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(72) Inventeurs/Inventors:
BARGIOTTI, ALBERTO, IT;
CARUSO, MICHELE, IT;
FAIARDI, DANIELA, IT;
SUARATO, ANTONINO, IT;
MONGELLI, NICOLA, IT

(73) Propriétaire/Owner:
PHARMACIA & UPJOHN S.P.A., IT

(74) Agent: FETHERSTONHAUGH & CO.

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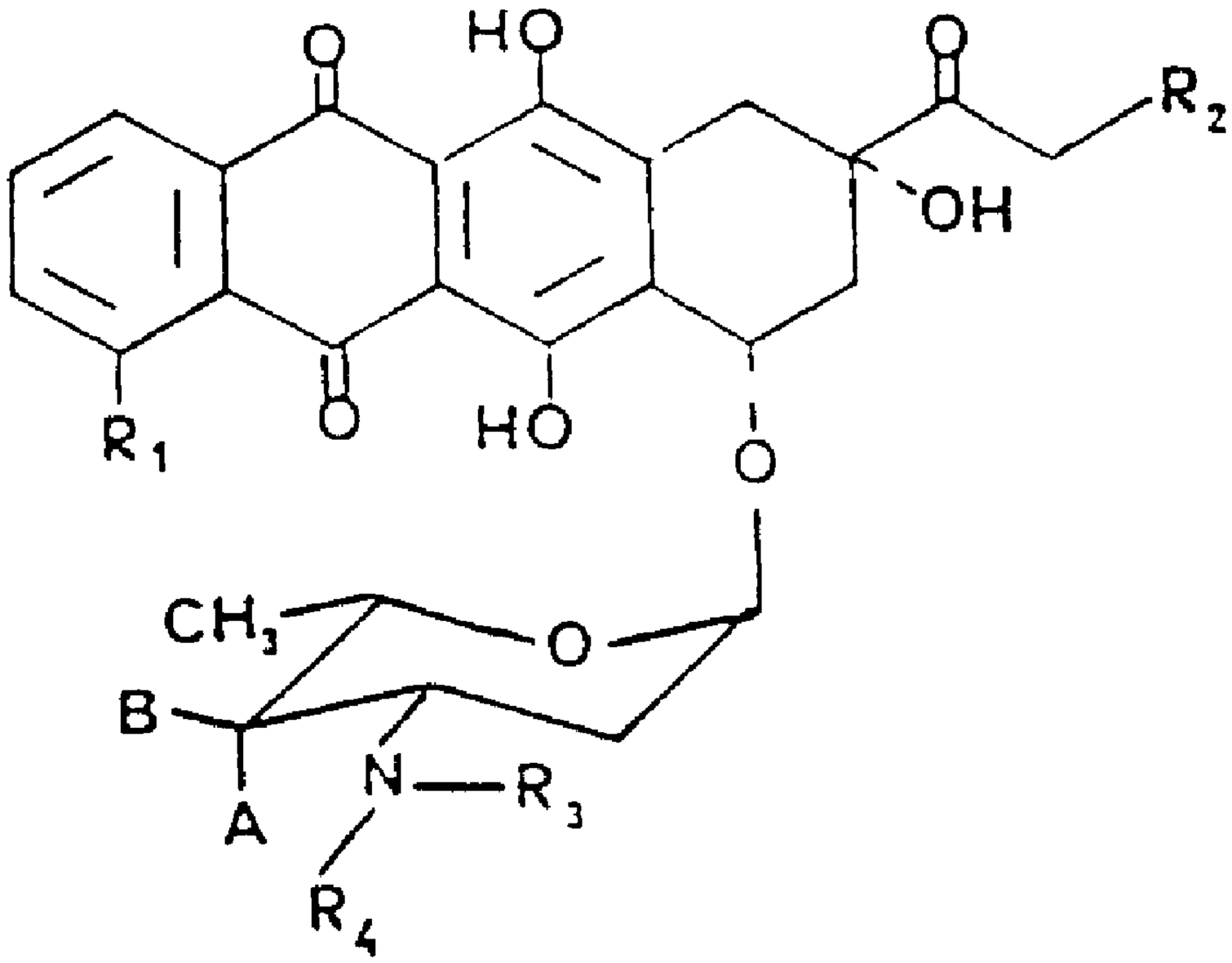

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(57) Abrégé/Abstract:
An anthracycline glycoside of general formula (1), wherein R_1 is hydrogen or methoxy group; R_2 is hydrogen or hydroxy group, A and B both represent hydrogen or one of A and B is hydrogen and the other is hydroxy or a group of formula $-OSO_2R_5$ in which R_5 is C_1-C_4 alkyl or aryl optionally substituted by C_1-C_4 alkyl, nitro, amino, methoxy or halogen; R_3 is a hydrogen atom or a group of formula (2) and R_4 is a group of formula (2): $-(CH_2)_n$-X, in which n is 2 or 3 and X is hydroxy group, a halogen or a group of formula $-OSO_2R_5$ in which R_5 is as defined above and with the proviso that if R_2, X and A are a hydroxy group and R_3=H, n must be 3; or a pharmaceutically acceptable salt thereof. Compounds of the invention have activity as antitumor agents. Processes for their preparation and pharmaceutical composition containing them are also disclosed.

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(71) Applicant: FARMITALIA CARLO ERBA S.R.L. [IT/IT]; Via Carlo Imbonati, 24, I-20159 Milan (IT).

(72) Inventors: BARGIOTTI, Alberto ; Via Donati, 8, I-20100 Milan (IT). CARUSO, Michele ; Via Desiderio, 3, I-20100 Milan (IT). FAIARDI, Daniela ; Via Bernardo Da Pavia, 6, I-27100 Pavia (IT). SUARATO, Antonino ; Via Degli Imbriani, 38, I-20138 Milan (IT). MONGELLI, Nicola ; Via Tertulliano, 38, I-20132 Milan (IT).

(74) Agents: WOODS, Geoffrey, Corlett et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

