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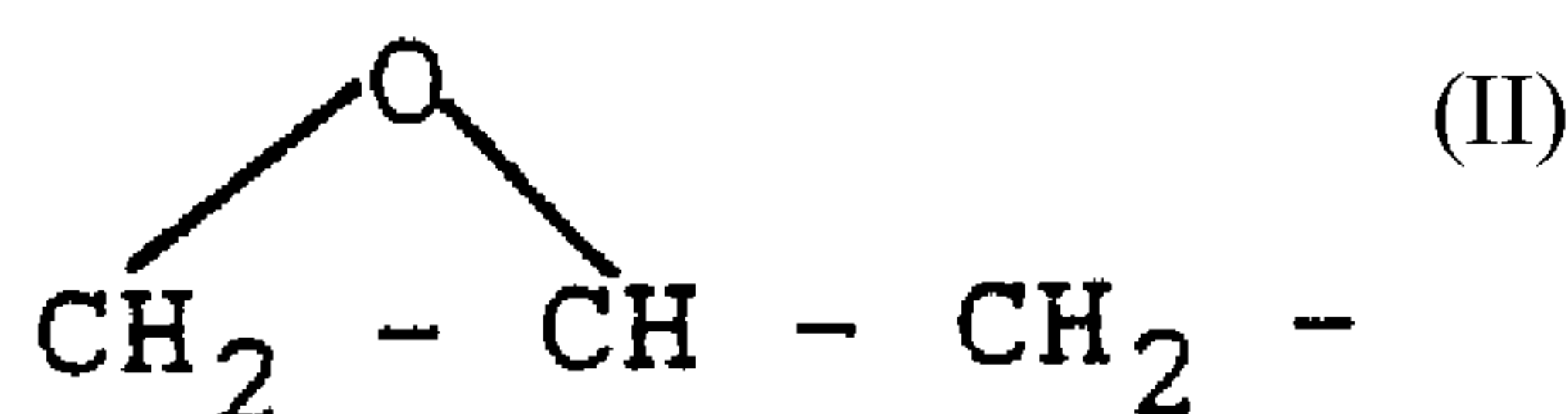
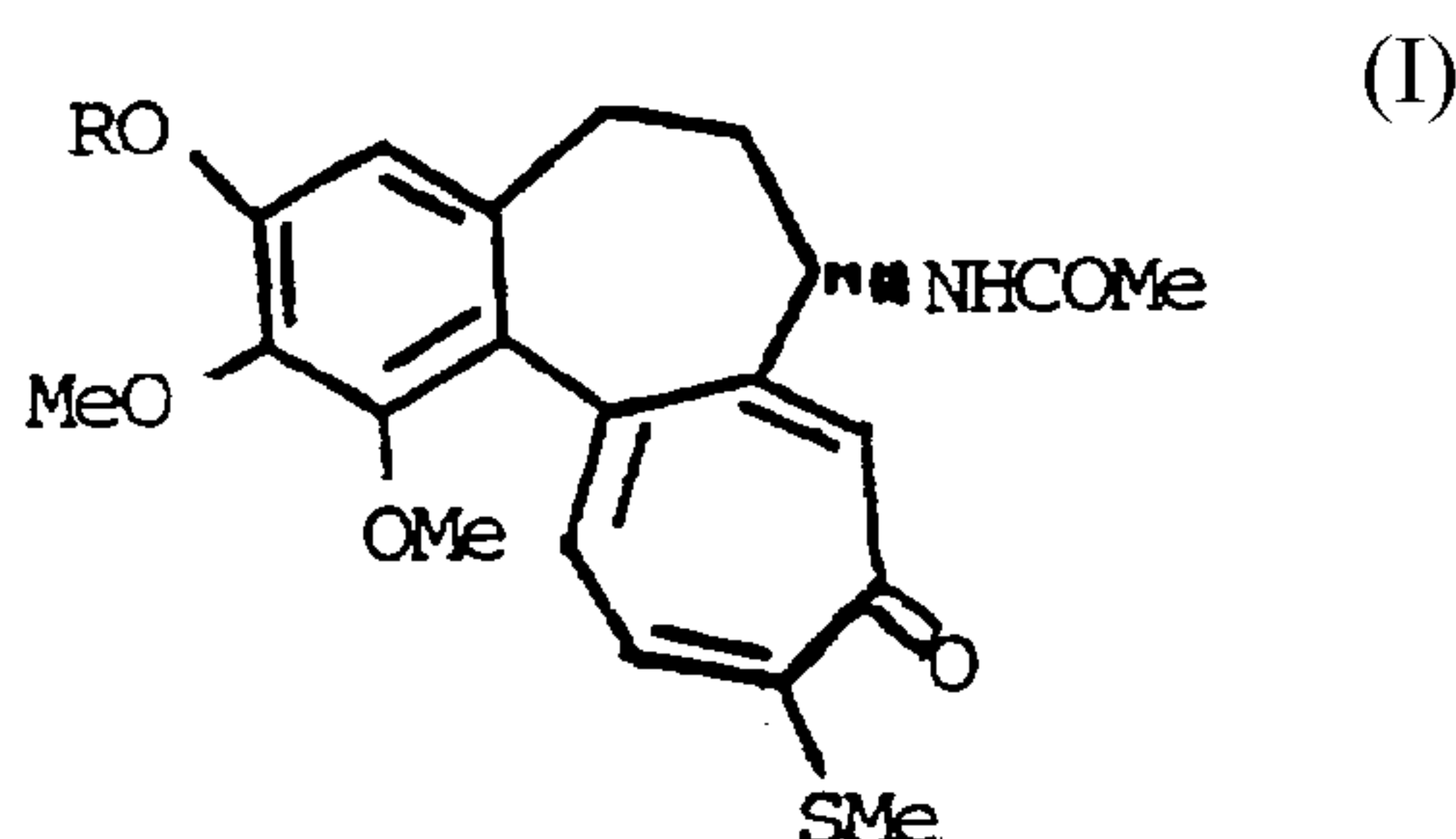
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(54) Titre : DERIVES DE COLCHICINE ET DE THI COLCHICINE EXERCANT UN EFFET MYORELAXANT ET ANTIINFLAMMATOIRE

(54) Title: COLCHICINE AND THI COLCHICINE DERIVATIVES WITH ANTIINFLAMMATORY AND MUSCLE RELAXANT ACTIVITIES



(57) Abrégé/Abstract:

