

## PATENT SPECIFICATION

(11) 1 511 680

- (21) Application No. 47559/75 (22) Filed 18 Nov. 1975  
 (23) Complete Specification filed 15 Nov. 1976  
 (44) Complete Specification published 24 May 1978  
 (51) INT CL<sup>2</sup> C07C 15/24; A61K 31/35, 31/535; C07C 15/26  
 (52) Index at acceptance

C2C 1253 1562 1672 22X 22Y 246 253 255 25Y 30Y 313  
 314 31Y 321 32Y 338 339 351 353 355 360 361 362  
 363 364 365 366 368 36Y 440 620 628 638 645 665  
 670 790 79Y LZ

- (72) Inventors FEDERICO ARCAMONE, LUIGI BERNARDI,  
 PIETRO GIARDINO and AURELIO DI MARCO

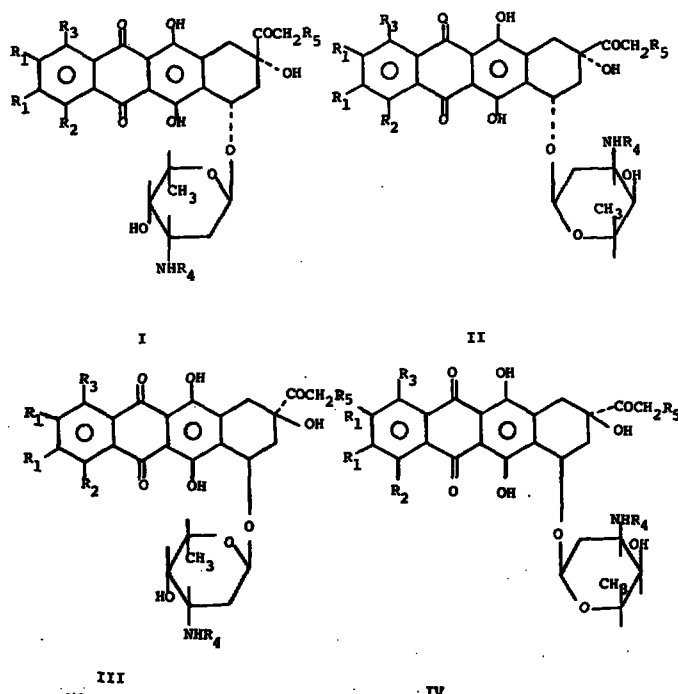


## (54) DAUNOSAMINYL ANTHRACYCLINONES

(71) We, SOCIETA FARMACEUTICI ITALIA S.p.A., a body corporate organised and existing under the laws of Italy, of 1/2 Largo Guido Donegani-1 20121 Milan, Italy, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to daunosaminyl anthracyclines, which have shown anti-tumour efficacy.

In British Patent Application No. 2691/75 (Serial No. 1,500,421) the preparation of daunosaminyl anthracyclines of general formulae I to IV is described and claimed:



wherein  $R_1$ ,  $R_2$  and  $R_3$  each represents a hydrogen, bromine or chlorine atom or a methyl or methoxy group with the proviso that, except when  $R_1$  and  $R_3$  represent hydrogen atoms and  $R_2$  represents a methoxy group,  $R_2$  and  $R_3$  represent the same atom or group,  $R_4$  represents a hydrogen atom or a trifluoroacetyl group, and  $R_5$  represents a hydrogen atom.

This invention provides as new compounds those of the above formulae I to IV wherein  $R_5$  represents a hydroxy group, an acyloxy group of the formula

OCOR<sub>6</sub>, an acylglycoloyloxy group of the formula OCOCH<sub>2</sub>OCOR<sub>6</sub> or a substituted amino group of the formula NR<sub>7</sub>R<sub>8</sub>, wherein R<sub>6</sub> represents a hydrocarbon residue having from 6 to 16 carbon atoms, and R<sub>7</sub> and R<sub>8</sub> each represents an alkyl group having from 1 to 4 carbon atoms or together with the nitrogen atom to which they are attached represent a 1-pyrrolidinyl, piperidino, morpholino or 4-methyl-1-piperazinyl group. These compounds are related to adriamycin and can be prepared by brominating compounds I to IV (R<sub>5</sub>=H) according to our British Patent Specification 1,217,133 to give the corresponding 14-bromo compounds I to IV (R<sub>5</sub>=Br). These are treated with sodium formate to make R<sub>5</sub>=OH, or with the sodium salt of a carboxylic acid to make R<sub>5</sub>=OCOR<sub>6</sub>, or with the sodium salt of an acylglycolic acid to make R<sub>5</sub>=OCOCH<sub>2</sub>OCOR<sub>6</sub>, or with an appropriate amine to make R<sub>5</sub>=NR<sub>7</sub>R<sub>8</sub>.

