Abstract: The present invention relates to colchicine derivatives, in particular to the 3-demethyl and 3-demethylthio-colchicine of the general formula (I) in which X is oxygen or sulfur, a method for the preparation thereof and pharmaceutical compositions containing them. The compounds of formula (I) have muscle relaxing, anti-inflammatory and anti-gout activity.
COLCHICOSIDE ANALOGUES

Field of the invention

The present invention relates to colchicine derivatives, in particular 3-demethyl- and 3-demethylthio-colchicine derivatives with muscle relaxant, anti-inflammatory and anti-gout activity.

Technological background

Relaxant drugs reduce muscle tone and are used in therapy for the treatment of contractures and muscle spasm. Muscle spasm is one of the main factors responsible for chronic pain; it characterises several pathologies of the locomotory apparatus as well as inflammatory-rheumatic and degenerative orthopaedic pathologies; when it affects articulations, further to pain, it causes rigidity, which reduces joint mobility and flexibility in the affected part. For these reasons, the study of molecules endowed with muscle relaxant and antispasmodic properties still raises remarkable clinical interest.

As it is known, colchicine is a pseudoalcaloid that has been widely used for some time for the treatment of gout. The use of 3-demethyl-thiolcolchicine glucoside, thiocolchicoside, is also widespread in therapy for treating contractures and inflammatory conditions that affect the muscular system (Ortopedia e traumatologia Oggi XII, n. 4, 1992). It has been recently shown that the activity of thiocolchicoside is due to its ability to interact with strychnine-sensitive glycine receptors; therefore, compounds having glycine-mimicking activity can be used in the rheumatologic-orthopaedic field, due to their muscle relaxant properties.

Disclosure of the invention

The present invention relates to colchicine derivatives of the general formula (I):
in which X is oxygen or sulfur.

For the purposes of the present description, the compound in which X is oxygen is referred to as (Ia), whereas the compound of formula (I) in which X is sulfur is referred to as (Ib). D and L isomers are comprised in the compounds of formula (I). The D and L isomers of compound (Ib), 3-O-β-D-xylopyranosyl-3-O-demethylthiocolchicine and 3-O-β-L-xylopyranosyl-3-O-demethylthiocolchicine are particularly preferred.

The compounds of the present invention are prepared by reaction of D- or L-xylopyranosyl-fluoride with 3-O-demethylcolchicine (IIa) and 3-O-demethylthiocolchicine (IIb)

\[
\text{IIa: } X = O \\
\text{IIb: } X = S
\]

according to the general method disclosed in EP 0 789 028.

In more detail, 3-O-demethylcolchicine (IIa) or 3-O-demethylthiocolchicine (IIb) are reacted with D- or L-xylopyranosyl-fluoride (III) or a protected form thereof, preferably peracetate. The reaction is carried out in polar aprotic solvents, preferably selected from acetonitrile and chlorinated solvents, at temperatures ranging from 0°C to the boiling temperature of the solvent, preferably at room temperature, and in the
presence of a base, preferably 1,1,3,3-tetramethylguanidine. The reaction is usually complete in a time ranging from 10 minutes to 2 hours. Hydrolysis of the protective groups can be carried out without recovery of the intermediates.

In particular, it has been observed that the β-D isomer of compound (Ib) has a significant muscle relaxant activity, higher than that of the corresponding thiocolchicoside isomer, and is also endowed with a significant anti-inflammatory and anti-gout activity.

Muscle relaxant activity was evaluated with the rota-rod test. Swiss male mice weighing 20-25 g were treated intraperitoneally with the β-D isomer of compound (Ib) at doses of 1-3-10 mg/kg, thirty minutes before the test. Relaxant activity on striated muscles was evaluated by testing the resistance of the mice to the stimuli of a rotating plane revolving at increasing rate, from 2 to 50 r.p.m.. The results reported in the following table show that the compound of the present invention is more active than thiocolchicoside used as the reference compound.

Table 1

<table>
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<tr>
<th>Treatment</th>
<th>Dose (mg/Kg i.p.)</th>
<th>Resistance time (sec. M±S.E.)</th>
<th>DE₅₀ mg/Kg</th>
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<tr>
<td>Controls</td>
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<td>400 ± 27</td>
<td></td>
</tr>
<tr>
<td>Compound (Ib) isomer β-D</td>
<td>1</td>
<td>270 ± 19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>175 ± 14</td>
<td>2.23 (1.84-2.82)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>80 ± 10</td>
<td></td>
</tr>
<tr>
<td>Thiocolchicoside isomer β-D</td>
<td>1</td>
<td>345 ± 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>265 ± 17</td>
<td>4.47 (3.16-7.01)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>110 ± 12</td>
<td></td>
</tr>
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</table>

Moreover, the compound of the invention is significantly less toxic. In fact, its DL₅₀ is 80 (63-94) mg/kg i.p., whereas the DL₅₀ of thiocolchicoside is 20 mg/kg. These results show that the compound of the invention, further to
being more active, has a toxic/active dose ratio significantly more favourable than thiocolchicoside.