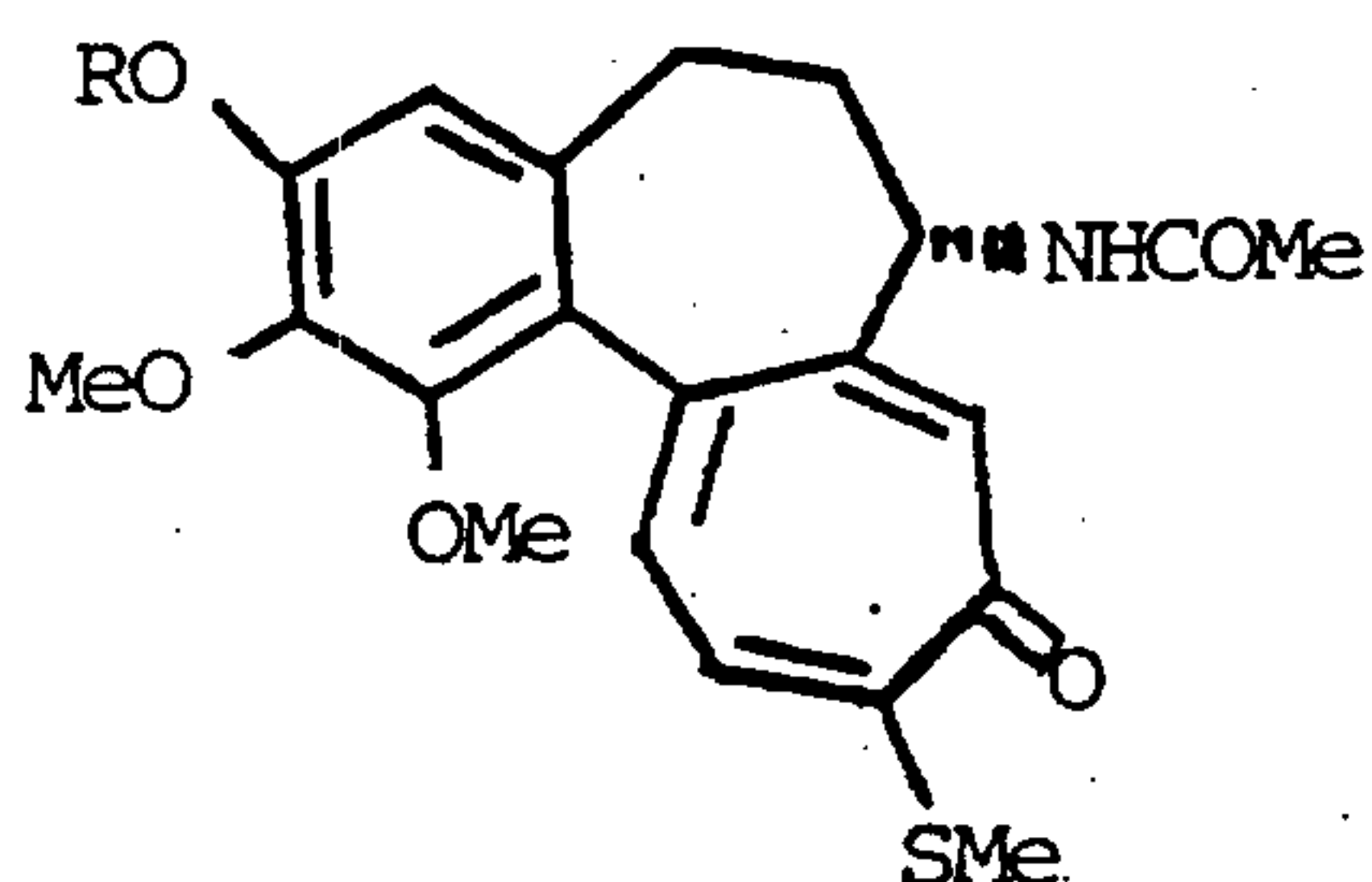
The present invention relates to 3-demethyl-thiocolchicine derivatives of general formula (I) (see formula I) in which R can be (see formula II) HOCH₂-CHOH-CH₂-, H₂NCH₂CHOHCH₂-, HOOCCH₂-, OH-CH₂-CHCl-CH₂-, to a process for the preparation thereof, to pharmaceutical compositions containing them and to the use thereof for the preparation of medicaments with muscle relaxant and antiinflammatory activities.

AbstractCOLCHICINE AND THIocolchicine DERIVATIVES WITH
ANTIINFLAMMATORY AND MUSCLE RELAXANT ACTIVITIES

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The present invention relates to 3-demethyl-thiocolchicine derivatives of general formula (I)

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(I)

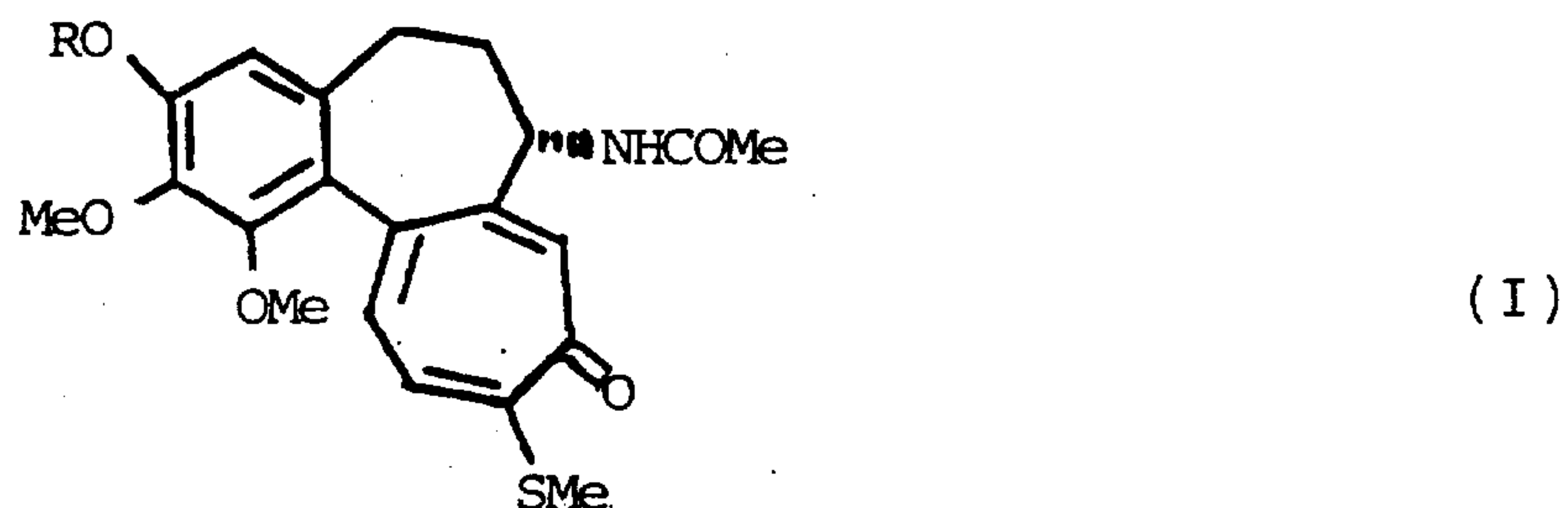
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in which R can be $\text{CH}_2 - \text{CH} - \text{CH}_2 -$, $\text{HOCH}_2 - \text{CHOH} - \text{CH}_2 -$, $\text{H}_2\text{NCH}_2\text{CHOHCH}_2 -$, $\text{HOOCCH}_2 -$, $\text{OH} - \text{CH}_2 - \text{CHCl} - \text{CH}_2 -$, to a process for the preparation thereof, to pharmaceutical compositions containing them and to the use thereof for the preparation of medicaments with muscle relaxant and antiinflammatory activities.

