

COLCHICINE DERIVATIVES AND THE THERAPEUTICAL USE THEREOF

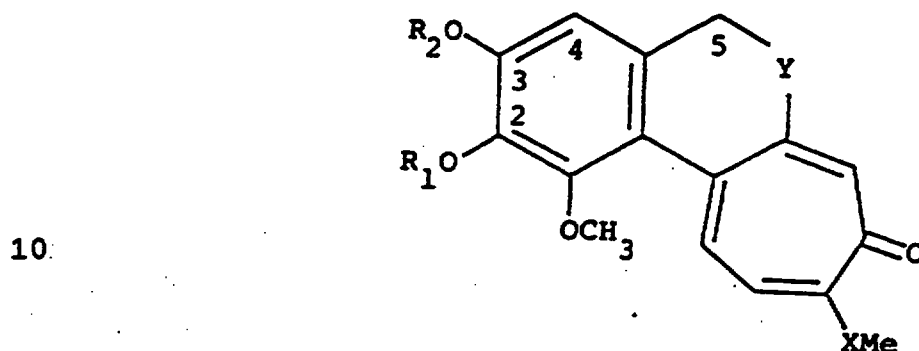
The present invention relates to novel colchicine derivatives having antiproliferative, antitumoral and antiinflammatory activities, the methods for the preparation thereof and the pharmaceutical formulations containing them. Colchicine is a known pseudo-alkaloid widely used for a very long time in therapy for the treatment of gout; it acts very quickly and specifically, even though it should be used for short times due to its toxicity. In addition, colchicine is a very potent antiproliferative agent, whose action is connected with a mechanism blocking the formation of the mitotic spindle in cell division; this latter aspect has been investigated thoroughly for any antitumoral activity and a great deal of colchicine derivatives have been prepared for this purpose.

Colchicine as such and a number of its prepared derivatives could not be used due to its high toxicity in terms of risk/benefit ratio. Only one colchicine derivative, demecolcine, is used in some degree in oncology for the treatment of some leukemia forms. As far as the use in the antiinflammatory field is concerned, the only marketed colchicine derivative is thiocolchicoside, bearing a thiomethyl moiety at C₁₀ and a glucose molecule at the hydroxyl in C₃; the therapeutic uses of this derivative are related to the muscle relaxant and antiphlogistic effects. The products of the invention differ from those of the prior art in the high therapeutical index. In the antitumoral field, researches have been focused on the research of products

2

having, besides a normal cytotoxicity, a cytotoxicity aimed at cell lines resistant to the usual antitubercular medicaments.

The derivatives of the present invention have the general formula 1

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wherein:

X can be an oxygen or sulfur atom;

15 R_1 and R_2 , which can be the same or different, are straight or branched alkyl groups or cycloalkyl groups, containing 1 to 6 carbon atoms; or saturated or unsaturated acyl groups, containing 16 to 22 carbon atoms, or a β -D-glucose residue as such or a β -D-glucose residue wherein the hydroxyl in positions 4 and 6 are protected as chetals with aliphatic or aromatic or heteroaromatic aldehydes; Y is a $-\text{CH}_2-\text{CH}-\text{NH}-\text{R}_3$ group, the methylene group of which being bound to the carbon in position 5 and the methine group, having the same absolute configuration as colchicine, is bound to the tropolone ring, or a $-\text{CH}-\text{CH}_2\text{OR}_4$ group, the methine group of which being bound to the carbon in position 5 and to the tropolone ring and having absolute configuration S; R_3 is an acyl group containing 2 to 6 carbon atoms bearing one to three halogen atoms, preferably fluorine or chlorine, or an acyl group from a natural amino acid,

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wherein the amino group can be free or protected as trifluoroacetamide or benzamide;

R_4 is an acyl residue of a dicarboxylic acid containing 4 to 6 carbon atoms, or an acyl residue of a natural amino acid, wherein the amino group can be free or protected as trifluoroacetamide or benzamide, or a glycoside residue consisting of D-glucose, D-galactose, L-fucose or L-rhamnose. In this case, the sugars act as chemotactic for melanocytes, hepatocytes, fibroblasts or they give the derivative the characteristic of a pro-drug.

Preferred compounds of formula 1 are those wherein X is sulfur.

R_1 and R_2 , which are the same or different, are preferably hydrogen or C_1 - C_6 alkyl.

Y is preferably a group of formula $-CH_2-CH-NHR_3$ as defined above.

