>1</sup> and R<sup>2</sup>, independently, are selected from hydrogen,

(11) AU-B-81148/87  
(10) 614068

-2-

unsubstituted and substituted  $C_1$ - $C_6$  alkyl, aralkyl having 1 to 3 carbons in the alkyl moiety and unsubstituted phenyl or phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, carboxyl, alkoxycarbonyl, and amino and mono- and di-lower-alkylamino groups in the aryl moiety, and aryl selected from unsubstituted phenyl and phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, amino, mono- and dialkylamino, nitro, carboxyl, alkoxycarbonyl, and cyano groups provided that both  $R^1$  and  $R^2$  are not each aryl and, when taken together,  $R^1$  and  $R^2$  may be selected from  $-(CH_2)_4-$  and  $(CH_2)_2-R^3-(CH_2)_2-$  to form a 5- or 6-membered ring together with the N-atom wherein  $R^3$  is selected from  $CH_2$ , NH, O and S;

X is selected from H, F, Cl, Br,  $C_1$ - $C_3$  alkyl, OH, carboxyl, alkoxycarbonyl and alkoxy wherein the alkyl moiety is  $C_1$ - $C_3$  alkyl, benzyloxy, amino, and mono- and dialkylamino; and

$R^4$  is selected from H and  $CH_3$ ; and pharmaceutically acceptable acid addition and base salts thereof.

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COMPLETE SPECIFICATION  
(ORIGINAL)

Class

Int. Class

Application Number:  
Lodged:

Complete Specification Lodged:  
Accepted:  
Published:

614068

Priority

Related Art:

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APPLICANT'S REFERENCE: CT-1866

Name(s) of Applicant(s):

~~Bristol-Myers Company~~ Bristol-Myers Squibb Company

Address(es) of Applicant(s):

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UNITED STATES OF AMERICA.



Address for Service is:

PHILLIPS ORMONDE & FITZPATRICK  
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367 Collins Street  
Melbourne 3000 AUSTRALIA

Complete Specification for the invention entitled:

REBECCAMYCIN ANALOGS

Our Ref : 73786  
POF Code: 1490/1490

The following statement is a full description of this invention, including  
the best method of performing it known to applicant(s):

## REBECCAMYCIN ANALOGS

### BACKGROUND OF THE INVENTION

#### Field of the Invention

5 This invention relates to novel compounds having antineoplastic properties, to their production, to a pharmaceutical composition containing an amount of at least one compound according to the invention which is effective to inhibit the growth of tumors in experimental animal systems, and to a method for therapeutically treating an experimental animal by administering an amount of at least one compound according to the invention which is effective to inhibit tumor growth in an experimental animal system.

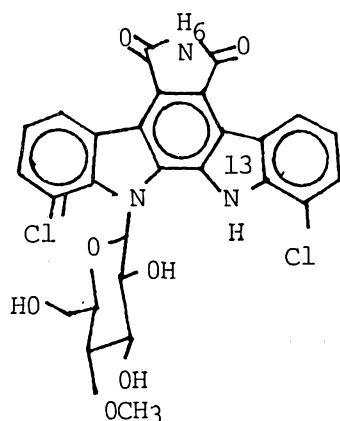
#### Background Art

U. S. Patents 4,487,925 and 4,552,842 disclose the anti-tumor agent designated rebeccamycin, and the 5'-N-methyl and 5',2",3",6"-tetraacetate derivatives thereof, and a process for producing the same agent by cultivating a rebeccamycin-producing strain of Nocardia aerocolonigenes, preferably Nocardia aerocolonigenes ATCC 39243, or a rebeccamycin-producing mutant thereof in an aqueous nutrient medium containing assimilable sources of carbon and nitrogen under submerged aerobic conditions until a substantial amount of rebeccamycin is produced.

### SUMMARY OF THE INVENTION

This invention comprises analogs of the antitumor agent

designated rebeccamycin (Formula I)

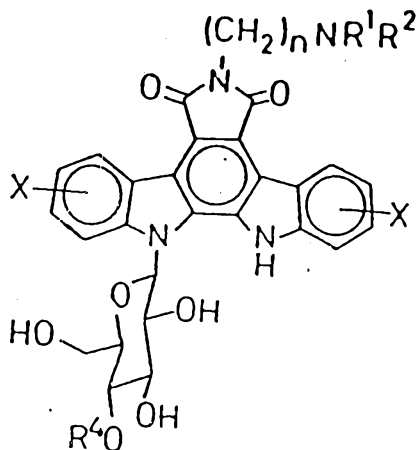


Formula I

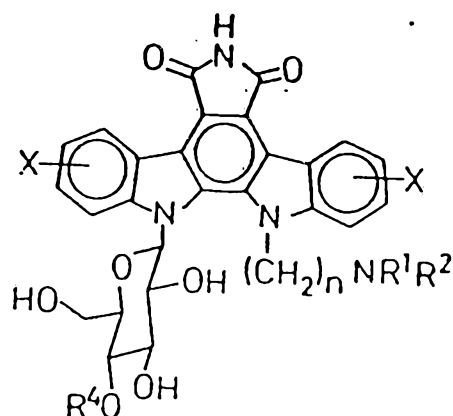
produced by first reacting rebeccamycin with a strong base to obtain a reactive intermediate and then reacting the reactive intermediate with an aminoalkyl halide. By the use of an amount of strong base slightly in excess, for example about 10% excess, of the molar equivalent amount of rebeccamycin followed by reacting the resulting intermediate with at least one molar equivalent based on rebeccamycin of an aminoalkyl halide as the alkylating agent, there is obtained the corresponding 6-aminoalkylrebeccamycin analog. By the use of an amount of strong base slightly in excess of two-times the molar equivalent amount of rebeccamycin, for example about 20% excess over two-times the molar equivalent amount of rebeccamycin, and then reacting the resulting intermediate with about one molar equivalent of an aminoalkyl halide as the alkylating agent, there is obtained the corresponding 13-aminoalkyl-rebeccamycin analog.