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COLCHICINE AND THIocolCHICINE DERIVATIVES WITH ANTI-
INFLAMMATORY AND MUSCLE RELAXANT ACTIVITIES

The present invention relates to 3-demethyl-thiocolchicine derivatives of general formula (I):



10 in which R can be $\text{CH}_2 - \text{CH} - \text{CH}_2 -$, $\text{HOCH}_2 - \text{CHOH} - \text{CH}_2 -$, $\text{H}_2\text{NCH}_2\text{CHOHCH}_2 -$, $\text{HOOCCH}_2 -$, $\text{OH} - \text{CH}_2 - \text{CHCl} - \text{CH}_2 -$, (which will be named hereinafter compound I, II, III, IV and V respectively), to a process for the preparation thereof, to pharmaceutical compositions containing them and to

15 the use thereof in the rheumathologic-orthopedic field, for the preparation of medicaments with muscle relaxant and antiinflammatory activities.

20 Muscle relaxant medicaments have the common characteristic of reducing the muscle tone, for example in muscle contractures.

Muscle contracture is a feature characterizing a number of pathologies of the locomotor apparatus, and it is one of the major factors responsible for the persistence of the painful condition related thereto.

25 Muscle contracture also occurs in the inflammatory-rheumathic and degenerative orthopedic pathologies; when affecting an articulation, it causes, in addition to pain, a stiffening which limits the mutual mobility of

the joint extremities and therefore the functionality of the part involved. Due to these reasons, there is a great interest in molecules characterized by remarkable muscle relaxant and antispastic properties.

5 Colchicine is known to be a pseudo-alkaloid used widely and for a very long time in therapy for the treatment of gout. Likewise diffused in therapy is the use of 3-demethyl-thiocolchicine glucoside, namely thiocolchicoside, as antispastic in the inflammatory
10 processes against skeletal muscles (Ortopedia e Traumatologia Oggi XII, n. 4, 1992). Recently, thiocolchicoside activity has been proved to be related to its interaction with strychnine-sensitive glycine receptors, therefore compounds having glycine-mimetic
15 activity can be used in the rheumathologic-orthopedic field thanks to their muscle relaxant characteristics.

Now it has been found that thiocolchicine derivatives of general formula (I) are capable of exerting an effective muscle relaxant action, evaluated
20 by studies both in vitro and in vivo, at remarkably more advantageous doses than those commonly used for similar known substances.

The compounds of the invention have shown in binding tests a higher affinity to glycine receptors
25 (Table I) than a structurally similar compound, i.e. thiocolchicoside.

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TABLE I

	Compound	μ M concentration	% displacement
5	thiocolchicoside	0.1	20
		0.5	45
		1	70
10	Compound I	0.1	40
		1	85
	Compound II	0.1	35
		1	65
15	Compound III	0.1	50
		1	75

The interaction with the receptors has been evaluated according to the procedure by A.B. Young and S.H. Snyder reported in Proc. Natl. Acad. Sci U.S.A. 71, 4002, 1974.

The inhibition of the polysynaptic reflexes induced by strychnine in the rabbit has been studied for the in vivo tests.

Using this model, the compounds of the invention, injected at doses of 1 mg/kg intramuscularly, were capable of reducing polysynaptic reflexes by 50% (compound I), by 60% (compound II) and by 65% (compound III), and of removing completely the potentiation of strychnine-induced reflexes at the same doses; the control molecule thiocolchiside has been used at the

minimum doses of 5 mg/kg to obtain comparable effects.