For the preparation of the products of the invention, the starting products used are the natural substances colchicine (1; $X=O$; $R_1=R_2=Me$; $Y=CH_2-CH-NHAc$), 2-O-demethylcolchicine (1; $X=O$; $R_1=H$; $R_2=Me$; $Y=CH_2-CH-NHAc$), 3-O-demethylcolchicine (1; $X=O$; $R_1=Me$; $R_2=H$; $Y=CH_2-CH-NHAc$), colchicoside (1; $X=O$; $R_1=Me$; $R_2=\beta$ -D-glucose; $Y=CH_2-CH-NHAc$) which can be recovered from vegetable materials according to procedures known in literature. These natural substances, by treatment with methylmercaptan in alkali solution, according to procedures also known in literature, yield the corresponding thioderivatives which are used as the syntones for the preparation of the derivatives of general formula 1, wherein X is sulfur.

For the preparation of the derivatives of general formula 1 wherein R_1 and R_2 are alkyl or acyl groups, the starting products used are the colchicine or thiocolchicine syntons demethylated in position 2 or 3.

5 These syntons are subjected to alkylation or acylation using the well known methods for the phenol derivatization. Analogously, the derivatives of general formula 1 wherein R_1 or R_2 are a β -D-glucose residue or a β -D-glucose residue wherein the hydroxyls in 4 and 6

10 are protected as chetals with aliphatic or aromatic aldehydes, are prepared from colchicine or thiocolchicine syntons demethylated in position 2 or 3. These syntons are subjected to a reaction with α -bromotetraacetyl-D-glucose or with 2,3-di-O-dichloroacetyl- β -

15 D-glucose containing a chetal group involving the hydroxyls in the positions 4 and 6 (cf. Canada Pat. N. 956939). After removing the protective acyl groups by known methods described in literature, the derivatives glucosidated in the position 2 or 3 according to the

20 present invention are obtained.

The derivatives of formula 1 wherein Y is a $-\text{CH}_2-\text{CH}-\text{NH}-\text{R}_3$ group are prepared subjecting to N-desacetylation by acid catalysis the colchicine or thiocolchicine syntons, bearing methoxy or hydroxy

25 groups in the positions 2 and 3, followed by acylation of the primary amino moiety with an acid reactive derivative containing one to three fluorine or chlorine atoms or a natural amino acid, the amino group of which can be free or protected as trifluoroacetamide or

30 benzamide. The derivatives of formula 1 wherein R_3 has the meaning defined above are thereby prepared. The

derivatives of formula 1 wherein Y is a $-\text{CH}-\text{CH}_2-\text{OR}_4$ group are obtained subjecting colchicine or thiocolchicine syntons, bearing methoxy or hydroxy groups in positions 2 and 3, to N-desacetylation followed by treatment with sodium nitrite and acetic acid which contracts of the cycloheptane ring with the formation of the syntons of formula 1 wherein $\text{Y}=-\text{CH}-\text{CH}_2\text{OH}$ (J. Med. Chem. 36, 544, 1993). The resulting primary alcohol moiety yields, by reaction with suitably activated dicarboxylic acids, or with activated natural amino acids, wherein the amino group can be free or protected as trifluoroacetamide or benzamide, or with the reactive form of the sugars D-glucose, D-galactose, L-fucose or L-rhamnose, the derivatives of general formula 1 wherein $\text{Y}=\text{CH}-\text{CH}_2\text{OR}_4$, R_4 having the meaning defined above.

The following table shows the antimitotic activity on tumoral cell lines of some derivatives of the invention. Taxol and colchicine are the comparison substances.

TABLE - In vitro cytotoxic activity of some novel colchicine derivatives.

Substances	IC ₅₀ (nM)				
	Cell lines				
	Ovary	Colon	Breast	Lung	Cell. resist.
Taxol	6.1	3.5	3.2	1.7	299
Colchicine	5.3	3.9	4.4	16	112
Compound IZ	4.2	2.6	6.2	8	26
" IIZ	9.4	1.8	5.0	19	11
" IVZ	6.1	2.2	4.1	12	7

15 This table evidences that the novel derivatives have significant advantages on the resistant cell lines, which is the main target for cytotoxic medicaments. The products of the present invention can be incorporated in pharmaceutical formulations useful for the

20 administration of the medicament. Formulations for the parenteral, oral, transdermal, epicutaneous administrations can be conveniently prepared.