**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DE$_{50}$ mg/kg i.p.</th>
<th>DL$_{50}$ mg/kg i.p.</th>
<th>DL$<em>{50}$/DE$</em>{50}$</th>
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<tbody>
<tr>
<td>Compound (Ib) isomer β-D</td>
<td>2.23</td>
<td>80</td>
<td>35.87</td>
</tr>
<tr>
<td>Thiocolchicoside isomer β-D</td>
<td>4.47</td>
<td>20</td>
<td>4.47</td>
</tr>
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</table>

The compounds of the invention can be incorporated in pharmaceutical formulations intended to oral, intravenous, intramuscular, transdermal and topical administration with conventional excipients and methods, such as those reported in Remington's Pharmaceutical Sciences Handbook, XVII Ed., Mack Pub., N.Y., U.S.A.. Among the excipients useful for the preparation of liposomal forms for the parenteral or topical administration, natural and synthetic phospholipids are particularly preferred. The doses can range from 5 to 50 mg a day depending on the disease and the administration route.

The invention will now be illustrated in greater detail by means of some examples.

**EXPERIMENTAL SECTION**

Melting points were measured with a Buchi 510 apparatus. NMR spectra were recorded with a Bruker AC 200.

**Example 1 - 3-O-(2',3',4'-O-triacetyl-β-D-xylopyranosyl)-3-O-demethylthiocolchicine**

3-O-Demethylthiocolchicine (IIb) (0.5 mmoles) and 2,3,4-O-triacetyl-α-D-xylopyranosyl fluoride (0.75 mmoles), prepared according to Hayashi et al. (*Chemistry Lett.* 1984, 1747), were suspended in dry acetonitrile at room temperature (10 ml), under nitrogen and with stirring. 1,1,3,3-Tetramethylguanidine (1.5 mmoles) was added and the suspension turned clear red. Boron trifluoride etherate (4 mmoles) was added, thereafter the
solution turned colourless. The reaction was monitored by TLC
(\text{CH}_2\text{Cl}_2:\text{MeOH} 9:1). After disappearance of the starting products (30 min),
the reaction was quenched adding a saturated sodium bicarbonate solution
(10 ml). The phases were separated and the aqueous one was extracted with
ethyl acetate (3 x 10 ml). The combined organic phases were washed with a
saturated potassium hydrogen sulfate solution (15 ml), brine (15 ml) and dried
over magnesium sulfate. After evaporation of the solvent, the reaction
products were separated by chromatography on silica gel. Alternatively, the
 crude was directly subjected to deprotection.

\textbf{H-NMR} (CDCl\textsubscript{3}) - $\delta$ (ppm) 7.06 (NH, d, 7.4 Hz), 7.06 (H12, d, 10.3 Hz),
7.27 (H11. d, 10.3 Hz), 7.33 (H8, s), 6.71 (H4, s), 4.71-4.55 (H7, m), 2.60-
1.90 (H5-H6, m), 3.90 (2-OMe, s), 3.66 (1-OMe, s), 2.44 (SMe, s), 2.00
(acetamide), 5.28-5.18, 5.08-4.98 (H1', H2', H3', H4', m), 4.30 (H5'a, ddd, 4.3,
7.0, 12.1 Hz), 3.58 (H5'b, ddd 4.3, 7.0, 12.1 Hz), 2.12 (OAc), 2.11 (OAc),
2.10 (OAc).

\textbf{Example 2 - 3-O-(2',3',4'-O-triacety1-\beta-L-xylopyranosyl)-3-O-
 demethylthiocolchicine}

3-O-Demethylthiocolchicine (II\textsubscript{b}) (0.5 mmoles) and 2,3,4-O-triacety1-
\alpha-L-xylopyranosyl fluoride (0.75 mmoles), prepared according to Takanashi
\textit{et al.} \textit{(Liebig's Ann. Chem.} 1997, 1081), were suspended in dry acetonitrile at
room temperature (10 ml), under nitrogen and with stirring. 1,1,3,3-
Tetramethylguanidine (1.5 mmoles) was then added and the suspension turned
clear red. After addition of boron trifluoride etherate (4 mmoles) the solution
turned colourless. The reaction was monitored by TLC (\text{CH}_2\text{Cl}_2:\text{MeOH} 9:1).

After disappearance of the starting material (2 hours), the reaction was
quenched by addition of a saturated sodium bicarbonate solution (10 ml). The
phases were separated and the aqueous one was extracted with ethyl acetate
(3 x 10 ml). The combined organic phases were washed with a potassium
hydrogen sulfate saturated solution (15 ml), brine (15 ml) and dried over magnesium sulfate. After evaporation of the solvent, the reaction products were separated by chromatography on silica gel. Alternatively, the crude was directly subjected to deprotection.