The new compounds of this invention have proved distinctly active in the inhibition of malignant or transformed cell growth; in particular they compare favourably with the well established anticancer drug adriamycin.

Daunomycin and Adriamycin are our Trade Marks and are used herein to mean daunorubicin and doxorubicin respectively.

This invention is illustrated by the following Examples in which all temperatures are indicated in degrees Celsius.

#### EXAMPLE 1.

4-Demethoxyadriamycin (I; R<sub>1</sub>=R<sub>2</sub>=R<sub>3</sub>=R<sub>4</sub>=H; R<sub>5</sub>=OH).

To 1 g (1.87 mM) of 4-demethoxydaunomycin hydrochloride dissolved in 14 ml of methanol and 38 ml of dioxan, 1 ml of ethyl orthoformate is added. The solution is kept at +10° and 1.96 mM of bromine in 3 ml of chloroform and 1.17 mM of 2.5 N hydrogen chloride in methanol are added at once. After one hour the solution is poured into a mixture of 100 ml of petroleum ether and 200 ml of diethyl ether. The precipitate is dissolved in 150 ml of dioxan and 150 ml of 0.25 N hydrobromic acid. The solution is kept overnight at 25°, and a solution of 11 g of sodium formate in 110 ml of water is added. The resulting red solution is kept at 25° for 24 hours. The solution is concentrated *in vacuo*. The residue is taken up in 200 ml of chloroform/methanol (13/6 by vol). The solution is washed with 5% aqueous sodium bicarbonate, and then with water. Evaporation of the chloroform leaves a residue. To the residue an equivalent of hydrogen chloride in diethyl ether is added to give, after filtration, 0.65 g of 4-demethoxyadriamycin, m.p. 186—188° as hydrochloride.

#### EXAMPLE 2.

4-Demethoxy-2,3-dimethyladriamycin (I; R<sub>2</sub>=R<sub>3</sub>=R<sub>4</sub>=H; R<sub>1</sub>=CH<sub>3</sub>; R<sub>5</sub>=OH).

Operating as in Example 1 and employing 1 g of 4-demethoxy-2,3-dimethyl-daunomycin (I; R<sub>2</sub>=R<sub>3</sub>=R<sub>4</sub>=R<sub>5</sub>=H; R<sub>1</sub>=CH<sub>3</sub>) 0.55 g of 4-demethoxy-2,3-dimethyl-adriamycin (I; R<sub>2</sub>=R<sub>3</sub>=R<sub>4</sub>=H; R<sub>1</sub>=CH<sub>3</sub>; R<sub>5</sub>=OH) are obtained. m.p. 205—207° as hydrochloride.

#### EXAMPLE 3.

4-Demethoxyadriamycin octanoate (I; R<sub>1</sub>=R<sub>2</sub>=R<sub>3</sub>=R<sub>4</sub>=H; R<sub>5</sub>=OCO(CH<sub>2</sub>)<sub>6</sub>(CH<sub>3</sub>)).

To 1 g of 4-demethoxydaunomycin hydrochloride dissolved in 14 ml of methanol and 38 ml of dioxan, 1 ml of ethyl orthoformate is added. The solution is kept at +10° and 1.96 mM of bromine in 3 ml of chloroform and 1.71 mM of 2.5 N hydrogen chloride in methanol are added at once. After one hour, the solution is poured into a mixture of 100 ml of petroleum ether and 200 ml of diethyl ether. The precipitate is dissolved in 150 ml of dioxan and 150 ml of 0.25 N hydrogen bromide. The solution is kept overnight at 25°. The solution is repeatedly extracted with chloroform which is discarded, and then with butanol. The butanol extracts are concentrated *in vacuo*, and on dilution with diethyl ether 1.1 g of 14-bromo-4-demethyl-daunomycin (I; R<sub>1</sub>=R<sub>2</sub>=R<sub>3</sub>=R<sub>4</sub>=H; R<sub>5</sub>=Br) are collected.