Among the excipients useful to prepare said formulations, natural and synthetic phospholipids proved

25 to be particularly useful for preparing liposomal forms for the parenteral, transdermal or epicutaneous routes; the latter two formulations being particularly useful in the treatment of arthrosic or peripheral venous inflammatory conditions; said formulations also being

30 useful in the topical treatment of cutaneous epitheliomas and in cutaneous hyperproliferative

conditions, such as psoriasis. In the specific antitumoral field, besides the phospholipids which allow for the administration of the medicament in liposomal form, some surfactants such as polyethoxylated castor oil, such as for example Cremoform L50, or polysorbate, such as for example Tween, acting synergistically with the active ingredient, turned out to be particularly useful. In oncology, the products are used at dosages from 1 to 100 mg/m², whereas as antiinflammatory the dosages range from 1 to 20 mg per unit dose, one to more times daily. All of the pharmaceutical formulations such as vials, capsules, creams etc. can be prepared with the main part of said derivatives.

The following examples further illustrate the invention.

Example I - Preparation of N-desacetyl-N-trifluoroacetyl-3-O-demethyl-3-O-cyclopentyl-thiocolchicine, compound IZ (1; X=S; R₁=Me; R₂=C₅H₉; Y=CH₂CHNH-COCF₃).

20 g of 3-O-demethylthiocolchicine (1; X=S; R₁=Me; R₂=H; Y=CH₂CHNHAc) are dissolved in 300 ml of 20% sulfuric acid and treated at 100°C under nitrogen atmosphere for 36h. The reaction mixture is neutralized, thus separating 12 g of N-desacetyl-3-O-demethylthiocolchicine (1; X=S; R₁=Me; R₂=H; Y=CH₂CHNH₂). This product is dissolved in acetone and reacted with 3 equivalents of trifluoroacetic anhydride under strong stirring in the presence of anhydrous Na₂CO₃. After 2h the reaction mixture is filtered and the solution is evaporated to dryness. The residue, consisting of 3-O-demethyl-N,3-O-bistrifluoroacetylthiocolchicine, is hydrolysed in methanol containing NH₄Cl. The reaction

mixture is evaporated to dryness under vacuum and the residue taken up in acetone. The acetone solution is filtered and refluxed for eight hours with 5 equivalents of cyclopentyl bromide in the presence of sodium carbonate. Salts are filtered off, the solution is evaporated to dryness and the residue is purified by chromatography on silica gel column using ethyl acetate as eluent. By crystallization from acetone/hexane. 8.6 g of product are obtained, M^+a m/z 523.

Example II - Preparation of N-desacetyl-N-trifluoroacetyl-3-O-demethyl-3-O-isopropyl-thiocolchicine, compound IIZ (1; X=S; R_1 =Me; R_2 =iPr; Y=CH₂-CH-NHCOCF₃).

For the preparation of this derivative, the procedure of example I is repeated, using as reagent isopropyl bromide instead of cyclopentyl bromide. After purification of the crude reaction product on silica gel and crystallization, 7.6 g of product are obtained, M^+a m/z 497.

Example III - Preparation of N-desacetyl-N-trifluoroacetylthiocolchicoside, compound IIIZ (1; X=S; R_1 =Me; R_2 =β-D-glucosyl; Y=CH₂-CH-NHCOCF₃).

10 g of N-desacetyl-thiocolchicoside (1; X=S; R_1 =Me; R_2 =β-D-glucose; Y=CH₂-CH-NH₂) are dissolved in acetone and treated for two hours at 10°C with three equivalents of trifluoroacetic anhydride. The mixture is evaporated to dryness and the residue is crystallized from isopropanol and subsequently from ethanol. 8.5 g of product are obtained, M^+a m/z 617.

Example IV - Preparation of N-(N-trifluoroacetyl-α-phenylglycyl)-desacetylthiocolchicine, compound IVZ (1; X=S; R_1 = R_2 =Me; Y=CH₂-CH-NH-CO-CH(NHCOCF₃)Ph).

400 mg of N-desacetyl-thiocolchicine (1; X=S; R₁=R₂=Me; Y=CH₂-CH-NH₂) (1.07 mmol) are dissolved together with 265 mg (1.07 mmol) of L-N-trifluoro-acetyl- α -phenyl-glycine in 10 ml of methylene chloride under nitrogen atmosphere. The solution is added 221 mg (1.07 mmol) of N,N-dicyclohexylcarbodiimide, stirring until disappearance of the reagents. The reaction mixture is cooled to -30°C and filtered to remove the precipitated urea. The chloromethylene solution is concentrated and purified by filtration on silica gel eluting with a methylene chloride/methanol 98:2 mixture. Upon crystallization from methylene chloride/ethyl ether, 350 mg of product are obtained, M⁺a m/z 602.