DETAILED DESCRIPTION OF THE INVENTION

In one generic aspect, this invention is a compound selected from the group consisting of the compounds having the formulas II and III below



Formula II



Formula III

wherein:  $n$  is an integer from 1 to 6;

$R^1$  and  $R^2$ , independently, are selected from hydrogen, unsubstituted and substituted  $C_1$ - $C_6$  alkyl, aralkyl having 1 to 3 carbons in the alkyl moiety and unsubstituted phenyl or phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, carboxyl, alkoxycarbonyl, and amino and mono- and di-lower-alkylamino groups in the aryl moiety, and aryl selected from unsubstituted phenyl and phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, amino, mono- and dialkylamino, nitro, carboxyl, alkoxycarbonyl, and cyano groups provided that both  $R^1$  and  $R^2$  are not each aryl and, when taken together,  $R^1$  and  $R^2$  may be selected from  $-(CH_2)_4-$  and  $(CH_2)_2-R^3-(CH_2)_2-$  to form a 5- or



6-membered ring together with the N-atom wherein  $R^3$  is selected from  $CH_2$ , NH, O and S;

X is selected from H, F, Cl, Br,  $C_1-C_3$  alkyl, OH, carboxyl, alkoxy carbonyl and alkoxy wherein the alkyl moiety is  $C_1-C_3$  alkyl, benzyloxy, amino, mon- and dialkylamino; and

$R^4$  is selected from H and  $CH_3$ ; and pharmaceutically acceptable acid addition and base salts thereof.

Preferred, compounds according to this invention having one of Formulas II and III above are those wherein n is an integer from 1 to 6,  $R^1$  and  $R^2$ , independently, are selected from unsubstituted and substituted  $C_1-C_6$  alkyl and, when taken together,  $R^1$  and  $R^2$  may be selected from  $-(CH_2)_4-$  and  $-(CH_2)_2-R^3-(CH_2)_2-$  to form a 5- or 6-membered ring together with the N-atom wherein  $R^3$  is selected from  $CH_2$ , NH, O and S, and wherein  $R^4$  is H or  $CH_3$  and X is selected from H, Cl, Br, OH,  $OCH_3$  and  $OCH_2C_6H_5$ .

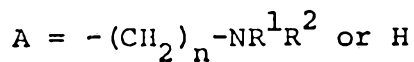
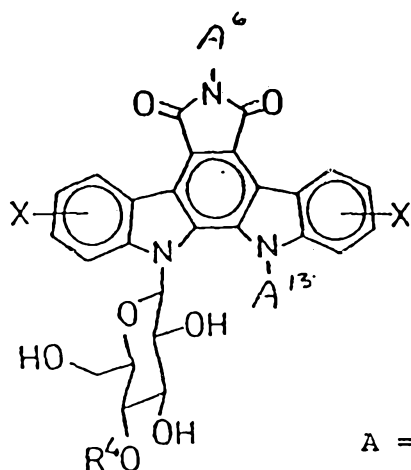
More preferred compounds according to this invention having one of Formulas II and III above are those wherein n is selected from the integers 1, 2 and 3;  $R^1$  and  $R^2$ , independently, are selected from H,  $C_1-C_3$  alkyl, and  $-(CH_2)_4-$ ;  $R^4$  is H or  $CH_3$ ; and X is selected from H, Cl, Br, OH,  $OCH_3$  and  $OCH_2C_6H_5$ .

Most preferred compounds according to this invention having one of Formulas II and III above are those wherein n is an integer selected from 2 and 3; X is Cl in each of the 1- and 11-positions of the ring system;  $R^1$  and  $R^2$  are each

$C_2H_5$ ; and  $R^4$  is H or  $CH_3$ .

The following Table 1 presents representative combinations of the many groups within the definition of X, n,  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  and many further combinations of such groups will be readily apparent to those skilled in the art. It is to be understood that the aminoalkyl group, A, bearing the groups n,  $R^1$  and  $R^2$  may be substituted at either or both the 6- and 13-positions (designated  $A^6$  and  $A^{13}$ ) of the rebeccamycin ring system. Of course, when only one of  $A^6$  or  $A^{13}$  is aminoalkyl, the other is H.

Table 1 - Representative Substituent Groups



<u>X</u>	<u>n</u>	<u>R<sup>1</sup></u>	<u>R<sup>2</sup></u>	<u>R<sup>4</sup></u>
1,11-dichloro	2	ethyl	ethyl	methyl
1,11-dichloro	3	ethyl	ethyl	methyl
3,9-diamino	3	methyl	methyl	H
2,10-diamino	4	propyl	propyl	H
3,9-dibenzoyloxy	5	i-propyl	H	methyl
4,8-dichloro	6	ethyl	H	methyl
3,9-dichloro	2	phenethyl	H	methyl
4,8-dihydroxy	3	benzyl	H	methyl
3,9-dihydroxy	2	$-(CH_2)_4-$		H
4,8-dimethoxy	3	$-(CH_2)_2-O-(CH_2)_2-$		methyl
3,9-dimethoxy	2	$-(CH_2)_2-NH-(CH_2)_2-$		methyl
4,8-dimethyl	3	$(CH_2)_5-$		H
3,9-dimethyl	1	$-(CH_2)_2-S-(CH_2)_2-$		methyl
2,10-dimethyl	2	hexyl	H	methyl
3,9-dihydroxycarbonyl	3	ethyl	ethyl	methyl
2,10-dibromo	2	$-(CH_2)_4-$		methyl
H	3	ethyl	ethyl	H

In other aspects, this invention is a pharmaceutical composition containing at least one of the compounds according to the invention and a method for therapeutically treating a mammalian host, for example, an experimental animal, affected by a malignant tumor by administering at least one of the compounds according to this invention in a tumor growth-inhibiting amount and, usually, by means of administering the compound in the form of the pharmaceutical composition.

By the expression " $C_1-C_6$  alkyl", more particularly unsubstituted  $C_1-C_6$  alkyl, is meant straight-chain or branched-chain or cyclic alkyl groups having a total of six carbon atoms. Examples of suitable straight-chain alkyl groups include methyl, ethyl, propyl, butyl, pentyl and hexyl groups. Examples of suitable branched-chain alkyl groups include isopropyl, sec-butyl, t-butyl, 2-methylbutyl, 2-pentyl, 3-pentyl and the like groups. Examples of suitable cyclic alkyl groups include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl groups. The alkyl groups may be substituted, generally with 1 or 2 substituents, with substituents selected from halo, hydroxy, alkoxy, amino, mono- and dialkylamino, nitro, carboxyl, alkoxycarbonyl, and cyano groups. By the expression "aralkyl" is meant benzyl, phenethyl (phenylethyl) and phenylpropyl groups wherein the phenyl moiety may be substituted. By the expression "aryl" is meant phenyl. The aralkyl or aryl group may contain substituted phenyl wherein the substituent may be from 1 to 3 alkyl, hydroxy, alkoxy, halo, amino, mono- and dialkylamino, nitro, carboxyl, alkoxycarbonyl, and cyano groups.

As used herein, the expression "halo" is meant in the conventional sense to include F, Cl, Br, and I.