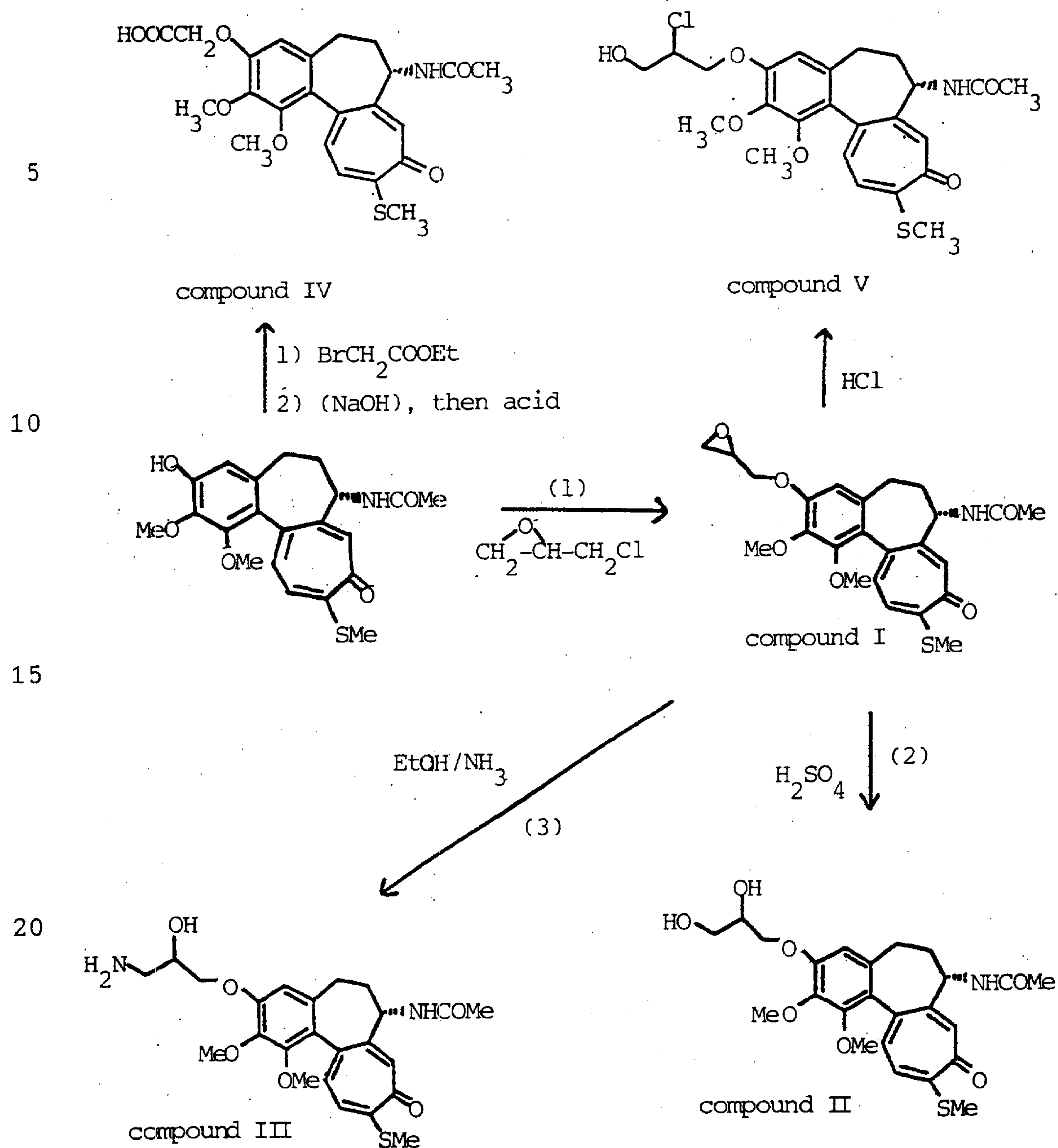
Moreover, the compounds of formula (I) have an acute toxicity significantly lower than thiocolchicoside. The DL_{50} of the compounds I-III is, in fact, higher than 30 mg/kg i.v. in the mouse, the DL_{50} of thiocolchicoside being 7.5 mg/kg.

In vitro cytotoxicity tests on cells of breast carcinoma and of other tumors proved that the compounds of the invention are not cytotoxic up to concentrations higher than 5000 nM, whereas parent thiocolchicine is cytotoxic even at a concentration of 0.6 nM.

In conclusion, the compounds of the invention are safe and therapeutically advantageous.

The compounds I-V can be prepared starting from 3-demethylthiocolchicine, according to the following general reaction scheme, using conventional reagents and synthetic procedures.

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Scheme 1

For the use in therapy, compounds I-V can be suitably formulated using pharmaceutically acceptable excipients and carriers, in forms such as capsules, tablets, granulates, suppositories, creams, injectable solutions, ointments, gels and others, more generally according to conventional techniques, such as those

described in "Remington's Pharmaceutical Sciences Handbook", Mack Publishing Company, New York, U.S.A., 17th Ed., 1985.

Therefore, the present invention further relates to pharmaceutical compositions containing a compound of general formula I for use as muscle relaxants, antispastics, antiinflammatories, antigouts, more generally in the rheumathologic-orthopedic field.

The following examples further illustrate the invention.

Example I

Synthesis of 3-demethyl-3-glycidylthiocolchicine

3-Demethylthiocolchicine (200 mg, 0.5 mmol) is suspended in CH_3CN (10 ml). The mixture is refluxed, then added with 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU, 153 μl , 1 mmol). The product solubilizes immediately, and the solution darkens. After the addition, ($\pm$)-epichlorohydrin (3 mmol, 190 μl) is added. The reaction is monitored via TLC (CCH_2Cl_2 -MeOH 9-1). After 7 hours, the starting product has reacted completely. The solvent is evaporated off and the reaction crude is purified by gravimetric chromatography on silica gel, eluting with a CH_2Cl_2 -MeOH 100-2 mixture. The resulting oily product (160 mg, 0.35 mmol, yield: 70%) is crystallized from acetone and identified on the basis of the ^1H - ^{13}C -NMR, COSY and NOESY spectra.

The formed product is a mixture of the two diastereomers (2'R, 7S, aS) and (2'S, 7S, aS).

m.p.: 241-241.5°C.

^1H -NMR: (CDCl_3) 7.84-7.79 (m, 1H), 7.42 (s, 1H), 7.31 (d, 1H, J 10.3), 7.08 (d, 1H, J 10.3), 6.57, 6.56 (2s,

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1H), 4.66-4.61 (m, 1H), 4.37-4.28 (m, 1H), 4.10-3.98 (m, 1H), 3.94 (s, 3H), 3.65 (s, 3H), 3.44-3.37 (m, 1H), 2.96-2.91 (m, 1H), 2.82-2.76 (m, 1H), 2.43 (s, 3H), 2.48-1.85 (m, 4H), 1.97 (s, 3H).

5 ¹³C-NMR: (CDCl₃) 162.5, 170.2, 158.4, 152.7, 152.0, 151.4, 142.3, 138.6, 135.0, 134.5, 128.4, 126.8, 126.7, 109.6, 109.5, 70.4, 70.1, 61.7, 61.5, 52.4, 50.3, 44.7, 36.4, 29.9, 22.9, 15.2.

Example II

10 Synthesis of 3-demethyl-3-(2,3-dihydroxypropyl)thiocolchicine

3-Demethyl-3-glycidylthiocolchicine (300 mg, 0.67 mmol) is dissolved in a dioxane-H₂O (1-1.5 ml) mixture and treated with a catalytic amount of 0.2 N H₂SO₄, then
15 heated to reflux. The reaction is monitored by TLC (CH₂Cl₂-MeOH 9-1). After 5 hours, the solvent is evaporated off and the reaction crude is purified by gravimetric chromatography on silica gel, eluting with a CH₂Cl₂-MeOH 100-3 mixture. The desired product
20 (identified on the basis of its spectroscopic properties: ¹H-¹³C-NMR and COSY) is obtained in a 73% yield (228 mg, 0.48 mmol) as a mixture of the two diastereomers (2'R, 7S, aS) and (2'S, 7S, aS).

m.p.: 149-150°C, dec.

25 ¹H-NMR: (CDCl₃) 7.28 (d, 1H, J 9.8), 7.26 (s, 1H), 7.06 (d, 1H, J 9.8), 6.58 (s, 1H), 6.48 (d, 1H, J 8.5), 4.71-4.60 (m, 1H), 4.20-4.11 (m, 4H), 3.94 (s, 3H), 3.85-3.82 (m, 1H), 3.65 (s, 3H), 2.60-1.92 (m, 4H), 2.44 (s, 3H), 1.99 (s, 3H).

