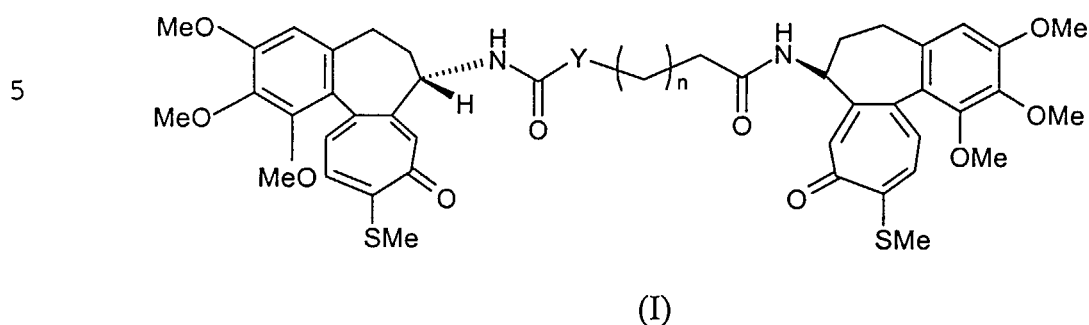


N-DEACETYLTHIOLCHICINE DERIVATIVES AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

The present invention relates to derivatives of N-deacetyl-thiocolchicine or of the isoster thereof of formula (I)



10 wherein:

n is an integer of 0 to 8;

Y is a CH_2 group or, when n is 1, can also be a group of formula NH.

Colchicines and thiocolchicines are known antiproliferative compounds capable of destabilizing microtubules through interaction with tubulin.

15 Colchicine is currently used in the therapy of gout and related inflammatory conditions, but its use is restricted to the acute phases due to its high gastro-intestinal toxicity.

A number of colchicine or thiocolchicine derivatives have been studied, in view of a possible use thereof as antitumor medicaments, but the efforts of
20 researchers have to date been unsuccessful due to the often very restricted therapeutic index of such compounds.

Only one colchicine derivative, demecolcine, has been used in the past in clinic for the treatment of leukemias, but with poor success.

It has now been found that the compounds of formula (I) have anti-
25 proliferative activity, in particular on cells expressing the MDR (multi-drug

resistance) phenotype, with an approximately 1:1 ratio of activity on sensitive cells to activity on resistant cells.

The compounds of the invention have in fact powerful antimitotic activity and are characterized by favorable therapeutic index which makes them suitable for the therapeutical treatment of various forms of tumors, as well as for degenerative rheumatoid arthritis, a disease characterized by excessive proliferation and abnormal migration of leukocytes.

Compounds (I) have cytotoxicity comparable to that of the most effective antitumor medicaments, while having a remarkably wider action spectrum, particularly against cells resistant to known drugs.

Compounds (I) wherein Y is CH₂ are prepared by reacting N-deacetyl-thiocolchicine with dicarboxylic acid reactive derivatives in dry solvents. Examples of suitable dicarboxylic acid reactive derivatives comprise chlorides, reactive anhydrides or esters, in particular N-hydroxysuccinyl diesters obtainable by reacting said acids with N-hydroxy-succinimide. The reaction is preferably carried out in solvents such as ethyl ether, dioxane or tetrahydrofuran in the presence of bases, for example triethylamine.

On the other hand, compounds (I) wherein Y is NH and n is 1 can be prepared by reacting N-deacetyl-thiocolchicine with N-hydroxy-succinimide in the presence of amines and condensing agents such as dicyclohexylcarbodiimide (DCC), in a suitable aprotic solvent, preferably a chlorinated hydrocarbon (methylene chloride, chloroform). Said compounds can also be obtained as side-products from the reaction between dicarboxylic acid N-hydroxysuccinyl diesters and N-deacetyl-thiocolchicine.

The activity of these compounds was evaluated on a wide number of resistant tumour cells expressing the MDR phenotype; these compounds proved to be particularly active on different sensitive colon lines expressing MDR.