When the expressions alkyl, alkoxy, alkoxy carbonyl, mono- and dialkylamino are used, these expressions usually are meant to include an alkyl moiety having 1 to 3 carbon atoms.

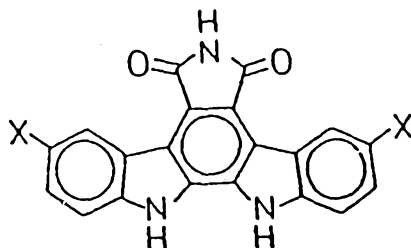
As mentioned above,  $R^1$  and  $R^2$  are not each aryl. Also,  $R^1$  and  $R^2$  generally are not each selected from the sterically larger of the  $C_1$ - $C_6$  alkyl and from aralkyl groups. Preferably, when  $R^1$  and  $R^2$  are each alkyl, they are each selected from  $C_1$ - $C_3$  alkyl groups.

As is mentioned above, rebeccamycin is a known antitumor agent disclosed in U.S. Patents 4,487,525 and 4,552,842. The Chemical Abstracts nomenclature for rebeccamycin is as follows: 5H-Indolo[2,3-a] pyrrolo[3,4-c] carbazole-5,7 (6H)dione, 1, 11-dichloro-12, 13-dihydro-12-(4-O-methyl-beta-D-glucopyranosyl). However, the name "rebeccamycin" is used throughout for simplicity although the Chemical Abstracts numbering systems is used to identify the various positions in the rebeccamycin ring system.

The starting material for the most preferred compounds according to this invention is rebeccamycin itself. Rebeccamycin, Formula I, may be produced by cultivating a rebeccamycin-producing strain of Nocardia aerocolonigenes, preferably a strain having the characteristics of Nocardia aerocolonigenes strain C38,383-RK2(ATCC39243) or a mutant thereof under submerged aerobic conditions in an aqueous nutrient medium as is described in U.S. Patents 4,487,925

and 4,552,842.

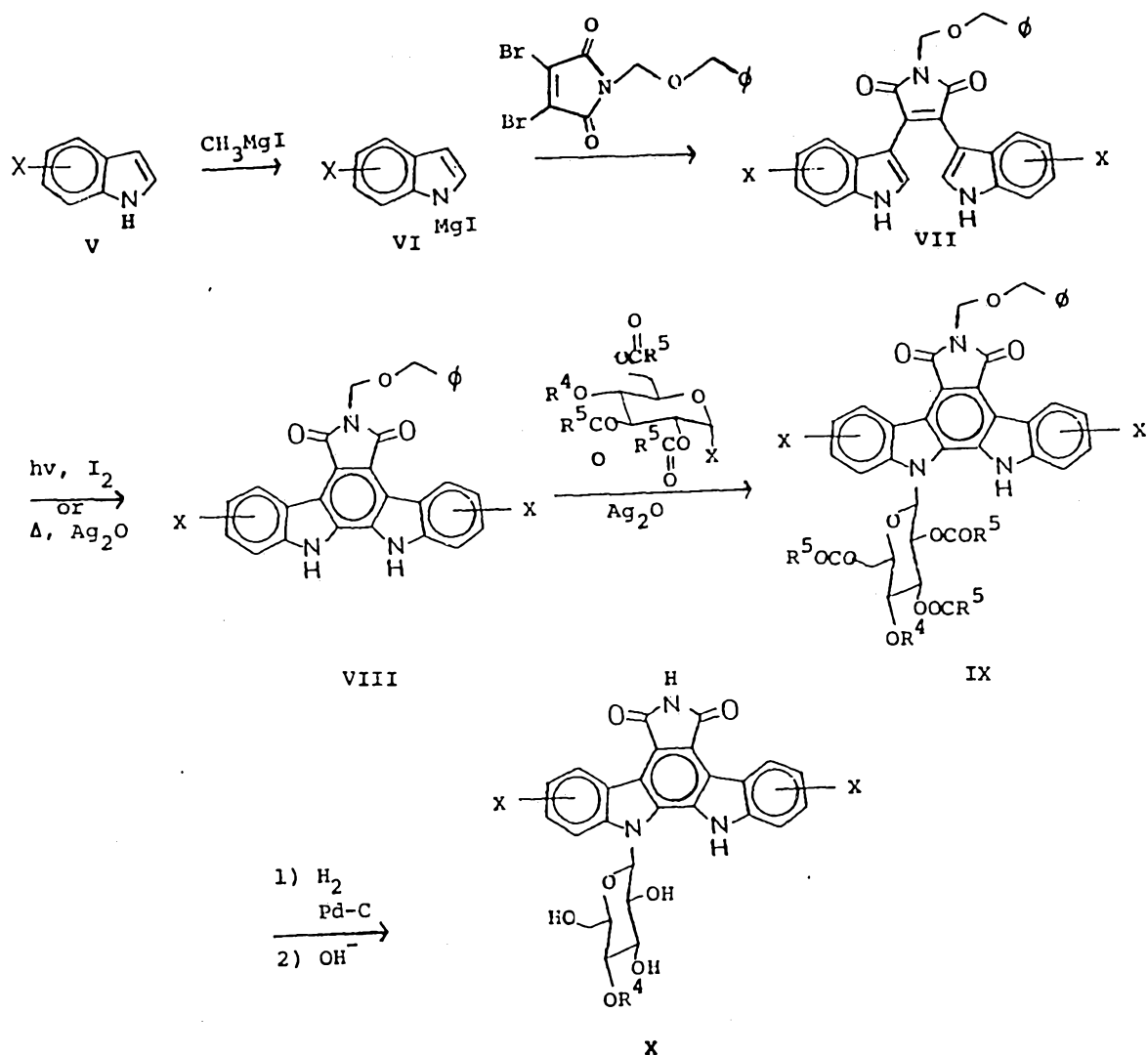
Derivatives of rebeccamycin having various groups substituted on the rebeccamycin ring system in place of the 1,11-dichloro substituents may be used as starting materials in the place of rebeccamycin to obtain compounds within the broad definition and preferred and more preferred embodiments according to the present invention. For example, didechlororebeccamycin can be produced by subjecting rebeccamycin to hydrogenolysis and the resulting intermediate may be subjected to alkylation using strong base followed by reaction of the intermediate from the strong base treatment with a suitable aminoalkyl halide. Other chromophores of rebeccamycin having various substituents on the rebeccamycin ring system can be produced using methods disclosed by T. Kaneko et al., Tetrahedron Letters, 26, 4015 (1985). For example, by using the procedures therein disclosed, compounds having the following formula, IV, were produced:



Formula IV

X = Cl  
X = OCH<sub>3</sub>  
X = OCH<sub>2</sub> Ph

The following more particularly describes a typical synthesis of analogs or chromophores of rebeccamycin having various substituents on the ring system in the place of the Cl groups. Treatment of a substituted indole with methyl magnesium tetrahydrofuran produces a solution of indole Grignard (VI). Two equivalents of this solution and one equivalent of N-benzyloxymethyl 3,4-dibromo-maleimide gives an adduct of formula VII. This can be either photochemically cyclized by irradiation at 300 nm in a benzene solution containing a small amount of  $I_2$  or thermally by refluxing a solution of the adduct in benzene in the presence of  $Ag_2O$ . The intermediate thus prepared (VIII) is then coupled with O-acylated 1-halosugar in the presence of  $Ag_2O$  to give N-glycoside IX. A catalytic hydrogenation to remove the N6 protecting group and a base hydrolysis of the sugar O-acyl groups gives the desired product X. Illustrative of the substituted indoles that can be used in this preparation are listed in Table 2.



X and  $\text{R}^4$  are as defined herein  
 $\text{R}^5$ , typically, is  $\text{CH}_3$



Table 2 - Starting Indoles

5-Aminoindole  
6-Aminoindole  
5-Benzyloxyindole  
4-Chloroindole  
5-Chloroindole  
4-Hydroxyindole  
5-Hydroxyindole  
Indole  
4-Methoxyindole  
5-Methoxyindole  
4-Methylindole  
5-Methylindole  
6-Methylindole  
Indole-5-carboxylic acid

Glycosidation of any one of the three chromophores represented by Formula IV or a chromophore represented by Formula X by reaction with an appropriate glycosyl halide provides a rebeccamycin derivative. And, reaction of the rebeccamycin derivative first with strong base followed by an aminoalkyl halide provides analogs of rebeccamycin according to this invention having one of Formulas II or III.

Having obtained rebeccamycin or a rebeccamycin derivative or chromophore, this starting material is first dissolved in a suitable inert solvent, for example dimethylformamide (DMF), dimethylsulfoxide (DMSO),

tetrahydrofuran (THF) or other anhydrous, aprotic solvent and then reacted with a strong base in an inert atmosphere, for example in an argon or nitrogen atmosphere. Although any strong base that is compatible with the starting material and solvent may be used, for example,  $\text{KNH}_2$  or  $\text{KH}$  or  $\text{NaNH}_2$  or  $\text{NaH}$  or lithium diisopropyl amide (LDA) or lithium hexamethyl disalazide or  $\text{KOtBu}$  or Grignard reagent such as  $\text{MeMgBr}$  or  $\text{NaNH}_2$  or  $\text{NaH}$  or equivalent base,  $\text{NaH}$  may be advantageously employed.

It has been discovered that by using strong base, for example  $\text{NaH}$ , in an amount slightly in excess of the molar equivalent of the amount of starting material, for example in the range of from about 5% to about 15% and preferably about 10% (9-11%) excess, followed by treatment with at least one molar equivalent based on rebeccamycin starting material of an appropriate aminoalkyl compound, there may be obtained a compound according to the invention having an aminoalkyl substituent on the N-atom in the 6-position of the rebeccamycin ring system.

Further, it has been discovered that by using strong base, for example  $\text{NaH}$ , in a relatively large excess amount such as an amount slightly in excess of two-times the molar equivalent of the amount starting material, for example, in the range of about 15% to about 25% and preferably about 20% (18-22%) excess, followed by treatment with an appropriate aminoalkyl compound in an amount about the molar equivalent of starting material, there may be obtained a compound according to the invention having an aminoalkyl substituent on the N-atom in the 13-position of the rebeccamycin ring

system.

When more than two equivalents of base are used, a dianion at N6 and N13 is formed. Since the N13 anion is more reactive than the N6 anion, a N13 aminoalkyl derivative is obtained when only one equivalent of aminoalkyl halide is used. When two equivalents of aminoalkyl halide is used, the N6,N13-diaminoalkyl derivative may be obtained.

Following the reaction of the starting material with a strong base, the resulting reactive intermediate is reacted in situ with an appropriate reactive aminoalkyl compound by adding the aminoalkyl compound to the mixture of starting material and strong base and intermediate reaction product thereof in inert solvent. Any aminoalkyl compound that is compatible with the starting material and product and solvent may be used, for example an aminoalkyl halide or sulfonate and the like, represented by the formula  $L-(CH_2)_n-NR^1R^2$  wherein  $n$ ,  $R^1$  and  $R^2$  are as defined above and  $L$  is a leaving group such as halide or methanesulfonate or p-toluenesulfonate. Generally an aminoalkyl halide such as, for example, diethylaminoethyl chloride and diethylamino-propyl chloride, may be advantageously employed.

Generally, the reaction of strong base with starting material may be carried-out advantageously at about room temperature, that is, at about 18°C to about 22°C. The mixture of starting material and strong base, generally, may be stirred for a period of a few minutes to several hours; however, the reaction is usually complete in about 20-30 minutes.

Then, there is added to the stirred mixture of starting material and strong base and resulting reactive intermediate reaction product thereof in inert solvent the appropriate aminoalkyl compound and the resulting mixture may be stirred at room temperature for about 20-24 hours or at about 4°C for a shorter period of time, for example, six hours until the reaction of the reactive intermediate with aminoalkyl compound is complete.

The compounds of Formulas II and III according to this invention may be provided as pharmaceutically acceptable acid addition and base salts provided that the anion or cation thereof did not contribute significantly to the toxicity of the salt and that the salts are compatible with the standard and conventional pharmaceutical carriers and other conventional adjuvants and excipients customarily employed in producing pharmaceutical compositions adapted for oral or parenteral administration. The acid addition salts are formed by conventional techniques involving reaction of compounds of Formulas II and III with a mineral acid or organic carboxylic and sulfonic acids. Examples of suitable mineral acids include hydrochloric acid, phosphoric acid, and the like. Examples of suitable organic acids include acetic acid, citric acid, maleic acid, succinic acid, benzoic acid, tartaric acid, ascorbic acid, methanesulfonic acid, p-toluenesulfonic acid, and the like.