A solution of this 1.1 g of 14-bromo-4-demethoxy-daunomycin in 500 ml of acetone is treated with 3 g of sodium octanoate at reflux temperature for one hour. Evaporation of the solvent leaves a residue which is taken up in chloroform. After washing with dilute sodium bicarbonate solution the solvent is evaporated *in vacuo* and the residue washed with ethyl acetate and diethyl ether to give 1 g of 4-demethyl-adriamycin 14-octanoate. IR spectrum: 1730 cm<sup>-1</sup>; 1620 and 1575 cm<sup>-1</sup> (CO; sharp).

EXAMPLE 4.

4 - Demethoxy - adriamycin 14 - hexanoylglycolate (I;  $R_1=R_2=R_3=R_4=H$ ;  $R_5=OCOCH_2OCO(CH_2)_4CH_3$ ).

Operating as in Example 3, 14-bromo-4-demethoxydaunomycin is treated with sodium hexanoylglycolate to give 4-demethoxy-adriamycin 14-hexanoylglycolate; Rf 0.32 in a chloroform:methanol:water: 14:6:1 system; I.R. spectrum  $1740\text{ cm}^{-1}$  (broad),  $1620$  and  $1575\text{ cm}^{-1}$  (sharp; CO).

EXAMPLE 5.

4-Demethoxy-14-morpholino-daunomycin (I;  $R_1=R_2=R_3=R_4=H$ ;  $R_5=\text{morpholino}$ ).

Operating as in Example 3, 14-bromo-4-demethoxy-daunomycin is prepared. Then 1 g thereof is dissolved in 50 ml of *t*-butanol and 50 ml of chloroform and treated with 3 ml of morpholine. After 3 hours at room temperature the solution is diluted with 500 ml of chloroform and washed repeatedly with water. Evaporation of the solvent leaves a residue. This is taken up in diethyl ether and filtered to give 0.6 g of 4-demethoxy-14-morpholino-daunomycin, electrophoretic mobility 1.73 at pH 1.2 (daunomycin=1).

EXAMPLE 6.

4-Demethoxy-1,4-dimethyladriamycin (I;  $R_1=R_4=H$ ;  $R_2=R_3=CH_3$ ;  $R_5=OH$ ).

Operating as in Example 1 and employing 1.5 g of 4-demethoxy-1,4-dimethyl-daunomycin (I;  $R_1=R_4=H$ ;  $R_2=R_3=CH_3$ ;  $R_5=H$ ), 0.62 g of 4-demethoxy-1,4-dimethyladriamycin are obtained as hydrochloride.

EXAMPLE 7.

4-Demethoxy-1,4-dichloroadriamycin (I;  $R_1=R_4=H$ ;  $R_2=R_3=Cl$ ;  $R_5=OH$ ).

Operating as in Example 1 and employing 1 g of 4-demethoxy-1,4-dichloro-daunomycin (I;  $R_1=R_4=H$ ;  $R_2=R_3=Cl$ ;  $R_5=H$ ), 0.6 g of 4-demethoxy-1,4-dichloro-adriamycin are obtained as hydrochloride.

EXAMPLE 8.

4-Demethoxy-2,3-dichloroadriamycin (I;  $R_2=R_3=R_4=H$ ;  $R_1=Cl$ ;  $R_5=OH$ ).

Operating as in Example 1 and employing 1.1 g of 4-demethoxy-2,3-dichloro-daunomycin (I;  $R_2=R_3=R_4=R_5=H$ ;  $R_1=Cl$ ) 0.42 g of 4-demethoxy-2,3-dichloro-adriamycin are obtained as hydrochloride.

*Biological activity of 4-demethoxyadriamycin in comparison with adriamycin.*

Table 1.

Biological activity on cultured HeLa cells. Data are expressed as 50% inhibiting dose (ng/ml) at different times of exposure.

| Compound              | ID <sub>50</sub> |      |      |
|-----------------------|------------------|------|------|
|                       | 2 h              | 8 h  | 24 h |
| 4-Demethoxyadriamycin | 1.5              | 0.34 | 0.14 |
| Adriamycin            | 125              | 28   | 12.5 |

Comment: 4-demethoxyadriamycin is one hundred fold more active than adriamycin as far as the inhibition of colony forming ability of HeLa cells "in vitro" is concerned.