30 ¹³C-NMR: (CDCl₃) 182.5, 170.0, 158.5, 152.6, 151.5, 142.2, 138.2, 134.8, 134.7, 128.4, 126.8, 126.7, 109.6,

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92.5, 71.7, 70.2, 63.8, 61.7, 52.3, 36.6, 29.9, 23.0, 15.3.

Example III

Synthesis of 3-demethyl-3-(3-amino-2-hydroxypropyl)thio- 5 colchicine

3-Demethyl-3-glycidylthiocolchicine (300 mg, 0.67 mmol) is dissolved in ammonia-saturated EtOH and heated to 60°C. After 1 hour the reaction is completed, and the reaction solvent is evaporated off to give the desired
10 product in a pure state, in an 83% yield (261 mg, 0.55 mmol), as a mixture of the two diastereomers (2'R, 7S, aS) and (2'S, 7S, aS). The product is identified on the basis of the its spectroscopical properties: ¹H-NMR.
m.p.: 144.8-145.5°C, dec.

15 ¹H-NMR: (CDCl₃) 7.28 (d, 1H, J 10.6), 7.26 (s, 1H), 7.06 (d, 1H, J 10.6), 4.72-4.58 (m, 1H), 4.12-3.90 (m, 4H), 3.94 (s, 3H), 3.65 (s, 3H), 3.05-1.5 (m, 5H), 2.44 (s, 3H), 1.99 (s, 3H).

Example IV

20 Synthesis of 2-(3-demethylthiocolchicine)acetic acid

3-Demethylthiocolchicine (401 mg, 1 mmol) is suspended in dry CH₃CN (10 ml) at room temperature. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (192 ml, 1.3 mmol) is added dropwise: the mixture solubilizes and darkens.
25 After the addition, ethyl bromoacetate (161 ml, 1.3 mmol) is added, the solution thereby slowly lightening. After about 2 hours, a further 60 ml of DBU and 70 ml of the ester are added. The reaction mixture is left at room temperature for 10 hours. TLC: CH₂Cl₂-MeOH=9/1.

30 The solvent is evaporated off under reduced pressure and the resulting crude is purified by

gravimetric chromatography with a polarity gradient, eluting with the CH_2Cl_2 -MeOH mixture. The desired ester (410 mg) is obtained in an 84% yield. The product is identified on the basis of its spectroscopical properties.

m.p.: 115°C

^1H NMR (CDCl_3): 1.31 (t, J 7.1, 3H, Me), 1.97 (s, 3H, MeCO), 1.8-2.5 (m, 4H, H-5, H-6), 2.43 (s, 3H, SMe), 3.66, 3.98 (two s, 6H, OMe), 4.25 (q, J 7.1, 2H, OCH_2Me), 4.58-4.70 (m, 1H, H-7), 4.72 (s, 2H, OCH_2), 6.46 (s, 1H, H-4), 7.08 (d, J 10.6, 1H, H-11), 7.29 (d, J 10.6, 1H, H-12), 7.27 (s, 1H, H-8), 7.85 (d, J 6.9, 1H, NH).

NaOH pellets (32 mg, 0.8 mmol) are dissolved in 5% aqueous EtOH (10 ml). 2-(3-Demethylthiocolchicine)ethyl acetate (300 mg, 0.62 mmol) is added, and the reaction is left at room temperature under magnetic stirring. After 1 hour, (TLC: CH_2Cl_2 -MeOH = 9/1), the solvent is evaporated off and the residue is dissolved in a HCl diluted aqueous solution. A yellow product precipitates, which is further purified by chromatography on silica gel, eluting with the CH_2Cl_2 -MeOH 9-1 mixture. 2-(3-Demethylthiocolchicine)acetic acid (260 mg) is obtained in a 92% yield.

m.p.: 189-190 dec.°C (acetone)

^1H NMR (CDCl_3): 1.95 (s, 3H, MeCO), 1.75-2.58 (m, 4H, H-5, H-6), 2.44 (s, 3H, SMe), 3.03 (s, 1H, COOH) 3.64, 3.97 (two s, 6H, OMe), 4.51-4.70 (m, 1H, H-7), 4.73 (s, 2H, OCH_2), 6.61 (s, 1H, H-4), 7.12 (d, J 10.7, 1H, H-11), 7.31 (d, J 10.7, 1H, H-12 and NH), 7.50 (s, 1H, H-8).

3-Demethyl-3-(2-chloro-3-hydroxypropyl)thiocolchicine has been obtained following a similar procedure to

10

that of the examples above.

m.p.: 118-119 dec. (acetone *i*-Pr₂O)

¹H NMR (CDCl₃): 1.99 (s, 3H, MeCO), 1.75-2.58 (m, 4H, H-5, H-6), 2.44 (s, 3H, SMe), 3.07 (t, 1H, OH, deuterable), 3.66, 3.94 (two s, 6H, OMe), 3.77-3.87, 4.15-4.32 (two m, 2+3H, CH₂CHCH₂), 4.57-4.70 (m, 1H, H-7), 6.58 (s, 1H, H-4), 7.14 (d, J10.6, 1H, H-11), 7.29 (d, J10.6, H-12), 7.35 (m, 1H, NH), 7.37 (s, 1H, H-8).

Example V

10 Example of formulation of the compounds of formula (I) in the form of vials.

Vials

Compound II	5	mg
Sodium chloride	15.8	mg
15 Water for injectable preparations	q.s. to	2 ml

Example VI

Example of formulation of the compounds of formula (I) in the form of capsules.