The base salts are formed by conventional techniques involving reaction of the compounds of Formulas II and III preferably with alkali (Na, K) and alkalkine earth (Ca, Zn, Ba, Mg, and Mn) metal bases, more preferably with alkali metal bases,



and by reaction with amines. Suitable bases include the hydroxide, carbonate or bicarbonate salts of the metals mentioned above such as, for example, sodium and potassium hydroxides, sodium and potassium carbonates, and sodium and potassium bicarbonates and the corresponding calcium and zinc salts. Additional suitable bases include ammonium salts those formed by reaction of compounds of formulas II and III with triethylamine, dibenzylamine, N, N'-dibenzylethylenediamine, procaine, and equivalent amines.

The pharmaceutical carrier may be solid or liquid to provide solid or liquid compositions. Solid form compositions suitable for oral administration include powders, tablets, capsules, caplets, dispersible granules, and cachets. Suitable solid carriers include at least one carrier substance which may function only as a carrier or may in addition serve a further function such as a diluent, flavoring agent, solubilizer, lubricant, suspending agent, binder, tablet disintegrating agent, encapsulating agent and the like. Inert solid carriers include, to name but a few, magnesium carbonate and stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, cellulosic materials, and the like. The compounds according to the invention may be provided as sterile soluble compounds or compositions, including solutions and suspensions and emulsions, thereof which can be dissolved in sterile water or other liquid medium for oral administration or for parenteral administration. Examples of liquid carriers suitable for oral administration include water, alcohol, polypropylene glycol, polyethylene glycol and mixtures of two or more of the above. Examples of liquid carriers suitable for parenteral use include water-for-injection, physiological

aline, and other suitable sterile injection media. Suitable buffers for use with the liquid carrier to provide, generally, a suitable buffered isotonic solution include trisodium orthophosphate, sodium bicarbonate, sodium citrate, N-methylglucamine, L(+)-lysine, and L(+)-arginine to name but a few representative buffering agents.

The pharmaceutical composition will contain an amount of active component, that is, compound of Formula II or III or mixture thereof, which may be varied or adjusted widely depending upon the particular application, the form, the potency of the particular compound used, and the desired concentration of compound in the composition. Generally, the amount of active component will range between about 0.5-90% by weight based on total weight of composition.

In therapeutic use for treating a mammalian host, for example an experimental animal host, affected by a malignant tumor, the compounds of this invention will be administered in an amount effective to inhibit the growth of the tumor, that is, a tumor growth-inhibiting amount of dosage. Generally, the tumor growth-inhibiting amount will be in the range of about 0.1 to about 15 mg/kg of animal body weight/day. It is to be understood that the actual preferred dosage of compound will vary widely depending upon the requirements of the animal being treated, the particular animal host and situs and disease being treated, the composition being used, and the route of administration. Many factors that modify the action of the anti-neoplastic agent will be taken into account by one skilled in the art to which this invention pertains including, for example, age, body weight and sex of the animal host; diet; time of

administration; rate of excretion; condition of the host; severity of the disease; and the like. Administration may be carried out simultaneously or periodically within the maximum tolerated dose. Optimal administration (or application) rates for a given set of conditions may be readily ascertained by those skilled in the art using conventional dosage determination tests.

The following examples are presented to illustrate but a few representative embodiments of this invention and are not to be construed as limiting in scope. All parts and percentages are by weight and all temperatures are in degrees Celsius unless otherwise indicated.

Rebeccamycin analogs according to this invention were tested for antitumor activity against the transplanted mouse leukemia P-388 according to the procedures of Geran *et al.* reported in Cancer Chemother. Rpts., 3, 1-103 (1972). Prolongation of survival of leukemic mice was observed at several dosage levels ranging from 6 mg/kg body wt./day to 100 mg/kg body wt./day. Results of the tests are shown in the accompanying Table 3. The standard, comparison agent is selected from olivomycin and mitomycin C. These results illustrate that the rebeccamycin analogs according to the invention possess useful antineoplastic activity.

Example 1. 6-(2-Diethylaminoethyl)rebeccamycin

DMF (100 mL) was added under argon to a mixture of rebeccamycin (990 mg, 1.74 mmol) and NaH (46 mg, 1.91 mmol). After 20 minutes of stirring at room temperature, 2-diethylaminoethyl chloride (524 mg, 3.86 mmol) was added.

The resulting mixture was stirred for 24 h. and the reaction was then quenched by addition of 1% aq HCl solution. The reaction mixture was basified by addition of saturated  $\text{NaHCO}_3$  solution and extracted with EtOAc.

The organic layer was collected and washed with brine and dried over  $\text{Na}_2\text{SO}_4$ . The residue obtained by evaporation of the solvent was chromatographed on silica gel (elution with EtOAc) to give 770 mg (66%) of the title compound: mp  $>250^\circ\text{C}$ ; NMR ( $\text{DMSO}-d_6$ )  $\delta$  10.68 (s, 1H), 9.26 (d, 1H,  $J = 7.8$  Hz), 9.08 (d, 1H,  $J = 7.9$  Hz), 7.73 (d, 1H,  $J = 8.9$  Hz), 7.70 (d, 1H,  $J = 8.9$  Hz), 7.45 (t, 2H,  $J = 7.8$  Hz), 6.94 (d, 1H,  $J = 9.1$  Hz), 5.43 (d, 1H,  $J = 5.6$  Hz), 5.31 (bs, 1H), 5.03 (d, 1H,  $J = 5.7$  Hz), 3.95 (s, 2H), 3.82 (m, 2H), 3.70 - 3.48 (m, 7H), 2.72 (m, 2H), 2.53 (m, 4H), 0.94 (t, 6H,  $J = 7.0$  Hz); IR (KBr) 3343, 1692, 1381, 1070, 760  $\text{cm}^{-1}$ ; FABMS 669 (M+1), 493, 217 m/e.

Example 2. 6-(2-Diethylaminoethyl)rebeccamycin hydrochloride

To a solution of 6-(2-diethylaminoethyl)rebeccamycin (770 mg, 1.15 mmol) in 30 mL of THF at  $0^\circ\text{C}$  was added one equivalent of 5.6M ethanolic HCl solution. After stirring 3 h at  $0^\circ\text{C}$  the resulting precipitate was collected by filtration and washed with diethyl ether to give 728 mg (90%) of the title compound: mp  $>250^\circ\text{C}$ ; NMR ( $\text{DMSO}-d_6$ )  $\delta$  10.7 (s, 1H), 10.16 (s, 1H), 9.23 (d, 1H,  $J = 8.1$  Hz), 9.05 (d, 1H,  $J = 8.0$  Hz), 7.74 (d, 1H,  $J = 8.5$ ), 7.72 (d, 1H,  $J = 9.3$  Hz), 7.46 (t, 2H,  $J = 8.1$  Hz), 6.94 (d, 1H,  $J = 9.1$  Hz), 5.47 (bs, 1H), 5.36 (bs, 1H), 5.08 (bd, 1H,  $J = 3.8$  Hz), 4.11 (t, 2H,  $J = 6.3$  Hz), 3.97 (bs, 1H), 3.85 (d, 1H,  $J = 9.5$  Hz),



3.66 - 3.57 (m, 7H), 3.49 - 3.20 (m, 7H), 1.26 (t, 6H,  $J = 7.0$  Hz); IR (KBr) 3336, 1699, 1416, 1381, 1084, 760  $\text{cm}^{-1}$ ; FABMS 669 (M+1), 635, 493 m/e.