12

4

1,511,680

4

Table 2.

Antitumour activity in mice bearing Sarcoma 180 ascites; single intraperitoneal treatment on day 1. The results are expressed as the time of survival of treated animals as a percentage of the controls (T/C%), the number of long term survivors (LTS) and toxic deaths (Tox).

5

5

| Compound              | Dose (mg/kg) | T/C %     | LTS  | Tox  |
|-----------------------|--------------|-----------|------|------|
| 4-Demethoxyadriamycin | 0.25         | above 583 | 7/10 | —    |
|                       | 0.5          | above 583 | 6/10 | —    |
|                       | 1.0          | 218       | —    | 6/9  |
| Adriamycin            | 2.5          | above 583 | 7/10 | —    |
|                       | 5.0          | 211       | 2/10 | 3/9  |
|                       | 10.0         | 163       | —    | 4/10 |

Comment: 4-Demethoxyadriamycin is at least ten times more active than adriamycin by this test.

Table 3.

10

Antitumour activity in various mice, as described in the Notes below, infected with various leukemias, also as described in the Notes below, and treated with 4-demethoxy-adriamycin and with adriamycin as control, also as described in the Notes below. The results are expressed in terms of T/C% and Tox, which have the meanings ascribed previously.

10

| Tumour                       | Compound               | Dose (mg/kg) | T/C % | Tox   |
|------------------------------|------------------------|--------------|-------|-------|
| L 1210 leukemia <sup>1</sup> | Adriamycin             | 2.5          | 155   |       |
|                              |                        | 5            | 166   | 1/10  |
|                              |                        | 10           | 155   | 4/10  |
|                              | 4-demethoxy-adriamycin | 0.25         | 155   |       |
|                              |                        | 0.5          | 166   |       |
|                              |                        | 1            | 133   | 10/10 |
| P 388 leukemia <sup>2</sup>  | Adriamycin             | 2.5          | 173   |       |
|                              |                        | 5            | 218   |       |
|                              |                        | 10           | 250   | 1/10  |
|                              | 4-demethoxy-adriamycin | 0.25         | 168   |       |
|                              |                        | 0.5          | 195   |       |
|                              |                        | 1            | 209   | 2/10  |
| Gross leukemia <sup>3</sup>  | Adriamycin             | 3.5          | 164   |       |
|                              |                        | 4.4          | 186   |       |
|                              |                        | 6.0          | 214   | 3/10  |
|                              | 4-demethoxy-adriamycin | 0.35         | 228   | 1/10  |
|                              |                        | 0.45         | 214   | 1/10  |
|                              |                        | 0.6          | 157   | 8/10  |

Notes:

1. BDF1 mice received  $10^5$  ascites cells intraperitoneally on Day 0 — Treatment intraperitoneal on Day 1.
2. BDF1 mice received  $10^6$  ascites cells intraperitoneally on Day 0 — Treatment intraperitoneal on Day 1.
3. C3H/Hc mice received  $2 \times 10^6$  leukemic cells intravenously on Day 0 — Treatment intravenous on Days 1, 2, 3.

20

20

Table 4.

Activity on L1210 leukemia.  
BDF1 mice were injected intraperitoneally with  $10^5$  ascites cells on Day 0 and treated intraperitoneally on Day 1.

| Compound                                | Dose<br>(mg/kg) | T/C % | LTS  |
|-----------------------------------------|-----------------|-------|------|
| Adriamycin                              | 2.9             | 141   |      |
|                                         | 4.4             | 152   | 1/10 |
|                                         | 6.6             | 158   |      |
|                                         | 10              | 164   | 1/10 |
| 2,3-Dimethyl-4-demethoxy-<br>adriamycin | 1.9             | 152   |      |
|                                         | 2.9             | 152   |      |
|                                         | 4.4             | 170   | 1/10 |

T/C and LTS mean the same as in Table 2.