Hard gelatin capsules

20 Compound II	10	mg
Lactose	212.3	mg
Starch	1.3	mg
Magnesium stearate	2.4	mg

Example VII

25 Example of formulation of the compounds of formula (I) in the form of cream.

Cream

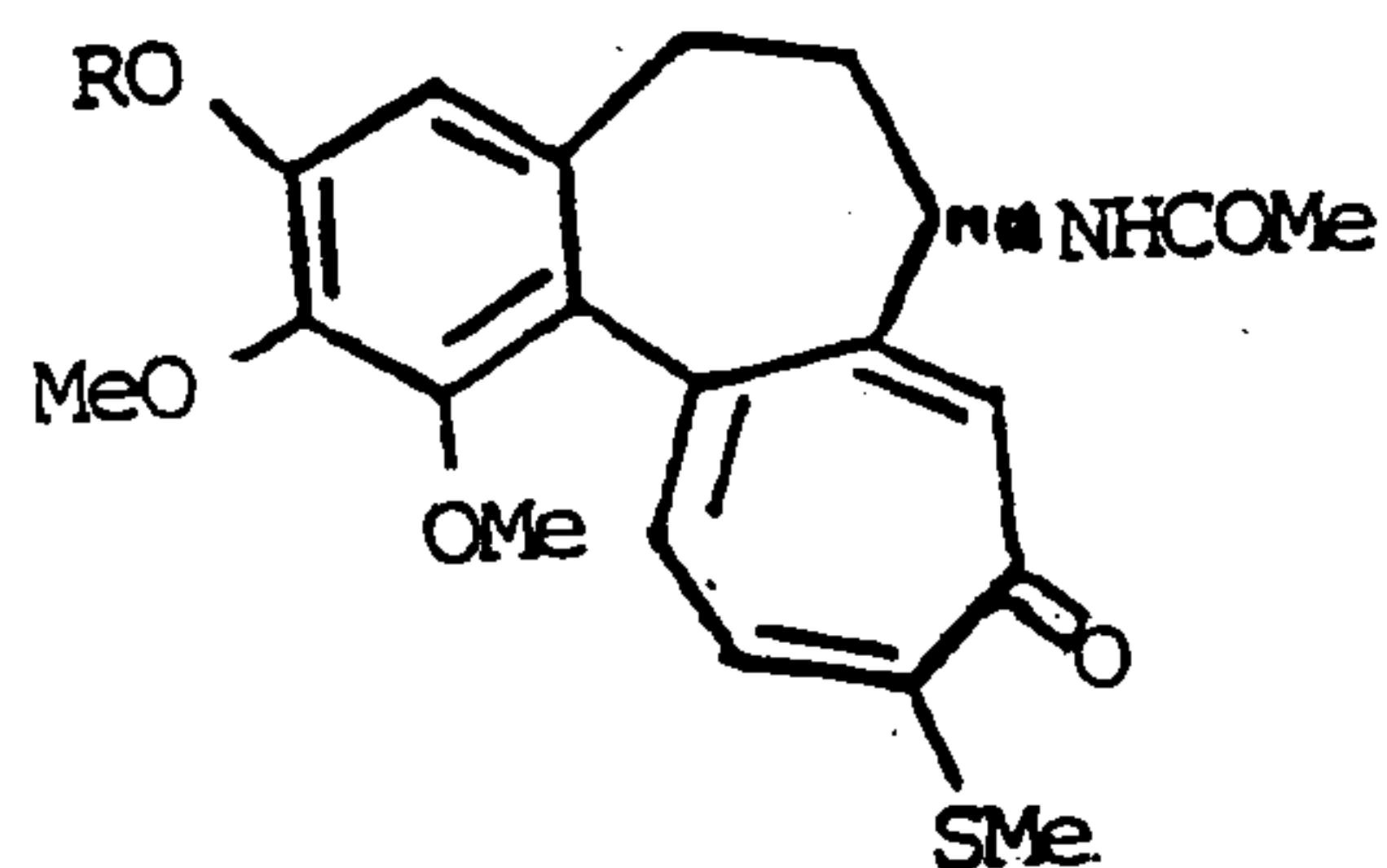
Compound II	0.5	g
Methyl p-hydroxybenzoate	0.14	g
30 Ethyl p-hydroxybenzoate	0.035	g
Polyoxyethylene-20-sorbitan monooleate	5	g

		11		
	Sodium lauryl sulfate		2	g
	Spermaceti		5	g
	Cetyl alcohol		7	g
	Hydrogenated lanolin		12.5	g
5	Stearic acid		8	g
	Sodium alginate		0.5	g
	Lavender oil		1	g
	Depurated water	q.s. to	100	g.

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CLAIMS

1. Compounds of general formula (I)



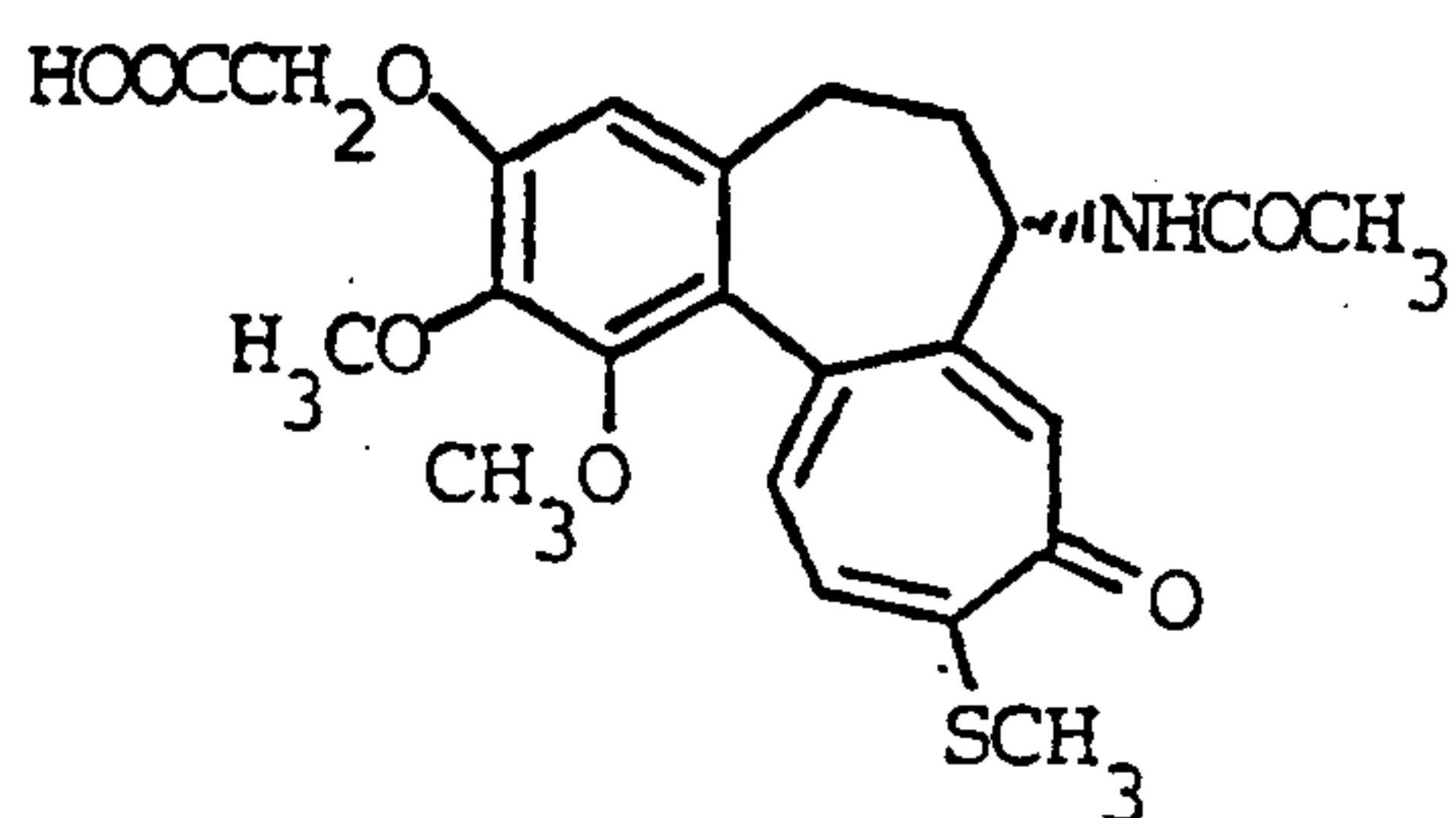
(I)