Example 3. 6-(3-Diethylaminopropyl)rebeccamycin

DMF (20mL) was added under argon to a mixture of rebeccamycin (162 mg, 0.28 mmol) and NaH (7.5 mg, 0.31 mmol). To this solution was added 110 mg (0.78 mmol) of 3-diethylaminopropyl chloride. The resulting solution was stirred and warmed to 40°C for 6 h. A workup similar to Example 1 gave 55 mg (28%) of the title compound: NMR (DMSO- $d_6$ )  $\delta$  9.28 (d, 1H,  $J = 7.8$  Hz), 9.10 (d, 1H,  $J = 8.1$  Hz), 7.73 (d, 1H,  $J = 9.4$  Hz), 7.70 (d, 1H,  $J = 9.4$  Hz), 7.45 (d, 1H,  $J = 7.8$  Hz), 6.94 (d, 1H,  $J = 9.1$  Hz), 5.42 (bd, 1H,  $J = 5.8$  Hz), 5.31 (bs, 1H), 5.03 (bs, 1H), 3.95 (bs, 2H), 3.82 - 3.59 (m, 9H), 2.44 (m, 6H), 1.81 (m, 2H), 0.90 (t, 6H,  $J = 7.0$  Hz); IR (KBr), 3328, 1691, 1376, 1052, 755  $\text{cm}^{-1}$ ; MS 682 (M), 653, 403, 393 m/e.

Example 4. 6-(3-Diethylaminopropyl)rebeccamycin hydrochloride

Treatment of the product from Example 3 with ethanolic HCl in a similar manner as Example 2 gave the title compound in 89% yield: mp >250°C; NMR (DMSO- $d_6$ )  $\delta$  10.70 (s, 1H), 9.81 (bs, 1H), 9.26 (d, 1H,  $J = 7.1$  Hz), 9.09 (d, 1H,  $J = 8.0$  Hz), 7.73 (d, 1H,  $J = 11.4$  Hz), 7.72 (d, 1H,  $J = 11.0$  Hz), 7.43 (t, 2H,  $J = 7.9$  Hz), 6.94 (d, 1H,  $J = 9.2$  Hz), 5.46 (bs, 1H), 5.34 (bs, 1H), 5.06 (bs, 1H), 3.95 (bs, 2H), 3.83 (m, 2H), 3.66 - 3.54 (m, 5H), 3.20 (m, 2H), 3.10 (m, 4H), 2.12 (m, 2H), 1.18 (t, 6H,  $J = 7.2$  Hz); IR (KBr) 3343, 1692, 1452, 1070, 760  $\text{cm}^{-1}$ ; FABMS 683, (M+1), 507, 406 m/e.

Example 5. 13-(3-Dethylaminopropyl)rebeccamycin

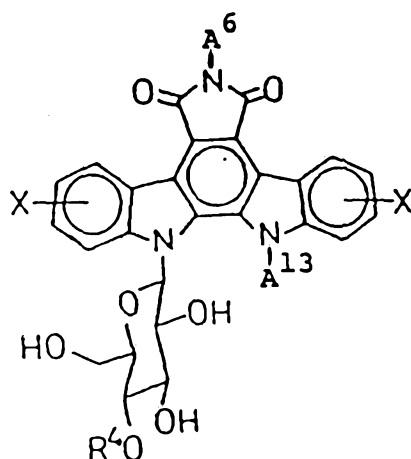
DMF (25 mL) was added under argon to a mixture of rebeccamycin (188 mg, 0.33 mmol) and NaH (17 mg 0.73 mmol). After a few minutes of stirring at room temperature, 3-diethylaminopropyl chloride (54 mg, 0.36 mmol) was added and the resulting solution was stirred at room temperature for 22 h. The reaction was quenched by addition of 10% HCl solution in water. Saturated  $\text{NaHCO}_3$  solution was added to basify the solution and the product was extracted with EtOAc. The organic layer was collected and washed with brine and dried over  $\text{MgSO}_4$ . The residue obtained after evaporation of the solvent was chromatographed on neutral alumina (10% MeOH-acetone) to give 76 mg (34%) of the title compound: mp 203-204°C; NMR ( $\text{DMSO-d}_6$ )  $\delta$  10.67 (s, 1H), 9.28 (d, 1H,  $J = 8.1$  Hz), 9.10 (d, 1H,  $J = 8.1$  Hz), 7.73 (d, 1H,  $J = 10.9$  Hz), 7.71 (d, 1H,  $J = 11.6$  Hz), 7.45 (t, 2H,  $J = 8.1$  Hz), 6.94 (d, 1H,  $J = 9.0$  Hz), 5.43 (d, 1H,  $J = 5.8$  Hz), 5.32 (t, 1H,  $J = 5.2$  Hz), 5.03 (d, 1H,  $J = 5.5$  Hz), 3.96 (m, 2H), 3.85-3.79 (m, 3H), 3.91-3.53 (m, 5H), 2.71 (m, 4H), 1.95 (m, 2H), 1.13 (m, 6H); IR (KBr) 3343, 1696, 1388, 1085, 760  $\text{cm}^{-1}$ ; MS 683 (M+1), 654, 506 m/e.