#### WHAT WE CLAIM IS:—

1. A compound of the general formula I, II, III or IV wherein  $R_1$ ,  $R_2$  and  $R_3$  each represents a hydrogen, bromine or chlorine atom or a methyl or methoxy group with the proviso that, except when  $R_1$  and  $R_3$  represent hydrogen atoms and  $R_2$  represents a methoxy group,  $R_2$  and  $R_3$  represent the same atom or group,  $R_4$  represents a hydrogen atom or a trifluoroacetyl group, and  $R_5$  represents a hydroxy group, an acyloxy group of the formula  $OCOR_6$ , an acylglycolloyloxy group of the formula  $OCOCH_2OCOR_6$  or a substituted amino group of the formula  $NR_7R_8$  wherein  $R_6$  represents a hydrocarbon residue having from 6 to 16 carbon atoms, and  $R_7$  and  $R_8$  each represents an alkyl group having from 1 to 4 carbon atoms or together with the nitrogen atom to which they are attached represent a 1-pyrrolidinyl, piperidino, morpholino or 4-methyl-1-piperazinyl group.
2. 4-Demethoxyadriamycin.
3. 4-Demethoxy-2,3-dimethyladriamycin.
4. 4-Demethoxy-1,4-dimethyladriamycin.
5. 4-Demethoxy-1,4-dichloroadriamycin.
6. 4-Demethoxy-2,3-dichloroadriamycin.
7. 4-Demethoxyadriamycin-14-octanoate.
8. 4-Demethoxyadriamycin-14-hexanoylglycolate.
9. 4-Demethoxy-14-morpholino-daunomycin.

ERIC POTTER AND CLARKSON,  
Chartered Patent Agents,  
25 The Crescent,  
Leicester.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1978.  
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from  
which copies may be obtained.

Table 4.

Activity on L1210 leukemia.  
BDF1 mice were injected intraperitoneally with 10<sup>5</sup> ascites cells on Day 0 and treated intraperitoneally on Day 1.

| Compound                            | Dose (mg/kg) | T/C % | LTS  |
|-------------------------------------|--------------|-------|------|
| Adriamycin                          | 2.9          | 141   |      |
|                                     | 4.4          | 152   | 1/10 |
|                                     | 6.6          | 158   |      |
|                                     | 10           | 164   | 1/10 |
| 2,3-Dimethyl-4-demethoxy-adriamycin | 1.9          | 152   |      |
|                                     | 2.9          | 152   |      |
|                                     | 4.4          | 170   | 1/10 |

T/C and LTS mean the same as in Table 2.

WHAT WE CLAIM IS:—

1. A compound of the general formula I, II, III or IV wherein R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> each represents a hydrogen, bromine or chlorine atom or a methyl or methoxy group with the proviso that, except when R<sub>1</sub> and R<sub>3</sub> represent hydrogen atoms and R<sub>2</sub> represents a methoxy group, R<sub>2</sub> and R<sub>3</sub> represent the same atom or group, R<sub>4</sub> represents a hydrogen atom or a trifluoroacetyl group, and R<sub>5</sub> represents a hydroxy group, an acyloxy group of the formula OCOR<sub>6</sub>, an acylglycolloyloxy group of the formula OCOCH<sub>2</sub>OCOR<sub>6</sub> or a substituted amino group of the formula NR<sub>7</sub>R<sub>8</sub> wherein R<sub>6</sub> represents a hydrocarbon residue having from 6 to 16 carbon atoms, and R<sub>7</sub> and R<sub>8</sub> each represents an alkyl group having from 1 to 4 carbon atoms or together with the nitrogen atom to which they are attached represent a 1-pyrrolidinyl, piperidino, morpholino or 4-methyl-1-piperazinyl group.
2. 4-Demethoxyadriamycin.
3. 4-Demethoxy-2,3-dimethyladriamycin.
4. 4-Demethoxy-1,4-dimethyladriamycin.
5. 4-Demethoxy-1,4-dichloroadriamycin.
6. 4-Demethoxy-2,3-dichloroadriamycin.
7. 4-Demethoxyadriamycin-14-octanoate.
8. 4-Demethoxyadriamycin-14-hexanoylglycolate.
9. 4-Demethoxy-14-morpholino-daunomycin.

ERIC POTTER AND CLARKSON,  
Chartered Patent Agents,  
25 The Crescent,  
Leicester.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1978.  
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.



\* C L M S \*