The following Table reports by way of example the activity of these two

compounds, comparing their biological activity to thiocolchicine and taxol as reference molecules.

TABLE

Compounds	IC ₅₀ nM		
	MCF7	MCF7-ADRR	MCF7-ADRR/MCF7
Tiocol 39 (Ex. 1)	12	43	3.58
Tiocol 43 (Ex. 4)	21	36	1.71
Tiocol 54 (Ex. 2)	2.6	2.8	1.07
Thiocolchicine	0.02	400	20000

5 The cytotoxic activity was evaluated according to M.C. Alley et al., *Cancer Research*, 48, 589-601, 1998.

The above-reported data evidence the high cytotoxic activity of the compounds of the invention on both sensitive cell lines and different drug-resistant cell lines to various antitumor drugs.

10 The compounds of the invention are therefore useful in the treatment of proliferative pathologies and in particular tumors of various origins, rheumatoid arthritis or other degenerative pathologies wherein antiproliferative and anti-inflammatory actions are indicated.

For this purpose, compounds (I) will be administered in the form of pharmaceutical compositions suitable to the oral, parenteral, epicutaneous or
15 transdermal administrations. The dosage of compounds (I) will range from 1 to 100 mg/m² body area, depending on the administration route. The compounds will preferably be administered orally.

Examples of compositions comprise capsules, tablets, vials, creams,
20 solutions, granulates.

The following examples illustrate the invention in greater detail.

EXAMPLE 1

Preparation of compound (I) wherein Y is CH₂ and n is 2 (Tiocol 39)

100 mg of N-deacetyl-thiocolchicine (M.W. = 373 g/mol, 0.27 mmol) are
5 dissolved in 6 ml of dry dioxane at room temperature under nitrogen atmosphere.
46 mg of adipic acid activated as N-hydroxy succinyl diester (M.W. = 340 g/mol,
0.135 mmol) and 40 µl of dry triethylamine (M.W. = 101 g/mol, d=0.726 g/ml,
0.27 mmol) are added. The mixture is stirred at room temperature under nitrogen
atmosphere for 48 hours (TLC control: CHCl₃: MeOH = 95:5). The solvent is
10 evaporated off and the product is recovered by flash chromatography on silica
(eluent: CHCl₃ : MeOH = 75:1).

Yield: 85%

EXAMPLE 2

Preparation of the compound (I) wherein Y is NH n is 1 (Tiocol 54)

15 A solution of 1 g of deacetyl-thiocolchicine in 40 ml of dry CH₂Cl₂ is
added with 154 mg of N-hydroxysuccinimide, 276 mg of DCC and 476 µl of
diisopropylethylamine. The mixture is refluxed under nitrogen for at least 2 days.
The progress of the reaction is monitored by TLC (CH₂Cl₂-EtOH=95/5). The
mixture is concentrated to small volume and the residue is taken up with ethyl
20 acetate. The product is left to crystallize, then further purified by flash
chromatography (eluent AcOEt - hexane 7/3 or (CH₂Cl₂-EtOH=95/5). 500 mg of
product are obtained.

¹H-NMR(DMSO-d₆-300Mhz): 8.80 d; 7.82 br s; 7.75-7.60 S; 7.37;7.18;
6.59; 4.90 m; 4.66 m; 4.52 dd; 3.96 s ppm.

25 ¹³C-NMR(CDCl₃): 182.5; 181.9; 172.2; 158.0; 175.5; 157.1; 153.8; 153.7;
153; 152.3; 151.3; 151.2; 141.6; 141.5; 139.4; 139.3; 135.5; 135.5 d, 134.8;
134.7; 129.0; 128.4 (d).

MS(m/z) 866.4 [(M+Na)+].