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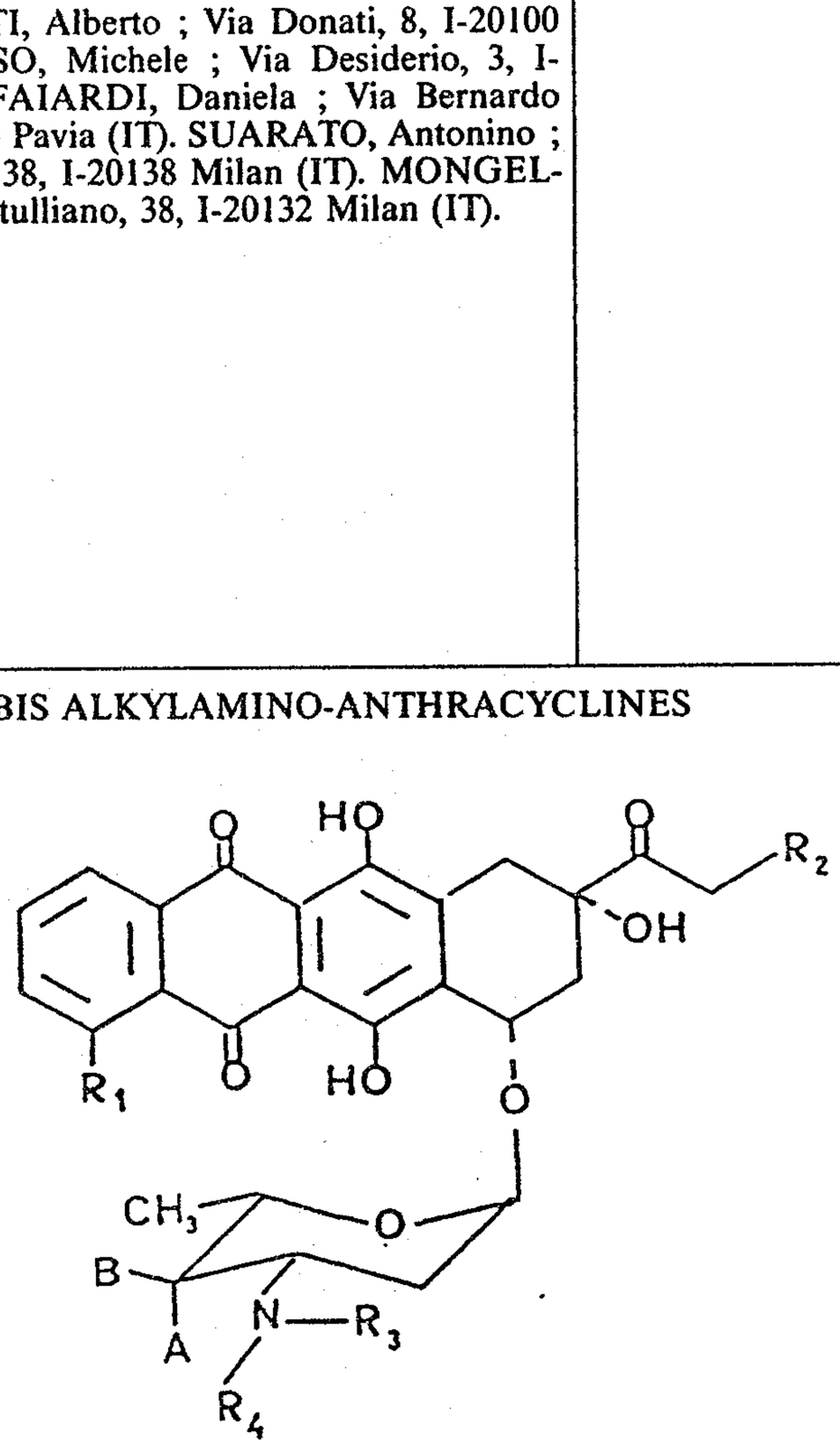

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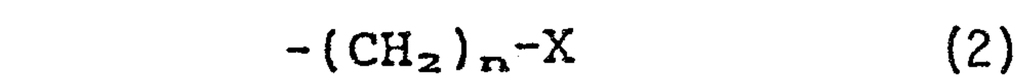


(2)

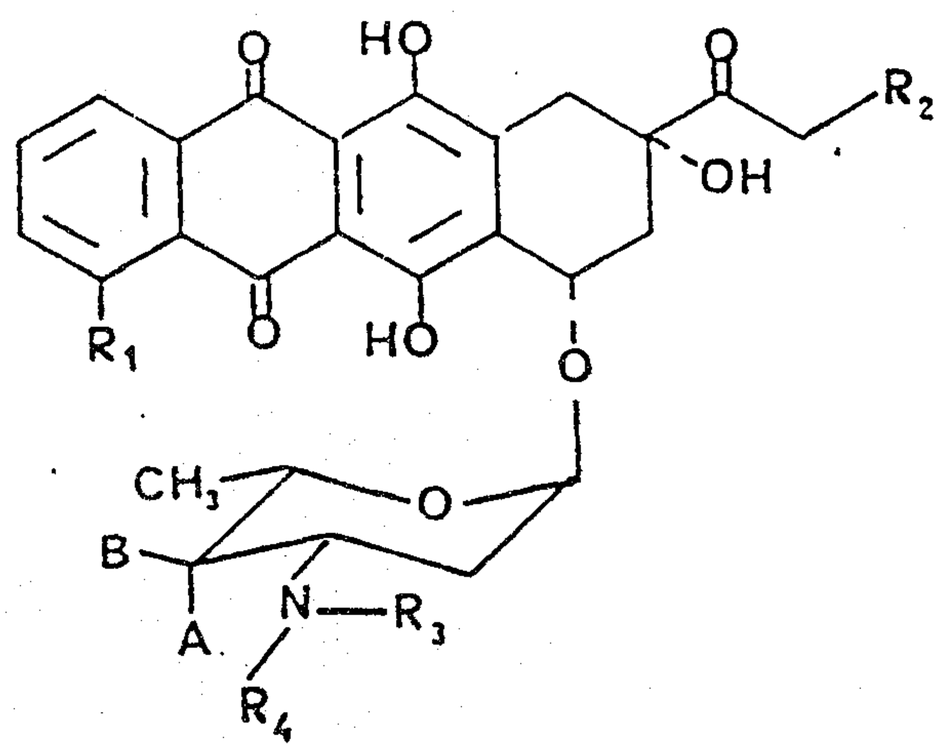
(57) Abstract

An anthracycline glycoside of general formula (I), wherein R_1 is hydrogen or methoxy group; R_2 is hydrogen or hydroxy group, A and B both represent hydrogen or one of A and B is hydrogen and the other is hydroxy or a group of formula $-OSO_2R_5$ in which R_5 is C_1-C_4 alkyl or aryl optionally substituted by C_1-C_4 alkyl, nitro, amino, methoxy or halogen; R_3 is a hydrogen atom or a group of formula (2) and R_4 is a group of formula (2): $-(CH_2)_n-X$, in which n is 2 or 3 and X is hydroxy group, a halogen or a group of formula $-OSO_2R_5$ in which R_5 is as defined above and with the proviso that if R_2, X and A are a hydroxy group and $R_3 = H$, n must be 3; or a pharmaceutically acceptable salt thereof. Compounds of the invention have activity as antitumor agents. Processes for their preparation and pharmaceutical composition containing them are also disclosed.

Mono and bis alkylamino-anthracyclines.

The present invention relates to new anthracycline glycosides, to processes for their preparation and to pharmaceutical compositions containing them.

The invention provides antracyclines glycosides of general formula 1 in which the amino group of the sugar moiety bears mono or bis alkyl-substituted chains:



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wherein R_1 is hydrogen or methoxy group; R_2 is hydrogen or hydroxy group, A and B both represent hydrogen or one of A and B is hydrogen and the other is hydroxy or a group of formula $-OSO_2R_5$ in which R_5 is C_1-C_4 alkyl or aryl optionally substituted by C_1-C_4 alkyl, nitro, amino, methoxy or halogen; R_3 is a hydrogen atom or a group of formula 2 and R_4 is a group of formula 2

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in which n is 2 or 3 and X is hydroxy group, a halogen or a group of formula $-OSO_2R_5$ in which R_5 is as defined above and with the proviso that if R_2, X and A are an hydroxy group and R_3=H, n must be 3; or a pharmaceutically acceptable salt thereof.

Compounds of the invention exibit antitumor activity.