TABLE 3 - EFFECT OF REBECCAMYCIN ANALOGS ON P-388 LEUKEMIA

<u>Compound</u>	<u>Dose, IP mg/kg/inj</u>	<u>Median Survival Time (MST) Days</u>	<u>% T/C</u>	<u>Avg. wt. change, gm day 4</u>
Olivomycin	0.8	17.0	162	-2.4
	0.4	13.5	129	-2.6
Ex. 1	100	13.0	124	-2.0
	50	17.5	167	-2.2
	25	14.0	133	-0.5
-----				
Mitomycin C	4.8	19.0	211	-2.0
	3.2	22.0	244	-1.5
Ex. 2	8.0	12.0	133	-0.4
	6.0	12.5	139	-0.0
Ex. 4	8.0	12.5	139	-0.9
	6.0	13.0	144	0.1

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A compound having the formula



wherein:

$n$  is an integer from 1 to 6;

$A^6$  and  $A^{13}$  are selected from H and  $-(CH_2)_nNR^1R^2$  and at least one of  $A^6$  and  $A^{13}$  is  $-(CH_2)_n-NR^1R^2$ ;

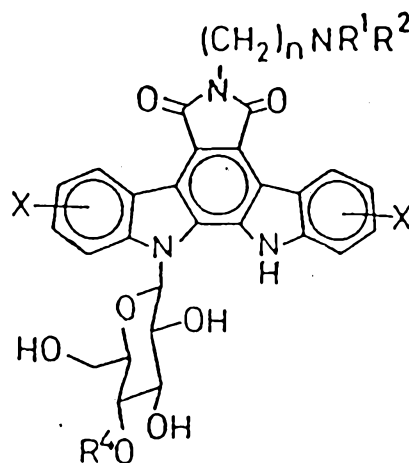
$R^1$  and  $R^2$ , independently, are selected from hydrogen, unsubstituted and substituted  $C_1-C_6$  alkyl, aralkyl having 1 to 3 carbons in the alkyl moiety and unsubstituted phenyl or phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, carboxyl, alkoxycarbonyl, and amino and mono- and di-lower-alkylamino groups in the aryl moiety, and aryl selected from unsubstituted phenyl and phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, amino, mono- and dialkylamino, nitro, carboxyl, alkoxycarbonyl, and cyano

groups provided that both  $R^1$  and  $R^2$  are not each aryl and, when taken together,  $R^1$  and  $R^2$  may be selected from  $-(CH_2)_4-$  and  $(CH_2)_2-R^3-(CH_2)_2-$  to form a 5- or 6-membered ring together with the N-atom wherein  $R^3$  is selected from  $CH_2$ ,  $NH$ ,  $O$  and  $S$ ;

$X$  is selected from  $H$ ,  $F$ ,  $Cl$ ,  $Br$ ,  $C_1-C_3$  alkyl,  $OH$ , carboxyl, alkoxycarbonyl and alkoxy wherein the alkyl moiety is  $C_1-C_3$  alkyl, benzyloxy, amino, and mono- and dialkylamino; and

$R^4$  is selected from  $H$  and  $CH_3$ ; and pharmaceutically acceptable acid addition and base salts thereof.

2. A compound according to Claim 1 having the formula



wherein:

$n$  is an integer from 1 to 6;

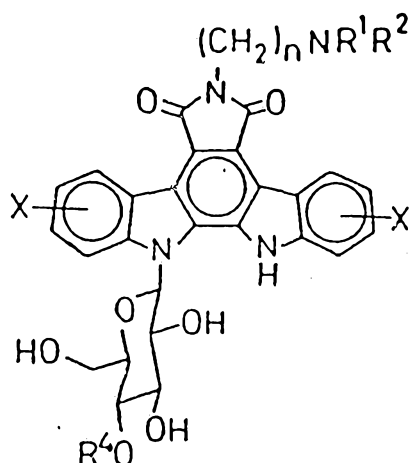
$R^1$  and  $R^2$ , independently, are selected from hydrogen, unsubstituted and substituted  $C_1-C_6$  alkyl, aralkyl having 1

groups provided that both  $R^1$  and  $R^2$  are not each aryl and, when taken together,  $R^1$  and  $R^2$  may be selected from  $-(CH_2)_4-$  and  $(CH_2)_2-R^3-(CH_2)_2-$  to form a 5- or 6-membered ring together with the N-atom wherein  $R^3$  is selected from  $CH_2$ ,  $NH$ ,  $O$  and  $S$ ;

$X$  is selected from  $H$ ,  $F$ ,  $Cl$ ,  $Br$ ,  $C_1-C_3$  alkyl,  $OH$ , carboxyl, alkoxycarbonyl and alkoxy wherein the alkyl moiety is  $C_1-C_3$  alkyl, benzyloxy, amino, and mono- and dialkylamino; and

$R^4$  is selected from  $H$  and  $CH_3$ ; and pharmaceutically acceptable acid addition and base salts thereof.

2. A compound according to Claim 1 having the formula



wherein:

$n$  is an integer from 1 to 6;

$R^1$  and  $R^2$ , independently, are selected from hydrogen, unsubstituted and substituted  $C_1-C_6$  alkyl, aralkyl having 1

to 3 carbons in the alkyl moiety and unsubstituted phenyl or phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, carboxyl, alkoxycarbonyl, and amino and mono- and di-lower-alkylamino groups in the aryl moiety, and aryl selected from unsubstituted phenyl and phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, amino, mono- and dialkylamino, nitro, carboxyl, alkoxycarbonyl, and cyano groups provided that both  $R^1$  and  $R^2$  are not each aryl and, when taken together,  $R^1$  and  $R^2$  may be selected from  $-(CH_2)_4-$  and  $(CH_2)_2-R^3-(CH_2)_2-$  to form a 5- or 6-membered ring together with the N-atom wherein  $R^3$  is selected from  $CH_2$ ,  $NH$ ,  $O$  and  $S$ ;

$X$  is selected from  $H$ ,  $F$ ,  $Cl$ ,  $Br$ ,  $C_1-C_3$  alkyl,  $OH$ , carboxyl, alkoxycarbonyl and alkoxy wherein the alkyl moiety is  $C_1-C_3$  alkyl, benzyloxy, amino, and mono- and dialkylamino; and

$R^4$  is selected from  $H$  and  $CH_3$ ; and pharmaceutically acceptable acid addition and base salts thereof.

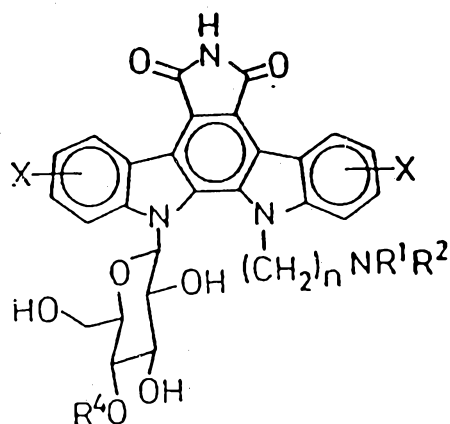
3. A compound according to Claim 2 wherein  $X$  is selected from  $H$ ,  $Cl$ ,  $Br$ ,  $OH$ ,  $OCH_3$  and  $OCH_2C_6H_5$ .