$^1$H-NMR (CDCl$_3$) - $\delta$ (ppm) 7.34 (NH, d, 7.9 Hz), 7.07 (H12, d, 10.7 Hz), 7.30 (H11, d, 10.7 Hz), 7.37 (H8, s), 6.71 (H4, s), 4.71-4.55 (H7, m), 2.60-1.80 (H5-H6, m), 3.88 (2-OMe, s), 3.64 (1-OMe, s), 2.44 (SMe, s), 2.00 (acetamide), 5.28-5.18 e 5.10-4.90 (H1', H2', H3', H4' m), 4.25 (H5'a, ddd, 4.3, 4.4, 12.1 Hz), 3.58 (H5'b, ddd 4.3, 4.4, 12.1 Hz), 2.14 (OAc), 2.11 (OAc), 2.10 (OAc).

**Example 3 - General method for deprotection in ethanol**

The crude product (0.5 theoretical mmoles) from example 1 or 2 was dissolved in ethanol (4 ml) and 1N NaOH (2 ml) at room temperature. The reaction was checked by TLC. After disappearance of the starting product the solvent was evaporated off and the residue was subjected to silica gel chromatography. The product can be further crystallized from methanol/isopropanol.

**Example 4 - General method for deprotection in acetone**

The crude product from example 1 or 2 (1 theoretical mmol) was suspended with potassium carbonate in acetone (30 ml) and water (10 ml). The mixture was refluxed until disappearance of the starting product. The solvent was evaporated off and the product recovered by chromatography. The product can be further crystallized from methanol and diisopropyl ether.

**Example 5 - 3-O-β-D-xylopyranosyl-3-O-demethylthiocolchicine**

The product was obtained according to the deprotection method of example 3 or 4 with 45% yield, after chromatography on silica gel eluting with a CH$_2$Cl$_2$/MeOH gradient.
m.p. 193°C; $[\alpha]_D^{22}$ -201 (c 1, MeOH);
\textbf{Example 6 - 3-\textit{O}-\textbeta-L-xylopyranosyl-3-\textit{O}-demethylthiocolchicine}

The product was obtained according to the deprotection method of example 3 or 4 with 45\% yield, after chromatography on silica gel eluting with a CH$_2$Cl$_2$/MeOH gradient.

m.p. 220\degree C; [\alpha]_D^{22} -176 (c 1, MeOH);

\begin{align*}
\text{\textbf{H-NMR} (CDCl$_3$): ppm} & \quad 8.64 \text{ (NH, d, 7.6 Hz)}, 7.15 \text{ (H12, d, 10.6 Hz)}, 7.28 \text{ (H11, d, 10.6 Hz)}, 7.03 \text{ (H8, s)}, 6.85 \text{ (H4, s)} 4.37-4.25 \text{ (H7, m)}, 2.60-1.80 \text{ (H5-H6, m)}, 3.84 \text{ (2-OME, s)}, 3.55 \text{ (1-OME, s)}, 2.42 \text{ (SMe, s)}, 1.86 \text{ (acetamide)}, 4.97 \text{ (H1, 6.6 Hz)}, 3.20-3.90 \text{ (H2',H3',H4',H5', m)}, 4.40-5.60 \text{ (OH)}.
\end{align*}
CLAIMS

1. Compounds of the general formula (I)

\[
\begin{align*}
\text{xylopyranosyl-O} & \quad \text{O-CH}_3 \\
\text{CH}_3\text{O} & \quad \text{CH}_3\text{O} \\
\text{CH}_3\text{O} & \quad \text{XCH}_3
\end{align*}
\]

(I),

in which X is oxygen or sulfur.

2. A compound as claimed in claim 1 wherein X is oxygen.

3. A compound as claimed in claim 1 wherein X is sulfur.

4. A compound selected from:

- 3-O-β-D-xylopyranosyl-3-O-demethylthiocolchicine and
- 3-O-β-L-xylopyranosyl-3-O-demethylthiocolchicine.

5. A compound of any one of claims 1 - 4 as a medicament.

6. Use of a compound of any one of claims 1 - 4 for the preparation of muscle relaxant medicaments.

7. Use of a compound of any one of claims 1 - 4 for the preparation of anti-inflammatory medicaments.

8. Use of a compound of any one of claims 1 - 4 for the preparation of anti-gout medicaments.

9. Pharmaceutical compositions containing a compound of any one of claims 1 - 4 in admixture with suitable excipients and/or carriers.


11. Pharmaceutical compositions as claimed in claim 9 for parenteral use.

12. Pharmaceutical compositions as claimed in claim 10 or 11 in which the excipients are selected from natural and synthetic phospholipids.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

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According to International Patent Classification (IPC) or to both national classification and IPC.

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic database consulted during the international search (name of database and, where practical, search terms used)

- EPO-Internal
- WPI Data
- CHEMABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>EP 0 789 028 A (INDENA SPA) 13 August 1997 (1997-08-13)the whole document</td>
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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"*" Special categories of cited documents:

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- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "X" document member of the same patent family

Date of the actual completion of the international search: 6 October 2004

Date of mailing of the international search report: 14/10/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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Fax. (+31-70) 340-3016

Authorized officer

de Wooy, A

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