Example of compounds of the invention include:

1a: N-(3-hydroxypropyl)daunorubicin
[R_1=OCH_3, R_2=R_3=H, A=OH, B=H, R_4=$(CH_2)_3$-OH]

1b: N-(3-hydroxypropyl)doxorubicin
[R_1=OCH_3, R_2=OH, R_3=H, A=OH, B=H, R_4=$(CH_2)_3$-OH]

1c: N,N-bis(3-hydroxypropyl)daunorubicin
[R_1=OCH_3, R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_3$-OH]

1d: 4-demethoxy-N-(2-hydroxyethyl)daunorubicin
[R_1=R_2=R_3=H, A=OH, B=H, R_4=$(CH_2)_2$-OH]

1e: N,N-bis(2-hydroxyethyl)daunorubicin
[R_1=OCH_3, R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-OH]

1f: N,N-bis(2-hydroxyethyl)doxorubicin
[R_1=OCH_3, R_2=OH, A=OH, B=H, R_3=R_4=$(CH_2)_2$-OH]

1g: 4-demethoxy-N,N-bis(2-hydroxyethyl)daunorubicin
[R_1=R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-OH]

1h: 4-demethoxy-4'-O-methansulfonyl-N,N-bis(2-chloroethyl)-daunorubicin
[R_1=R_2=H, A=OSO_2CH_3, B=H, R_3=R_4=$(CH_2)_2$-Cl]

1i: 4-demethoxy-N,N-bis(2-chloroethyl)daunorubicin
[R_1=R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-Cl]

1j: 4'-O-methansulfonyl-N,N-bis(2-chloroethyl)daunorubicin
[R_1=OCH_3, R_2=H, A=OSO_2CH_3, B=H, R_3=R_4=$(CH_2)_2$-Cl]

1k: N,N-bis(2-chloroethyl)daunorubicin
[R_1=OCH_3, R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-Cl]

1l: 4'-epi-4'-O-methansulfonyl-N,N-bis(2-chloroethyl)-daunorubicin
[R_1=OCH_3, R_2=H, A=H, B=OSO_2CH_3, R_3=R_4=$(CH_2)_2$-Cl]

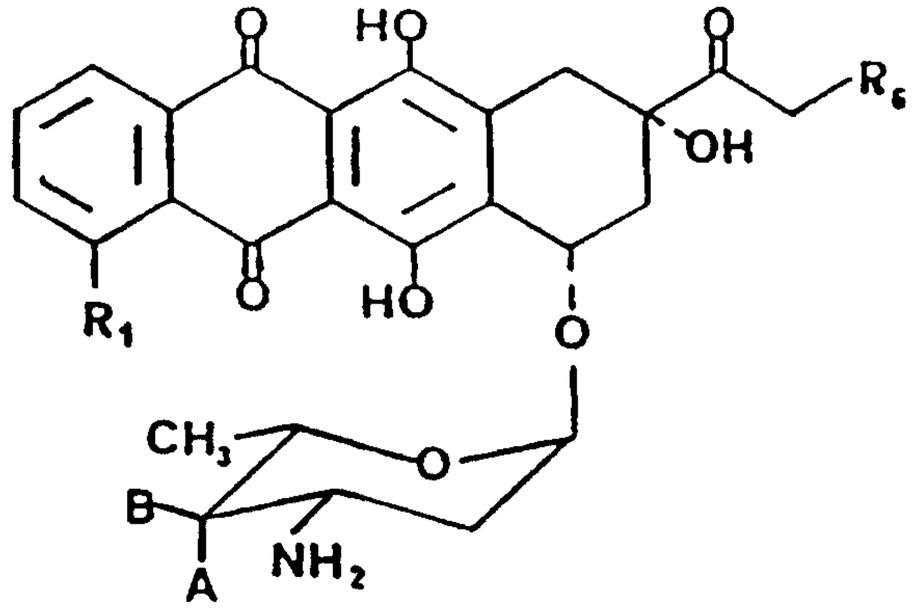
1m: 4'-epi-N,N-bis(2-chloroethyl)daunorubicin
[R_1=OCH_3, R_2=H, A=H, B=OH, R_3=R_4=$(CH_2)_2$-Cl]

1n: 4-demethoxy-4'-epi-4'-O-methansulfonyl-N,N-bis(2-chloro-ethyl)daunorubicin
[R_1=R_2=H, A=H, B=OSO_2CH_3, R_3=R_4=$(CH_2)_2$-Cl]

1o: N,N-bis(2-chloroethyl)doxorubicin
[R_1=OCH_3, R_2=OH, A=OH, B=H, R_3=R_4=$(CH_2)_3$-Cl]

and pharmaceutically acceptable salts thereof, such as the hydrochloride salt.

The compounds of the present invention can be prepared by several methods starting from compounds of formula 3



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wherein R_1, A and B are as defined above for formula 1, R_6 is hydrogen, a hydroxyl group or an acidic sensitive masked group for the hydroxy group. Examples of suitable protecting groups include those described in International Patent Application PCT/EP91/01449, published as WO92/02255, entitled "New Linker for Bioactive Agents".

Examples of the starting compounds of formula 3 include daunorubicin [3a: R_1=OCH_3, R_2=H, A=OH, B=H], 4-demethoxy-daunorubicin [3b: R_1=R_2=H, A=OH, B=H], doxorubicin [3c: R_1=OCH_3, R_2=OH, A=OH, B=H], 4-demethoxy-4'-epi-daunorubucin [3d: R_1=R_2=H, A=H, B=OH], 4'-epi-daunorubicin [3e: R_1=OCH_3, R_2=H, A=H, B=OH], or masked derivative of doxorubucin at C-14.

The compounds of general formula 1 can be prepared by alkylating the amino group of the sugar moiety using conventional methods. For instance, mono 2- or 3-hydroxy-alkylamino derivatives, which are compounds of formula 1 as defined above wherein R_3 and R_4 are as defined above wherein X is a hydroxy group, can be prepared by a process comprising

(i) reacting a compound of formula 3 as defined above, the

compound of formula 3 being dissolved in a polar aprotic solvent, with an alkylating agent of general formula 4

$$X-(CH_2)_n-Hal \qquad 4$$

wherein n is 2 or 3, X is hydroxy and Hal represents an halogen; and, if desired,

(ii) purifying the resulting anthracycline glycosides of formula 1 on a chromatographic column; and/or, if desired,

(iii) converting the anthracycline glycoside of formula 1 into a pharmaceutically acceptable acid addition salt thereof. The anthracycline glycoside of formula 1 may be converted into its hydrochloride salt by, for example, treatment with anhydrous hydrogen chloride.

Preferably the halogen in formula 4 is iodine or bromine. The polar aprotic solvent is preferably dry and is, for example, dimethylformamide or acetonitrile. The reaction is suitably conducted at a temperature from 20 to 30°C, typically for a time of from four to twenty four hours. The purification step (ii) is typically performed on a silica gel column using, as eluent, methylene chloride:methanol (80:20 v/v).

Bis 2- or 3-hydroxy-alkylamino derivatives, which are compounds of formula 1 as defined above wherein R_3 and R_4 are a group of formula 2 as defined above wherein X is a hydroxy group, can also be prepared by the process as described above. The reaction time for step (i) is then typically 1 to 3 weeks.