4. A compound according to Claim 3 wherein  $n$  is selected from the integers 1, 2, and 3 and  $R^1$  and  $R^2$ , independently, are selected from  $H$ ,  $C_1-C_3$  alkyl, and  $-(CH_2)_4-$ .

5. A compound according to Claim 4 wherein  $X$  is  $Cl$  in each of the 1- and 11-positions.

6. A compound according to Claim 5 wherein  $n$  is an integer selected from 2 and 3; and  $R^1$  and  $R^2$  are each  $C_2H_5$ .

7. A compound having the formula



wherein:  $n$  is an integer from 1 to 6;

$R^1$  and  $R^2$ , independently, are selected from hydrogen, unsubstituted and substituted  $C_1$ - $C_6$  alkyl, aralkyl having 1 to 3 carbons in the alkyl moiety and unsubstituted phenyl or phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, carboxyl, alkoxycarbonyl, and amino and mono- and di-lower-alkylamino groups in the aryl moiety, and aryl selected from unsubstituted phenyl and phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, amino, mono- and dialkylamino, nitro, carboxyl, alkoxycarbonyl, and cyano groups provided that both  $R^1$  and  $R^2$  are not each aryl and, when taken together,  $R^1$  and  $R^2$  may be selected from  $-(CH_2)_4-$  and  $-(CH_2)_2-R^3-(CH_2)_2-$  to form a 5- or 6-membered ring together the N-atom wherein  $R^3$  is selected from  $CH_2$ ,  $NH$ ,  $O$  and  $S$ .



X is selected from H, F, Cl, Br, C<sub>1</sub>-C<sub>3</sub> alkyl, OH, carboxyl, alkoxycarbonyl and alkoxy wherein the alkyl moiety is C<sub>1</sub>-C<sub>3</sub> alkyl, benzyloxy, amino, and mono- and dialkylamino; and

R<sup>4</sup> is selected from H and CH<sub>3</sub>; and pharmaceutically acceptable acid addition and base salts thereof.

8. A compound according to Claim 7 wherein R<sup>4</sup> is CH<sub>3</sub> and X is selected from Cl, Br, OH, OCH<sub>3</sub> and OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> in each of the 1- and 11-positions of the ring system.

9. A compound according to Claim 8 wherein n is selected from the integers 1, 2, and 3 and R<sup>1</sup> and R<sup>2</sup>, independently, are selected from H, C<sub>1</sub>-C<sub>3</sub> alkyl, and -(CH<sub>2</sub>)<sub>4</sub>-.

10. A compound according to Claim 9 wherein X is Cl in each of the 1- and 11-positions.

11. A compound according to Claim 10 wherein n is an integer selected from 2 and 3; and R<sup>1</sup> and R<sup>2</sup> are each C<sub>2</sub>H<sub>5</sub>.



Formula III

$R^1$  and  $R^2$ , independently, are selected from hydrogen, unsubstituted and substituted  $C_1$ - $C_6$  alkyl, aralkyl having 1 to 3 carbons in the alkyl moiety and unsubstituted phenyl or phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, carboxyl, alkoxycarbonyl and amino and mon- and di-lower-alkylamino groups in the aryl moiety, and aryl selected from unsubstituted phenyl and phenyl substituted with 1 to 3 alkyl, alkoxy, hydroxy, halo, amino, mono- and dialkylamino, nitro, carboxyl, alkoxycarbonyl and cyano groups provided that both  $R^1$  and  $R^2$  are not each aryl and, when taken together,  $R^1$  and  $R^2$  may be selected from  $-(CH_2)_4-$  and  $(CH_2)_2-R^3-(CH_2)_2-$  to form a 5- or 6-membered ring

together with the N-atom wherein  $R^3$  is selected from  $CH_2, NH, O$  and  $S$ ;

X is selected from  $H, F, Cl, Br, C_1-C_3$  alkyl,  $OH$ , carboxyl, alkoxy carbonyl and alkoxy wherein the alkyl moiety is  $C_1-C_3$  alkyl, and benzyloxy; and

5  $R^4$  is selected from  $H$  and  $CH_3$ ; and pharmaceutically acceptable acid addition and base salts thereof, and a pharmaceutically acceptable carrier, the salts being compatible with the pharmaceutically  
10 acceptable carrier.

13. A method for thereapeutically treating a mammalian host affected by a malignant tumor, sensitive to rebeccamycin comprising administering to the host a tumor growth-inhibiting amount of a compound according to any  
15 one of claims 1 to 11.

14. A method for therapeutically treating a mammalian host affected by a malignant tumor, sensitive to rebeccamycin comprising administering to the host a pharmaceutical composition according to claim 12 in an  
20 amount sufficient to effect tumor-growth inhibition.

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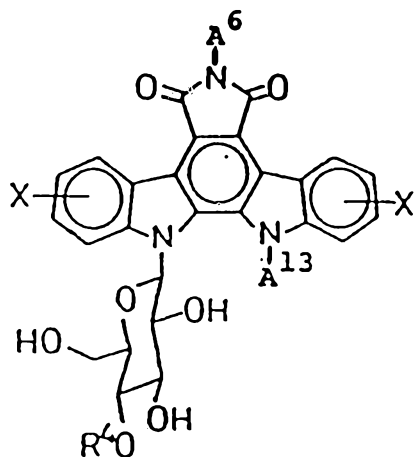
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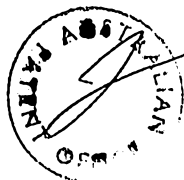


15. A process for producing a compound according to claim 1 having the formula

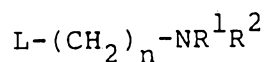


comprising the steps of:

- (a) a providing as the starting material one of rebeccamycin or a derivative thereof;
- (b) reacting the starting material from step (a), dissolved in a suitable inert solvent and held in an inert atmosphere, with an amount of strong base effective to obtain a reactive intermediate selected from (i) a rebeccamycin amion having the negative charge at the 6-position and (ii) a rebeccamycin dianion having negative charges at both the 6- and 13-positions;
- (c) reacting the reactive intermediate from step (b)



with an aminoalkyl compound represented by the formula



wherein L is a chemical leaving group and n, R<sup>1</sup> and R<sup>2</sup> are as defined hereinabove; and

(d) recovering the resulting aminoalkylated derivative of rebeccamycin from the mixture from step (c).

16. A compound <sup>according to claim 1</sup> substantially as hereinbefore described with reference to any one of the examples.

17. A pharmaceutical composition substantially as hereinbefore described with reference to any one of the examples.

18. A process for preparing a compound according to claim 1 substantially as hereinbefore described with reference to any one of the examples.

Dated: 6 November 1987

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