Example V - Preparation of N-(N-trifluoroacetyl-L-alanyl)-desacetylthiocolchicine, compound VZ (1; X=S; R₁=R₂=Me; Y=CH₂-CH-NH-CO-CH(NHCOCF₃)CH₃).

400 mg of N-desacetyl-thiocolchicine (1.07 mmol) are treated with one equivalent of N-trifluoroacetyl-L-alanine and one equivalent of N,N-dicyclohexylcarbodiimide in 10 ml of methylene chloride and under nitrogen atmosphere until disappearance of the reagents. The reaction mixture is cooled to -30°C and filtered to remove the precipitated urea. The chloromethylene solution is concentrated and purified by filtration on silica gel eluting with a methylene chloride/methanol 98:2 mixture. Upon crystallization from methylene chloride/ethyl ether, 94 mg of product are obtained, M⁺a m/z 540.

Example VI - Preparation of N-(N-trifluoroacetyl-methionyl)-desacetylthiocolchicine, compound VIZ (1; X=S; R₁=R₂=Me; Y=CH₂-CH-NHCO-CH(NHCOCF₃)CH₂-CH₂-SMe).

The procedure of example IV is repeated, reacting N-trifluoroacetylmethionine. By fractional crystallization, upon chromatographic purification of the reaction residue, from 400 mg of N-desacetylthiocolchicine, 84 mg of product are obtained, M^+a m/z 600.

Example VII - Preparation of N-(α -phenylglycyl)desacetylthiocolchicine, compound VIIIZ (1; X=S; $R_1 = R_2 = \text{Me}$; $Y = \text{CH}_2 - \text{CH} - \text{NHCO} - \text{CHNH}_2 - \text{Ph}$).

400 mg of the product obtained in example IV are dissolved in 5 ml of 50% acetone in the presence of 120 mg of potassium carbonate and heated at 60°C with stirring for 5 hours. The reaction mixture is cooled, saturated with NaCl and extracted with chloroform. The organic phase is dried over anhydrous sodium sulfate, then concentrated to dryness and the residue is chromatographed on silica gel with a methylene chloride/methanol 98:2 mixture. 160 mg of product are obtained, M^+a m/z 506.

Example VIII - Preparation of N-desacetyl-N-trifluoroacetyl-3-O-demethyl-3-O-ximeninylthiocolchicine, compound VIIIZ (1; X=S; $R_1 = \text{Me}$; $R_2 = \text{CO}(\text{CH}_2)_7\text{C}=\text{C}-\text{CH}=\text{CH}-(\text{CH}_2)_5\text{CH}_3$; $Y = \text{CH}_2 - \text{CH} - \text{NH} - \text{CO} - \text{CF}_3$).

500 mg of N-desacetyl-N-trifluoroacetyl-3-O-demethylthiocolchicine (1; X=S; $R_1 = \text{Me}$; $R_2 = \text{H}$; $Y = \text{CH}_2 - \text{CH} - \text{NHCOCF}_3$) are dissolved in 2.5 ml of pyridine and added at 0°C with 500 mg of ximeninic acid chloride. The reaction mixture is left to stand overnight at room temperature, then is poured onto ice. The resulting precipitate is separated and crystallized from acetone/hexane, M^+a m/z 715.

Example IX - Preparation of 5,6-dihydro-6(S)-[(β -D-glu-

copyranosyloxy)methyl]-1,2,3-trimethoxy-9-(methylthio)-
8H-cyclohepta[a]naphthalen-8-one, compound IXZ (1; X=S;
R₁=R₂=Me; Y=CH-CH₂-β-D-glucose).