Bis 2-hydroxyethylamino compounds of general formula 1 can also be prepared by a process comprising (i) reacting a compound of formula 3 as defined above, preferably as free base, with ethylene oxide; and, if desired, (ii) purifying the resulting anthracycline glycoside of formula 1 on a chromatographic column; and/or, if desired, (iii) converting the anthracycline glycoside of formula 1 into a pharmaceutically acceptable acid addition salt thereof. Preferably the compound of formula 3 is first dissolved in a solvent comprising methanol and methylene chloride. Typically step (i) is conducted in the dark, and at a starting temperature of about -40°C. The temperature is then

typically increased gradually to room temperature. Suitably it remains at room temperature for up 3 days.

Anthracycline glycosides of general formula 1 as defined above in which R_2 is hydrogen and one or both of R_3 and R_4 represent a group of formula $(CH_2)_n$-OSO_2R_5, as defined above, can be prepared by a process comprising treating the corresponding mono or bis-hydroxyamino derivatives of formula 1 with a sulfonyl chloride of formula 5

$$Cl\text{-}SO_2R_5 \qquad 5$$

wherein R_5 is a residue as defined above; and, if desired, (ii) purifying the resulting anthracyline glycoside of formula 1 on a chromatographic column. Typically step (i) is conducted in an aprotic solvent, such as methylene chloride. Suitably it is conducted in the presence of a tertiary amine, such as trietylamine or pyridine.

It is also possible to prepare anthracycline glycoside derivatives of general formula 1 in which R_2 is a hydroxy group from the corresponding mono or bis-hydroxyamino compounds of formula 1 in which the C-14 hydroxy group, R_2=OH is masked with an acid sensitive protecting group. Mild acidic treatment of the latter produces the desired sulfonylalkylamino anthracycline.

Compounds of general formula 1 in which X is a halogen can be prepared by a process comprising (i) dissolving an anthracycline glycoside of formula 1 as defined above, wherein one or both of R_3 and R_4 is a group of formula OSO_2R_5 wherein R_5 is alkyl or aryl group, in an aprotic solvent and reacting the resulting solution with a corresponding halide salt; and, if desired, (ii) purifying the resulting anthracycline glycoside on a chromatographic column; and/or, if desired, (iii) isolating the desired compound as the corresponding hydrohalide. When X is chlorine, the corresponding chlorine salt may be, for

example. pyridinium chloride or n-tetrabutylammonium chloride. The aprotic solvent is, for example acetone or dimethylformamide. The reaction is preferably conducted at room temperature.

Anthracycline glycosides of general formula 1 as defined above in which X is chlorine, R_2 is hydrogen, both A and B represent hydrogen or one of A or B represent the group $-OSO_2R_5$ wherein R_5 is as defined above can be prepared by a process comprising (i) treating the corresponding mono or bis-hydroxyalkyl amino derivative of formula 1 as defined above, in dry pyridine, with sulfonyl chloride of formula 5; and if desired (ii) purifying the resulting anthracycline glycoside on a chromatographic column; and/or, if desired, (iii) isolating the desired compound as the corresponding hydrohalide. Typically step (i) is conducted in the dark and at a temperature of 0°C. Suitably it is conducted under nitrogen. The temperature remains at 0°C for up to 16 hours.

It is also possible to prepare anthracycline glycoside derivatives of general formula 1 in which R_2 is a hydroxy group from the corresponding mono or bis-hydroxyalkyl amino compounds of formula 1 in which the C-14 hydroxy group, R_2=OH, is masked with an acid sensitive protecting group. Mild acidic treatment of the latter produces the desired chloroalkyl amino anthracycline derivative.

The compounds of the invention have activity as antitumor agents. A mammal, for example a human, can therefore be treated by a method comprising administering thereto, by an oral or parenteral route, a pharmaceutically effective amount of an anthracycline glycoside of formula 1 as defined above or a pharmaceutically acceptable acid addition salt thereof.

The invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and, as an active principle, an anthracycline glycoside of formula 1, or a pharmaceutically acceptable salt thereof. Conventional carriers or diluents may be

used. The composition may be formulated and administred, for example intravenously, in conventional manner.

The following Examples illustrate the invention without limiting it.

Example 1

Preparation of: N-(3-hydroxypropyl)daunorubicin [R_1=OCH_3, R_2=R_3=H, A=OH, B=H, R_4=$(CH_2)_3$-OH] (1a)

To a solution of daunorubicin (3a) (0.2g, 0.38 mmol) in anhydrous dimethylformamide (2 ml) was added 3-bromo-1-propanol [4: X=OH, Hal=Br, n=3] (200µl, 2.16 mmol) at room temperature under nitrogen and stirring was continued for five days. After that the solvent was removed in vacuo; the crude oil was dissolved in methylene chloride (10 ml) and trifluoroacetic anhydride (425 µl, 3 mmol) added. The mixture was stirred for one hour at 0°C then poured into satured aqueous sodium hydrogen carbonate and extracted with methylene chloride. The combined organic extracts were washed with water and the organic solvent was removed under reduced pressure. The crude oil was dissolved in methanol (50 ml) and stirred for one hour at 40°C, concentrated to small volume and purified by flash chromatography on silicic acid column using as eluting system a mixture of methanol and methylene chloride (10/90 by volume) to give after treatment with methanolic anhydrous hydrochloric acid the title compound 1a (0.12 g, yield 54%) as hydrochloride salt.

TLC on Kieselgel Plate F_{254} (Merck), eluting system: methylene chloride, methanol, acetic acid, water (80:20:7:3 by volume) R_f=0.42

FD-MS: m/e 569 (M^+)

^{1}HNMR (200 MHz, DMSO d_6) δ:

1.16 (d, J=6.4Hz, 3H, CH_3-5'); 1.5-1.8 (m, 4H, CH_2CH_2, CH_2-2')

2.15 (m, 2H, $\underline{CH_2}$-8); 2.25 (s, 3H, CO$\underline{CH^3}$); (2.6-2.9 (m, 2H, $\underline{CH_2}NH$); 2.99, 2.89 (ABq, J=18.0Hz, 2H, $\underline{CH_2}$-10); 3.31 (m, 1H, $\underline{H}$-3'); 3.41 (m, 2H, $\underline{CH_2}$-OH); 3.63 (m, 1H, $\underline{H}$-4'); 3.92 (s, 3H, O$\underline{CH_3}$); 4.14 (q, J=6.4Hz, 1H, $\underline{H}$-5'); 4.96 (m, 1H, $\underline{H}$-7); 5.29 (m, 1H, $\underline{H}$-1'); 5.49 (s, 1H, $\underline{OH}$-9); 7.6-7.9 (m, 3H, $\underline{aromatic}$ H's).

$\underline{Example\ 2}$

Preparation of: $\underline{N,N\text{-bis(3-hydroxypropyl)daunorubicin}}$ [$R_1=OCH_3$, $R_2=OH$, A=OH, B=H, $R_3=R_4=(CH_2)_3$-OH] ($\underline{1c}$)

The title compound was prepared by keeping daunorubicin ($\underline{3a}$) (0.2 g, 0.38 mmol) and 3-bromo-1-propanol (200 µl, 2.16 mmol) in anhydrous dimethylformamide for three weeks under nitrogen. After that the solvent was removed in vacuo and the crude oil was purified by flash chromatography on silicic acid column using as eluting system a mixture of methanol and methylene chloride (10/90 by volume) to give after treatment with methanolic anhydrous hydrochloric acid N,N-bis(3-hydroxypropyl)daunorubicin ($\underline{1c}$) (0.10 g, yield 50%) as hydrochloride salt.