EXAMPLE 3**Preparation of compound (I) wherein Y is CH₂ and n is 6 (Tiocol 33)**

200 mg of N-deacetyl-thiocolchicine (M.W. = 373 g/mol, 0.54 mmol) are dissolved in 12 ml of dry dioxane at room temperature under nitrogen atmosphere. 91.8 mg of sebacic acid activated as N-hydroxy succinyl diester (M.W. = 396 g/mol, 0.27 mmol) and 75 µl of dry triethylamine (M.W. = 101 g/mol, d= 0.726 g/ml, 0.54 mmol) are added. The mixture is stirred at room temperature under nitrogen atmosphere for 48 hours (TLC control: CHCl₃:MeOH = 95:5), then after 20 hours is heated to 50°C and the solvent is evaporated off. The reaction crude is purified by flash chromatography on silica (eluent: CHCl₃:MeOH=40:1), to obtain 30 mg of a mixture of the title compound (with Rf:=0.3) and of the compound of example 2.

EXAMPLE 4**Preparation of compound (I) wherein Y is CH₂ and n is 0 (Tiocol 43)*****Procedure A***

190 mg of N-deacetyl-thiocolchicine (M.W.=373 g/mol, 0.512 mmol) are dissolved in 6 ml of dry dioxane at room temperature under nitrogen atmosphere. 80 mg of succinic acid activated as N-hydroxy succinyl diester (M.W. = 312 g/mol, 0.256 mmol) and 70 µl of dry triethylamine (M.W. = 101 g/mol, d=0.726 g/ml, 0.512 mmol) are added. The mixture is stirred at room temperature under nitrogen atmosphere for 48 hours (TLC control: CHCl₃: MeOH = 95:5). The solvent is evaporated off, the residue is taken up with AcOEt to remove the residual N-deacetyl thiocolchicine and triethylamine (the product is insoluble).

Yield: 45%

Procedure B

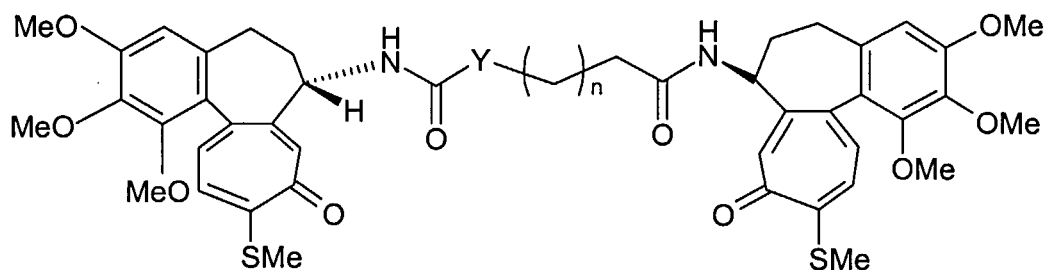
100 mg of N-deacetyl-N-succinyl-thiocolchicine (M.W.=473 g/mol, 0.21 mmol) are dissolved in 8 ml of dry CH₂Cl₂ at room temperature under nitrogen atmosphere. 93 mg of BOP (M.W. = 442,3 g/mol, 0.21 mmol) and 60 µl of dry

triethylamine (M.W. = 101 g/mol, d=0.726 g/ml, 0.42 mmol) are added. After 10 minutes, 80 mg of N-deacetyl thiocolchicine (M.W. = 101 g/mol, d=0.726 g/ml, 0.42 mmol) are added to the mixture, which is stirred at room temperature under nitrogen atmosphere for 48 hours (TLC control: CHCl₃: MeOH = 95:5). The
5 solvent is evaporated off and the residue is taken up with AcOEt to remove the residual N-deacetyl thiocolchicine and triethylamine (the product is insoluble).

Yield: 45%

CLAIMS

1. Compounds of formula (I)



(I)

- 5 wherein:

n is an integer of 0 to 8;

Y is a CH₂ group or, when n is 1, can also be a group of formula NH.

2. Compounds as claimed in claim 1 wherein Y is CH₂.
3. Compositions as claimed in claim 1 wherein Y is NH.
- 10 4. Pharmaceutical compositions containing a compound of claims 1-3.
5. The use of the compounds of claims 1-3 for the preparation of medicaments for the treatment of tumors and rheumatoid arthritis.