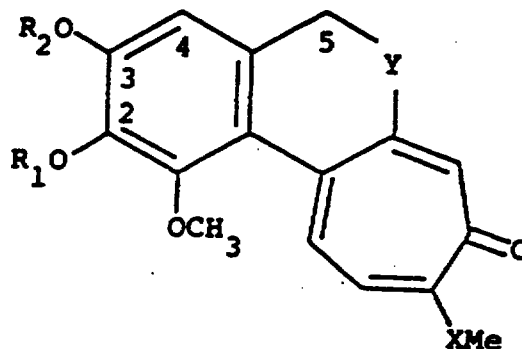
10 g of N-desacetylthiocolchicine are treated with
5 sodium nitrite to give 4 g of 5,6-dihydro-6(S)-
(hydroxymethyl)-1,2,3-trimethoxy-9-methylthio-8H-cyclo-
hepta[a]naphthalen-8-one, according to the process
described in J. Med. Chem., 36, 544, 1993. The resulting
product is treated for 12 hours under reflux in
10 acetonitrile with 26 g of α-bromotetraacetylglucose in
the presence of 85 g of mercuric cyanide. Salts are
filtered off and the solution is evaporated to dryness,
taken up with 70% acetone and treated for two hours with
15% sodium carbonate. The mixture is neutralized,
15 extracted with ethyl acetate and chromatographed on
silica gel eluting with a methylene chloride-ethanol 9:1
mixture. 2.1 g of product are obtained, M⁺a m/z 536.

Example X - Preparation of 5,6-dihydro-6-(S)-(hydroxy-
methyl)-1,2,3-trimethoxy-9-(methylthio)-8H-cyclohepta-
20 [a]naphthalen-8-one succinyl ester, compound XZ (1; X=S;
R₁=R₂=Me; Y=CH-CH₂-OCOCH₂CH₂CO₂H).

10 g of N-desacetylthiocolchicine are treated as in
example IX. The resulting naphthalen-8-one is dissolved
in pyridine and treated under reflux for 24 hours with a
25 succinic anhydride excess. The reaction mixture is
cooled, poured into abundant water and extracted with
methylene chloride. The organic phase is concentrated to
small volume and purified on silica gel eluting with a
methylene chloride-water-methanol 70:30:5 mixture. After
30 crystallization from methanol, 7 g of product are
obtained, M⁺a m/z 474.

CLAIMS

1. Compounds of formula 1



wherein:

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X can be an oxygen or sulfur atom;

R₁ and R₂, which can be the same or different, are straight or branched alkyl groups or cycloalkyl groups, containing 1 to 6 carbon atoms; or saturated or unsaturated acyl groups, containing 16 to 22 carbon atoms, or a β-D-glucose residue as such or a β-D-glucose residue wherein the hydroxyls in positions 4 and 6 are protected as chetals with aliphatic or aromatic or

heteroaromatic aldehydes; Y is a -CH₂-CH-NH-R₃ group, the methylene group of which being bound to the carbon in position 5 and the methine group, having the same absolute configuration as colchicine, is bound to the tropolone ring, or a -CH-CH₂OR₄ group, the methine group of which being bound to the carbon in position 5 and to the tropolone ring and having absolute configuration S;

R₃ is an acyl group containing 2 to 6 carbon atoms bearing one to three halogen atoms, preferably fluorine or chlorine, or an acyl group from a natural amino acid, wherein the amino group can be free or protected as trifluoroacetamide or benzamide;





R_4 is an acyl residue of a dicarboxylic acid containing 4 to 6 carbon atoms, or an acyl residue of a natural amino acid, wherein the amino group can be free or protected as trifluoroacetamide or benzamide, or a glycoside residue consisting of D-glucose, D-galactose, L-fucose or L-rhamnose.

2. Compounds according to claim 1, wherein X is sulfur.

3. Compounds according to claim 1, wherein X is oxygen.

4. Compounds according to claim 2 or claim 3, wherein R_1 and R_2 , which are the same or different, are straight or branched alkyl groups or cycloalkyl groups containing 1 to 6 carbon atoms.

5. Compounds according to any one of the above claims, wherein Y is a group of formula $-\text{CH}_2-\text{CH}-\text{NH}-\text{R}_3$ as defined in claim 1.

6. A process for the preparation of the compounds of claims 1-5, which comprises subjecting colchicine, 2-O-demethylcolchicine, 3-O-demethylcolchicine, colchicoside to one or more of the following reactions:

a) reaction with methylmercaptan in alkali solution;
b) alkylation, acylation or glycosylation of the phenol groups;

c) N-desacetylation by acid catalysis and subsequent acylation of the primary amino function with a $\text{R}_3\text{-COOH}$ acid or a derivative thereof, wherein R_3 is as defined in claim 1;

d) N-desacetylation followed by treatment with sodium nitrite and acetic acid to give compounds 1 wherein Y is $\text{Y-CH-CH}_2\text{-OH}$ and subsequent reaction with



derivatives of dicarboxylic acids of amino acids or with reactive derivatives of D-glucose, D-galactose, L-fucose, L-rhamnose.