TLC on Kieselgel Plate F_{254} (Merck), eluting system: methylene chloride, methanol, acetic acid, water (30:4:1:0.5 by volume) R_f=0.14

FD-MS: m/e 627 (M^+)

^{1}HNMR (200 MHz, $CDCl_3$) δ:

1.29 (d, J=6.4Hz, 3H, $\underline{CH_3}$-5'); 1.8-1.5 (m, 5H, 2xNHCH$_2\underline{CH_2}$, $\underline{H}$-2'eq); 2.04 (m, 1H, $\underline{H}$-2'ax); 2.09 (m, 1H, $\underline{H}$-8ax); 2.34 (m, 1H, $\underline{H}$-8eq); 2.41 (s, 3H, CO$\underline{CH_3}$); 2.5-2.9 (m, 5H, 2xNH$\underline{CH_2}$, $\underline{H}$-3'); 2.95 (d, J=19.0Hz, 1H, $\underline{H}$-10ax); 3.21 (dd, J=1.5, 19.0Hz, 1H, $\underline{H}$-10eq); 3.8-3.6 (m, 5H, 2x$\underline{CH_2}$OH, $\underline{H}$-4'); 4.07 (s, 3H, O$\underline{CH_3}$); 4.09 (q, J=6.4Hz, 1H, H-5'); 5.29 (m, 1H, $\underline{H}$-7); 5.57 (d, J=3.3Hz, 1H, $\underline{H}$-1'); 7.38 (d, J=8.4Hz, 1H, $\underline{H}$-3); 7.77 (dd, J=7.4, 8.4Hz, 1H, $\underline{H}$-2); 8.01 (d, J=7.4Hz, 1H, $\underline{H}$-1); 14.0, 13.3 (broad signals, 2H, 2xphenolic -OH).

Example 3

Preparation of: N,N-bis(2-hydroxyethyl)doxorubicin [R_1=OCH_3, R_2=OH, A=OH, B=H, R_3=R_4=$(CH_2)_2$-OH] (1f)

A mixture of doxorubicin (3c) (0.15g, 0.258mmole), methanol and methylene chloride (25ml, 1:1 by volume) was poured in a well stopped round bottomed flask, cooled at -40°C and added with ethylene oxide (15ml). The reaction mixture was slowly brought at room temperature and kept for two days in the dark. After that, the solvents were removed under reduced pressure and the residue purified by flash chromatography on silicic acid column using as eluting system a mixture of methylene chloride and methanol (80:20 by volume). The title compound 1f (0.1g, yield 60%) was converted into hydrochloride salt by treatment with methanolic anhydrous hydrochloric acid.

TLC on Kieselgel Plate F_{254} (Merck), eluting system: methylene chloride, methanol, acetic acid, water (80:20:7:3 by volume) R_f=0.36.

FD-MS: m/e 631 (M^+)

^{1}HNMR (200 MHz, DMSO d_6) δ:

1.13(d, J=6.6Hz, 3H, CH_3-5'); 1.53 (m, 1H, H-2'eq); 1.94 (m, 1H, H-2'ax); 2.16 (m, 2H, CH_2-8); 2.65 (m, 4H, $N(CH_2CH_2OH)_2$); 2.82 (m, 1H, H-3'); 2.98 (m, 2H, CH_2-10); 3.33 (m, 4H, $N(CH_2CH_2OH)_2$); 3.57 (m, 1H, H-4'); 4.00 (s, 3H, OCH_3-4); 4.02 (dq, J=<2, 6.6Hz, 1H, H-5'); 4.35 (bm, 3H, OH-4', $N(CH_2CH_2OH)_2$); 4.56 (m, 2H, CH_2-14); 4.84 (t, J=6.2Hz, 1H, OH-14); 4.98 (m, 1H, H-7); 5.30 (m, 1H, H-1'); 5.40 (s, 1H, OH-9); 7.67 (m, 1H, H-2); 7.93 (m, 2H, H-1, H-3); 13.28 (bs, 1H, OH-11); 14.06 (s, 1H, OH-6).

Example 4

Preparation of: 4-demethoxy-N,N-bis(2-hydroxyethyl)-daunorubicin [R_1=R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-OH] (1g)

Free base 4-demethoxydaunorubicin (3b) (0.23 g, 0.5 mmol) was converted into the title compound 1g following the procedure described in Example 3. Yield: 0.15 g as hydrochloride salt after treatment with methanolic anhydrous hydrochloric acid.

TLC on Kieselgel Plate F_{254} (Merck), eluting system: methylene chloride, methanol, acetic acid, water (80:20:7:3 by volume) R_f=0.38.

FD-MS: m/e 585 (M^+)

^{1}HNMR (200 MHz, DMSO d_6) δ:

1.13 (d, J=6.6Hz, 3H, CH_3-5'); 1.55 (m, 1H, H-2'eq); 1.93 (m, 1H, H-2'ax); 2.17 (m, 2H, CH_2-8); 2.26 (s, 3H, $COCH_3$); 2.65 [m, 4H, $N(CH_2CH_2OH)_2$]; 2.82 (m, 1H, H-3'); 2.99 (m, 2H, CH_2-10); 3.30 [m, 4H, $N(CH_2CH_2OH)_2$]; 3.59 (m, 1H, H-4'); 4.06 (dq, J=<2, 6.6Hz, 1H, H-5'); 4.35 [bm, 3H, OH-4', $N(CH_2CH_2OH)_2$]; 4.96 (m, 1H, H-7); 5.30 (m, 1H, OH-9); 8.00 (m, 2H, H-2, H-3); 8.30 (m, 2H, H-1, H-4); 13.35 (s, 1H, OH-11); 13.55 (s, OH-6).

Example 5

Preparation of N,N-bis(2-hydroxyethyl)daunorubicin [R_1=OCH_3, R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-OH] (1e)

The title compound 1e was prepared from daunorubicin (3a) following the same procedure described in Example 4.

TLC on Kieselgel Plate F_{254} (Merck), eluting system: methylene chloride, methanol, acetic acid, water (80:20:7:3 by volume) R_f=0.28

FD-MS: m/e 615 (M^+)

Example 6

Preparation of 4-demethoxy-4'-O-methansulfonyl-N,N-bis(2-chloroethyl)daunorubicin.

[$R_1=R_2=H$, $A=OSO_2CH_3$, $B=H$, $R_3=R_4=(CH_2)_2-Cl$] (1h)

4-demethoxy-N,N-bis(2-hydroxyethyl)daunorubicin (1g) (0.3 g, 0.5 mmol) prepared as described in Example 4, was dissolved with dry pyridine (15 ml) cooled at 0°C and added with methansulfonyl chloride (1 ml) and kept overnight at 0°C under stirring with a nitrogen blanket. After that, the reaction mixture was poured into water/ice and extracted with methylene chloride. The organic layer was washed with cold water, dried over anhydrous sodium sulphate, and concentrated under reduced pressure. The crude material was purified by flash chromatography on silicic acid column using as eluting system a mixture of methylene chloride and acetone (97:3 by volume) to give the title compound 1h (0.1g, yield 30%).

TLC on Kieselgel Plate F_{254} (Merck), eluting system: methylene chloride, acetone (20:1 by volume) $R_f=0.48$.

FD-MS: m/e 680 (M^+)

^{1}HNMR (200 MHz, $CDCl_3$) δ:

1.35 (d, J=6.5 Hz, 3H, CH_3-5'); 1.8 - 2.4 (m, 4H, H-2'eq, H-2'ax, H-8ax, H-8eq); 2.42 (s, 3H, CH_3-CO); 3.06 (t, 4H, J=7.1 Hz, CH_2-N-CH_2); 3.17 (m, 1H, H-3'); 3.43 (m, 4H, 2xCH_2-Cl); 4.16 (m, 1H, H-5'); 4.40 (s, 1H, OH-9); 4.91 (s, 1H, H-4'); 5.30 (dd, J=2.1, 3.7 Hz, 1H, H-7); 5.60 (d, 1H, J=3.4 Hz, H-1'); 7.85 (m, 2H, H-2, H-3); 8.37 (m, 2H, H-1, H-4); 13.55 (s, 1H, OH-11); 13.64 (s, 1H, OH-6).

Example 7

Biological Assays

4-demethoxy-4'-O-methansulfonyl-N,N-bis(2-chloroethyl)-daunorubicin (compound 1h) was tested "in vitro" as inhibitor of colony growth on two human cell lines: LoVo (colon adenocarcinama) and LoVo/DX (colon adenocarcinoma resistant to Doxorubicin) in comparison with 4-demethoxy-daunorubicin (3b) and Doxorubicin (3c) (Table 1).
When compared with 4-demethoxydaunorubicin and Doxorubicin a striking higher activity on the doxorubicin-resistant cell line was observed for compound 1h.

Compound 1h was also evaluated "in vivo" against P388 murine Leukemias, sensitive (Table 2) and resistant to Doxorubicin (Johnson). When tested on resistant Leukemia, compound 1h showed high activity (Table 3).

Table 1: "in vitro" cytotoxic activity.

Compounds	Cytotoxicity (IC_{50} = ng/ml)(1)		
	LoVo	LoVo/DX	R.I.(2)
1h	4.3	14.0	3.3
4-demethoxy-daunorubicin	4.0	48.0	12.0
Doxorubicin	82.5	4975	60.3

Colony assay: 4 hr treatment
(1) IC_{50} = concentration inhibiting 50% of colony formation
(2) R.I. = Resistance Index = (IC_{50} LoVo/DX)(IC_{50} LoVo)

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Table 2: Antitumor Activity Against Ascitic P388 Leukemia(3)

Compounds	Dose(4) (mg/kg)	T/C(5) %	TOX(6)
1h	1.0	184	0/12
	1.4	217	0/6
	2.0	233	1/6
	2.8	78	6/6
4-demethoxy-daunorubicin	0.33	142	0/27
	0.5	160	0/28
	0.75	163	4/28
Doxorubicin	10.0	299	0/10
	15.0	90	3/6

(3) 10^6 cell/mouse were injected i.p. on day 0.

(4) Compounds were suspended in Tween 80 10% and injected i.p. one day after tumor transplantation.

(5) Median survival time of treated mice/Median survival time of controls x 100.

(6) No. of toxic deaths/total No. of mice.

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Table 3: Antitumor Activity Against Disseminated P388/DX Johnson Leukemia(7)

Compounds	Dose(4) (mg/kg)	T/C(5) %	TOX(6)
1h	1.2	133	0/6
	1.6	144	0/6
	2.1	185	0/6
	2.8	220	0/6
	3.7	250	0/6
4-demethoxy-daunorubicin	1.9	106	0/10
	2.5	89	0/10
Doxorubicin	16.9	106	0/6
	22.0	94	3/6

(7) 10^5 cell/mouse were injected i.v. on day 0.

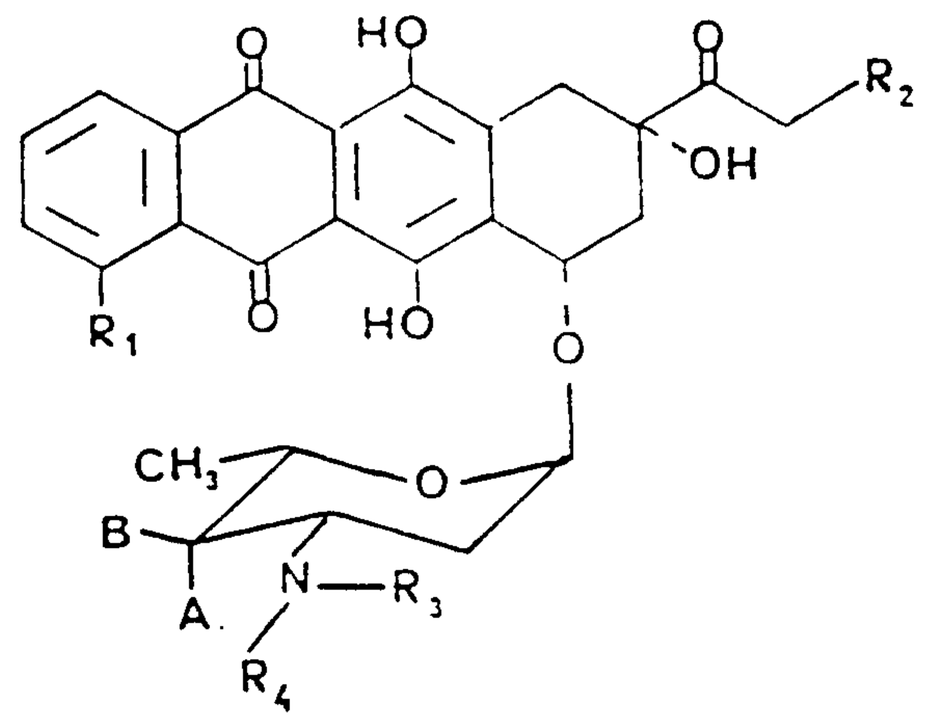
(4) Compounds were suspended in Tween 80 10% and injected i.p. one day after tumor transplantation.

(5) Median survival time of treated mice/Median survival time of controls x 100.

(6) No. of toxic deaths/total No. of mice.

CLAIMS

1. An anthracycline glycoside of general formula 1:



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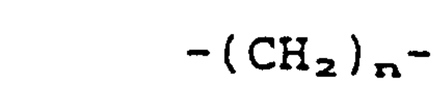
wherein R_1 is hydrogen or methoxy group; R_2 is hydrogen or hydroxy group, A and B both represent hydrogen or one of A and B is hydrogen and the other is hydroxy or a group of formula $-OSO_2R_5$ in which R_5 is C1-C4 alkyl or aryl optionally substituted by C1-C4 alkyl, nitro, amino, methoxy or halogen; R_3 is a hydrogen atom or a group of formula 2 and R_4 is a group of formula 2



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in which n is 2 or 3 and X is hydroxy group, a halogen or a group of formula $-OSO_2R_5$ in which R_5 is as defined above and with the proviso that if R_2, X and A are an hydroxy group and R_3=H, n must be 3; or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein, in formula 2, X is chlorine.