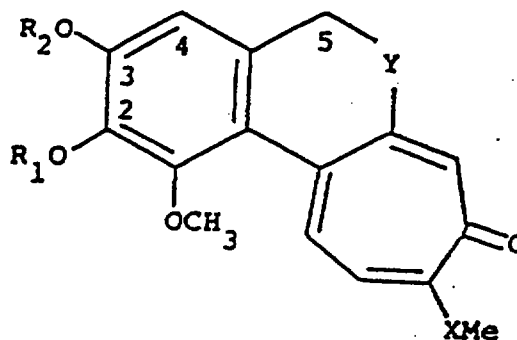
7. Pharmaceutical compositions containing as active
5 ingredient a compound of claims 1-5 in admixture with a suitable carrier, particularly natural or synthetic phospholipids.
8. The use of the compounds of claims 1-5 for the
10 preparation of medicaments having antiproliferative, antitumoral and antiinflammatory activities.



12

CLAIMS

1. Compounds of formula 1



wherein:

X can be an oxygen or sulfur atom;

R_1 and R_2 , which can be the same or different, are straight or branched alkyl groups or cycloalkyl groups, containing 1 to 6 carbon atoms; or saturated or unsaturated acyl groups, containing 16 to 22 carbon atoms, or a β -D-glucose residue as such or a β -D-glucose residue wherein the hydroxyls in positions 4 and 6 are protected as chetals with aliphatic or aromatic or heteroaromatic aldehydes; Y is a $-\text{CH}_2-\text{CH}-\text{NH}-R_3$ group, the methylene group of which being bound to the carbon in position 5 and the methine group, having the same absolute configuration as colchicine, is bound to the tropolone ring, or a $-\text{CH}-\text{CH}_2\text{OR}_4$ group, the methine group of which being bound to the carbon in position 5 and to the tropolone ring and having absolute configuration S; R_3 is an acyl group containing 2 to 6 carbon atoms bearing one to three halogen atoms, preferably fluorine or chlorine, or an acyl group from a natural amino acid, wherein the amino group can be free or protected as trifluoroacetamide or benzamide

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12A

with the proviso that when R1 and R2 are methyl, X is oxygen or sulfur atom, R3 is not $-\text{COCH}_2\text{F}$, $-\text{COCH}_2\text{Cl}$, $-\text{COCH}_2\text{Br}$, $-\text{COCH}_2\text{I}$;

when R1 and R2 are methyl, X is oxygen atom, R3 is not -
5 COCl_3 , $-\text{COCHCl}_2$, $-\text{COCHF}_2$, $-\text{COCH}_2\text{CHClCH}_3$; -
 $\text{COCH}_2\text{CH}_2\text{CHBrCH}_3$;

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(21) International Application Number: PCT/EP95/03823 (22) International Filing Date: 27 September 1995 (27.09.95) (30) Priority Data: MI94A002026 / 5 October 1994 (05.10.94) / IT (71) Applicant (for all designated States except US): INDENA S.P.A. [IT/IT]; Via Ripamonti, 99, I-20141 Milano (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): BOMBARDELLI, Ezio [IT/IT]; Via Ripamonti, 99, I-20141 Milano (IT). GABETTA, Bruno [IT/IT]; Via Ripamonti, 99, I-20141 Milano (IT). (74) Agent: MINOJA, Fabrizio; Studio Consulenza Brevettuale, Via Rossini, 8, I-20122 Milano (IT).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MX, NO, NZ, PL, PT, RO, RU, SE, <u>SG</u> , SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published With international search report.

(54) Title: COLCHICINE DERIVATIVES AND THE THERAPEUTICAL USE THEREOF

(57) Abstract

The present invention relates to novel colchicine derivatives of formula (1), where Y is a $-\text{CH}_2\text{-CH-NH-R}_3$ group or a $-\text{CH-CH}_2\text{OR}_3$ group and the other variables are as defined in claim 1, having antiproliferative, antitumoral and anti-inflammatory activities. The novel compounds have a cytotoxicity on human tumoral cell lines comparable with colchicine, but, in comparison with the latter, they are less toxic and more selective, particularly on cells resistant to the usual medicaments. Some compounds have a marked activity on TNF and interleukine 2, and therefore are very potent anti-inflammatory agents. They can be included in pharmaceutical formulations useful for the parenteral, oral and topical administrations.