3. A compound according to claim 1 which is selected from:

N-(3-hydroxypropyl)daunorubicin

[R_1=OCH_3, R_2=R_3=H, A=OH, B=H, R_4=$(CH_2)_3$-OH]

N-(3-hydroxypropyl)doxorubicin

[R_1=OCH_3, R_2=OH, R_3=H, A=OH, B=H, R_4=$(CH_2)_3$-OH]

N,N-bis(3-hydroxypropyl)daunorubicin

[R_1=OCH_3, R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_3$-OH]

4-demethoxy-N-(2-hydroxyethyl)daunorubicin

[R_1=R_2=R_3=H, A=OH, B=H, R_4=$(CH_2)_2$-OH]

N,N-bis(2-hydroxyethyl)daunorubicin

[R_1=OCH_3, R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-OH]

N,N-bis(2-hydroxyethyl)doxorubicin

[R_1=OCH_3, R_2=OH, A=OH, B=H, R_3=R_4=$(CH_2)_2$-OH]

4-demethoxy-N,N-bis(2-hydroxyethyl)daunorubicin

[R_1=R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-OH]

4-demethoxy-4'-O-methansulfonyl-N,N-bis(2-chloroethyl)-daunorubicin

[R_1=R_2=H, A=OSO_2CH_3, B=H, R_3=R_4=$(CH_2)_2$-Cl]

4-demethoxy-N,N-bis(2-chloroethyl)daunorubicin

[R_1=R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-Cl]

4'-O-methansulfonyl-N,N-bis(2-chloroethyl)daunorubicin

[R_1=OCH_3, R_2=H, A=OSO_2CH_3, B=H, R_3=R_4=$(CH_2)_2$-Cl]

N,N-bis(2-chloroethyl)daunorubicin

[R_1=OCH_3, R_2=H, A=OH, B=H, R_3=R_4=$(CH_2)_2$-Cl]

4'-epi-4'-O-methansulfonyl-N,N-bis(2-chloroethyl)daunorubicin

[R_1=OCH_3, R_2=H, A=H, B=OSO_2CH_3, R_3=R_4=$(CH_2)_2$-Cl]

4'-epi-N,N-bis(2-chloroethyl)daunorubicin

[R_1=OCH_3, R_2=H, A=H, B=OH, R_3=R_4=$(CH_2)_2$-Cl]

4-demethoxy-4'-epi-4'-O-methansulfonyl-N,N-bis(2-chloro-ethyl)daunorubicin

[R_1=R_2=H, A=H, B=OSO_2CH_3, R_3=R_4=$(CH_2)_2$-Cl]

N,N-bis(2-chloroethyl)doxorubicin

[R_1=OCH_3, R_2=OH, A=OH, B=H, R_3=R_4=$(CH_2)_3$-Cl]

and pharmaceutically acceptable salts thereof.

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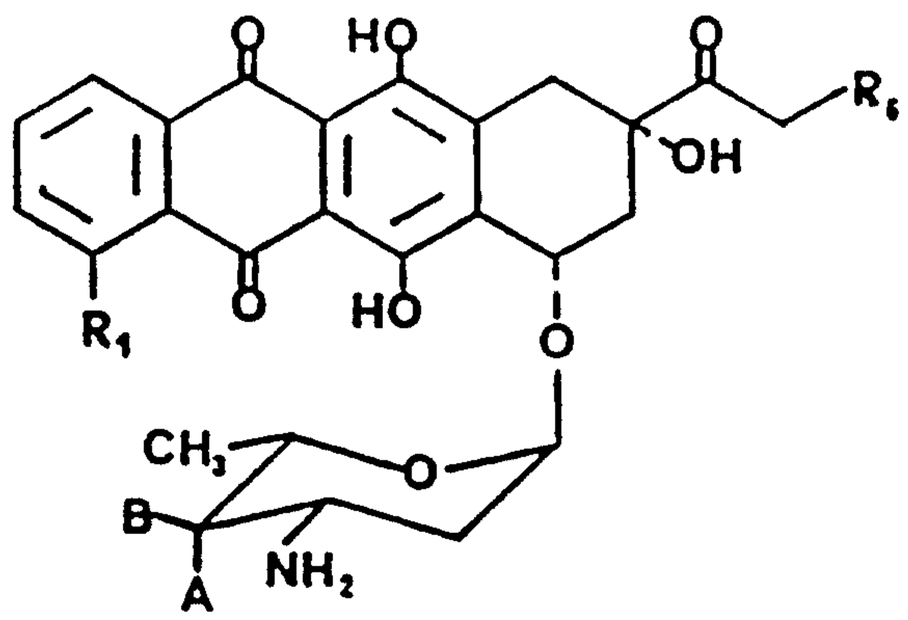
4. A compound according to any of claims 1 to 3 which is a hydrochloride salt.

5. A process for the preparation of an anthracycline glycoside of formula 1 as defined in claim 1 wherein R_3 and R_4 are as defined in claim 1 wherein X is a hydroxy group, or both R_3 and R_4 are a group of formula 2 as defined in claim 1 wherein X is a hydroxy group, or a salt thereof; the process comprising:

(i) reacting a compound of formula 3:



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wherein A and B are as defined in claim 1 R_1 is hydrogen or a methoxy group and R_6 is hydrogen, a hydroxy group or an acid sensitive group, the compound of formula 3 being dissolved in a polar aprotic solvent, with an alkylating agent of formula 4:

$$X\text{-}(CH_2)_n\text{-}Hal \qquad 4$$

wherein n is 2 or 3, X is hydroxy group and Hal is a halogen; and, if desired,

(ii) purifying the resulting anthracycline glycosides of formula 1 on a chromatographic column; and/or, if desired,

(iii) converting the anthracycline glycoside of formula 1 into a pharmaceutically acceptable acid addition salt thereof.

6. A process according to claim 5 wherein the polar aprotic solvent is dimethylformamide or acetonitrile.

7. A process according to claim 5 or 6 wherein step (i) is conducted at a temperature of from 20°C to 30°C for 4 to 24 hours.

8. A process according to claim 5 or 6 wherein step (i) is conducted at a temperature of from 20°C to 30°C for 1 to 3 weeks.

9. A process according to any of claims 5 to 8 wherein, in formula 4, the halogen denoted by Hal is bromine or iodine.

10. A process according to any of claims 5 to 9 wherein step (ii) is performed on silica gel column using, as eluent, methylene chloride:methanol (80:20 v/v).

11. A process according to any one of claims 5 to 10 wherein, in step (iii), the anthracycline glycoside is converted into its hydrochloride salt by treatment with anhydrous hydrogen chloride.

12. A process for the preparation of an anthracycline glycoside of formula 1 as defined in claim 1, wherein both R_3 and R_4 are a group of formula 2 as defined in claim 1 in which n is 2 and X is a hydroxy group, or a salt thereof; the process comprising:

(i) reacting a compound of formula 3, as defined in claim 5, with ethylene oxide; and, if desired,

(ii) purifying the resulting anthracycline glycoside of formula 1 on a chromatographic column; and/or, if desired,

(iii) converting the anthracycline glycoside of formula 1 into a pharmaceutically acceptable acid addition salt thereof.

13. A process according to claim 12 wherein, in step (i), the compound of formula 3 is first dissolved in a methylene chloride:methanol solvent.

14. A process according to claim 12 or 13 wherein step (i) is conducted in the dark at a starting temperature of -40°C, the temperature being subsequently increased to room temperature.

15. A process according to claim 14 wherein the temperature is maintained at room temperature for up to 3 days.

16. A process for the preparation of an anthracycline glycoside of formula 1, or salt thereof, as defined in claim 1, wherein R_2 is hydrogen and one or both of R_3 and R_4 represent a group of formula $-(CH_2)_n-OSO_2R_5$ wherein R_5 is C1-C4 alkyl or aryl optionally substituted by C1-C4 alkyl, nitro, amino, methoxy or halogen, and n is as defined in claim 1 the process comprising

(i) reacting an anthracycline glycoside of formula 1, as defined in claim 1 wherein one or both of R_3 and R_4 is a group of formula 2, in which X is a hydroxy group, with a sulfonyl chloride of formula 5:

$$Cl-SO_2-R_5 \qquad 5$$

wherein R_5 is as defined above; and, if desired,

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(ii) purifying the resulting anthracycline of formula 1 on chromatographic column.

17. A process according to claim 16 wherein step (i) is conducted in an aprotic solvent in the presence of a tertiary amine.

18. A process according to claim 17 wherein the tertiary amine is triethylamine or pyridine.

19. A process for the preparation of an anthracycline glycoside of formula 1, as defined in claim 1 wherein one or both of R_3 and R_4 represent a group of formula 2 as defined in claim 1 wherein X is a chlorine atom, or a salt thereof the process comprising

(i) dissolving an anthracycline glycoside of formula 1 as defined in claim 1 wherein one or both of R_3 and R_4 is a group of formula $-(CH_2)_n-OSO_2R_5$, wherein R_5 is a C1-C4 alkyl or aryl optionally substituted by C1-C4 alkyl, nitro, amino, methoxy or halogen and n is as defined in claim 1, in an aprotic solvent and reacting the resulting solution with a halide salt and, if desired,

(ii) purifying the resulting anthracycline glycoside on a chromatographic column; and/or, if desired,

(iii) isolating the desired compound as the corresponding hydrohalide.

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20. A process according to claim 19 wherein, in step (i), the aprotic solvent is acetone or dimethylformamide.

21. A process according to claim 19 or 20 wherein step (i) is performed at room temperature.

22. A process according to any one of claims 19 to 21

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wherein, in step (i), the halide salt is n-tetrabytylammonium chloride.

23. A process for the preparation of an anthracycline glycoside of formula 1 as defined in claim 1 wherein one or both of R_3 and R_4 represent a group of formula 2 wherein X is a chlorine atom and both A and B represent hydrogen or one of A or B represents a group of formula $-OSO_2R_5$ wherein R_5 is C1-C4 alkyl or aryl optionally substituted, or a salt thereof the process comprising

(i) dissolving an anthracycline glycoside of formula 1 as defined in claim 1 wherein one or both of R_3 and R_4 is a group of formula 2 as defined in claim 1 in which X is hydroxy group, in dry pyridine and reacting the resulting solution with a sulfonyl chloride of formula 5:

$$Cl\text{-}SO_2R_5 \qquad 5$$

wherein R_5 is as defined above; and, if desired

(ii) purifying the resulting anthracycline glycoside on a chromatographic column; and/or, if desired,

(iii) isolating the desired compound as the corresponding hydrohalide.

24. A process according to claim 23 wherein, in step (i), the compound of formula 1 is first dissolved in dry pyridine.

25. A process according to claim 23 or 24 wherein step (i) is conducted in the dark at 0°C.

26. A process according to claim 25 wherein the

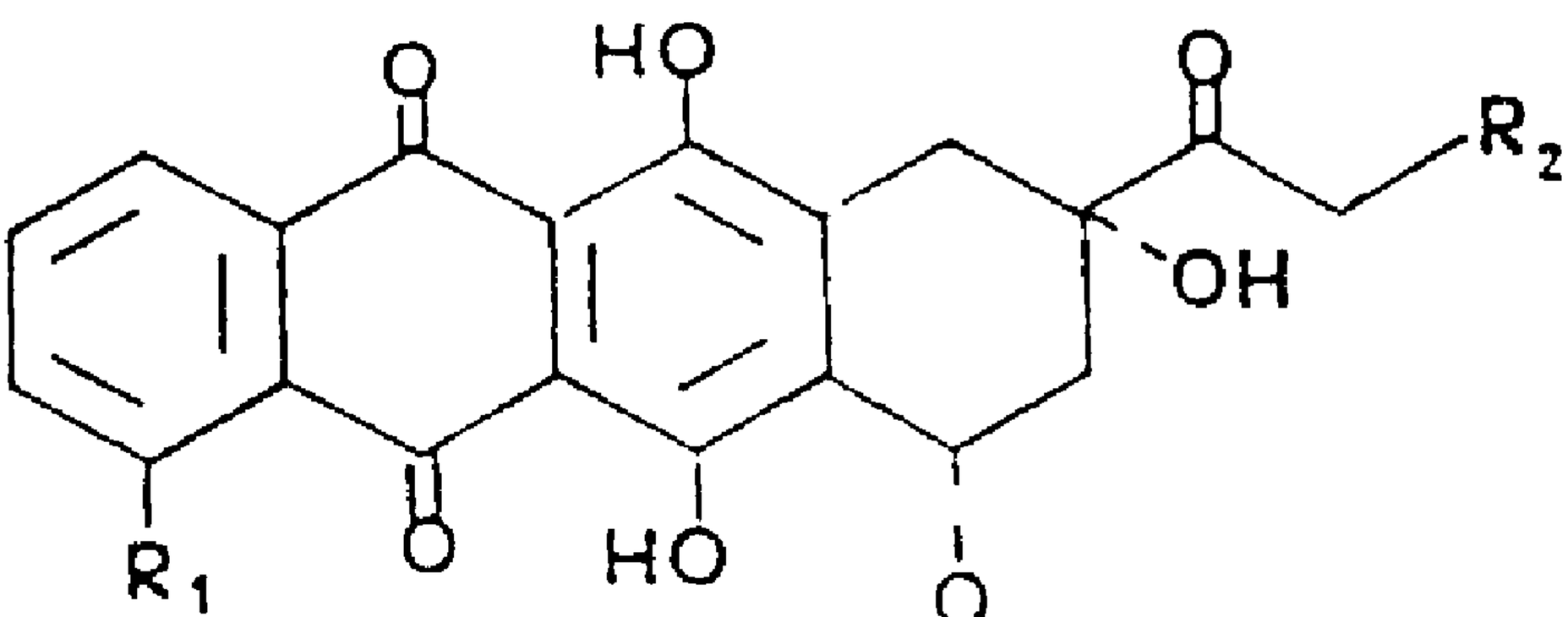
temperature is maintained at 0°C for up 16 hours.

27. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and, as active principle, an anthracycline glycoside of formula 1, or a pharmaceutically acceptable salt thereof, as defined in claim 1.

28. An anthracycline glycoside of formula 1 or a pharmaceutically acceptable salt thereof, as defined in claim 1, for use in treatment of the human or animal body by therapy.

29. An anthracycline glycoside or salt thereof, according to claim 28, for use as an anti-tumor agent.

Fetherstonhaugh & Co.,
Ottawa, Canada
Patent Agents



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