in which R represents $\text{CH}_2 - \text{CH} - \text{CH}_2 -$, $\text{HOCH}_2 - \text{CHOH} - \text{CH}_2 -$, $\text{H}_2\text{NCH}_2\text{CHOHCH}_2 -$, $\text{HOOCCH}_2 -$, $\text{OH} - \text{CH}_2 - \text{CHCl} - \text{CH}_2 -$.

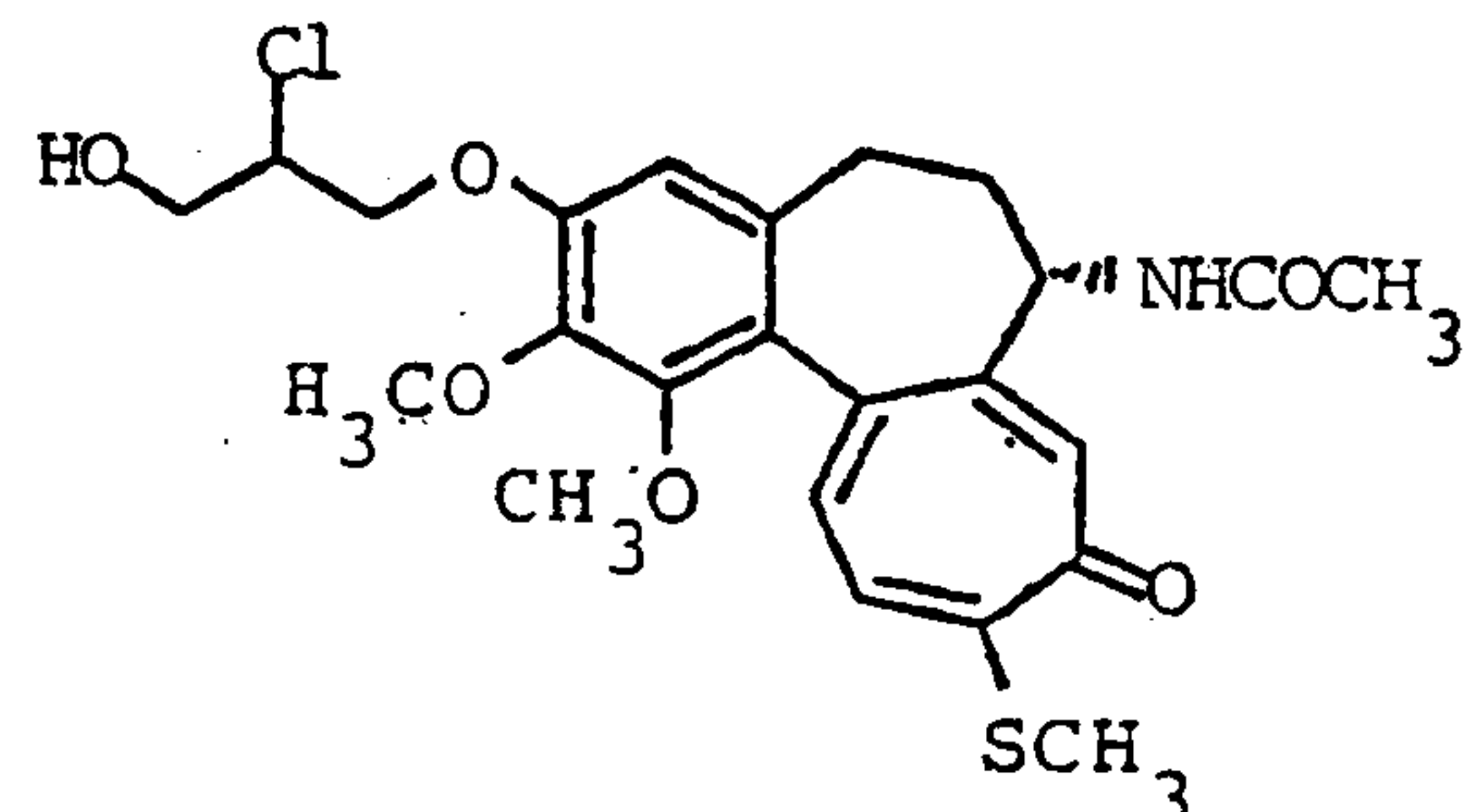
2. A process for the preparation of the compounds of claim 1, according to the following scheme 1:

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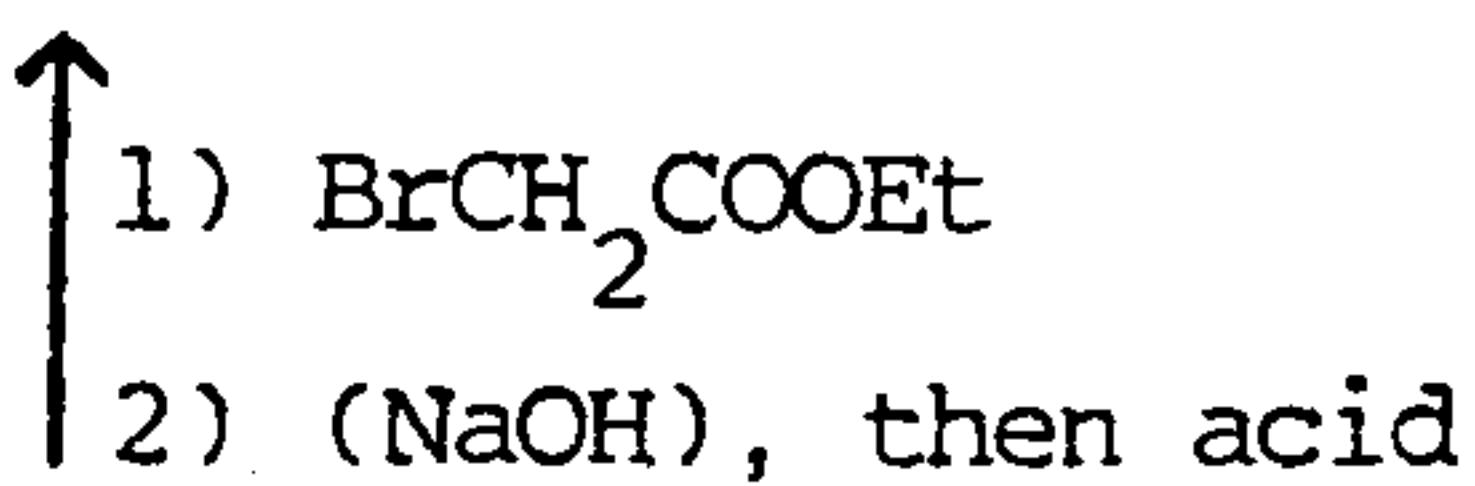
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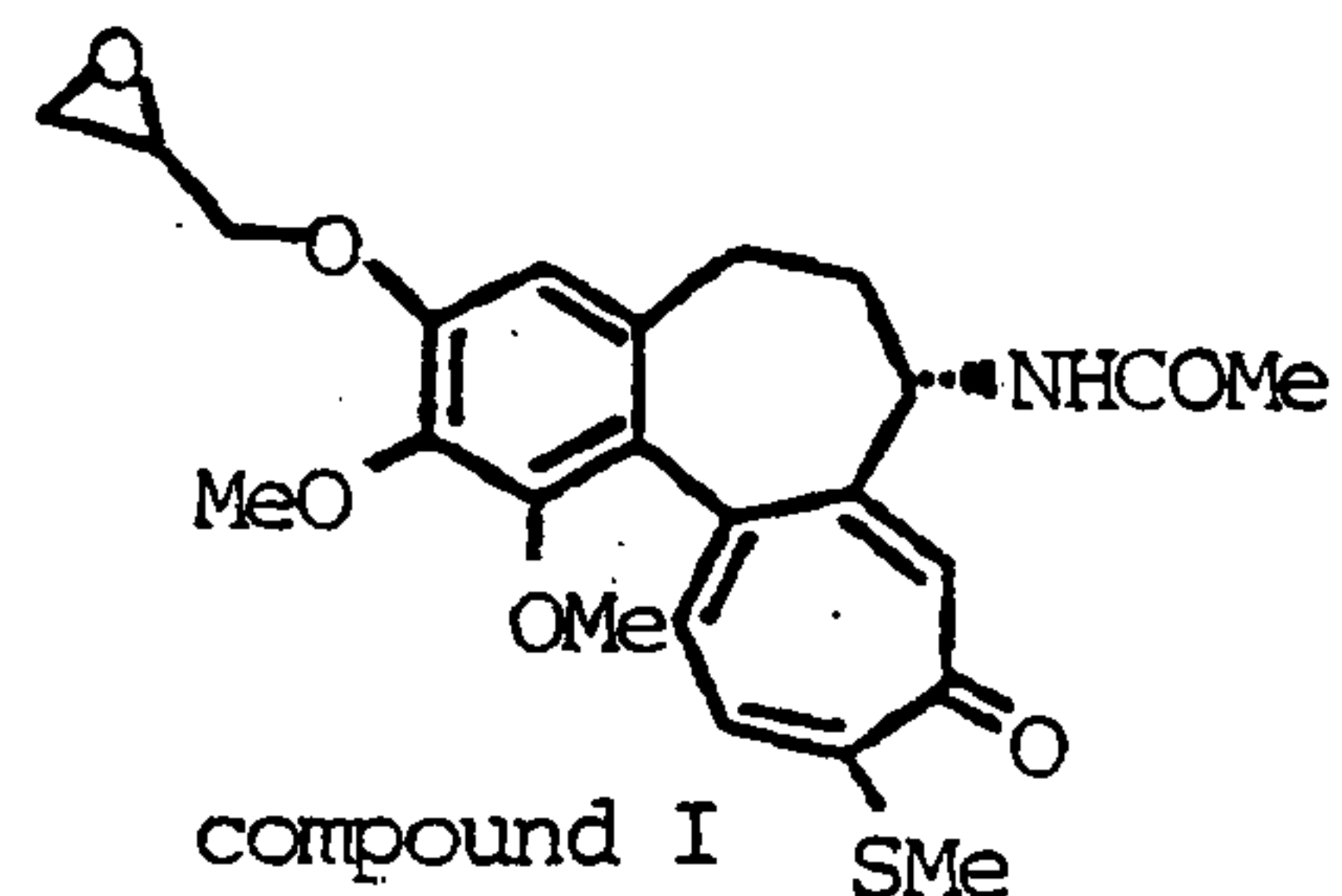
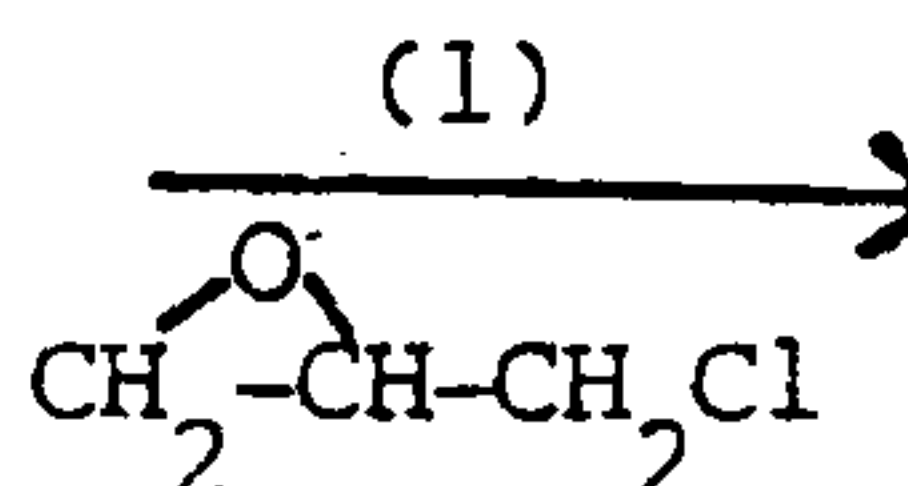
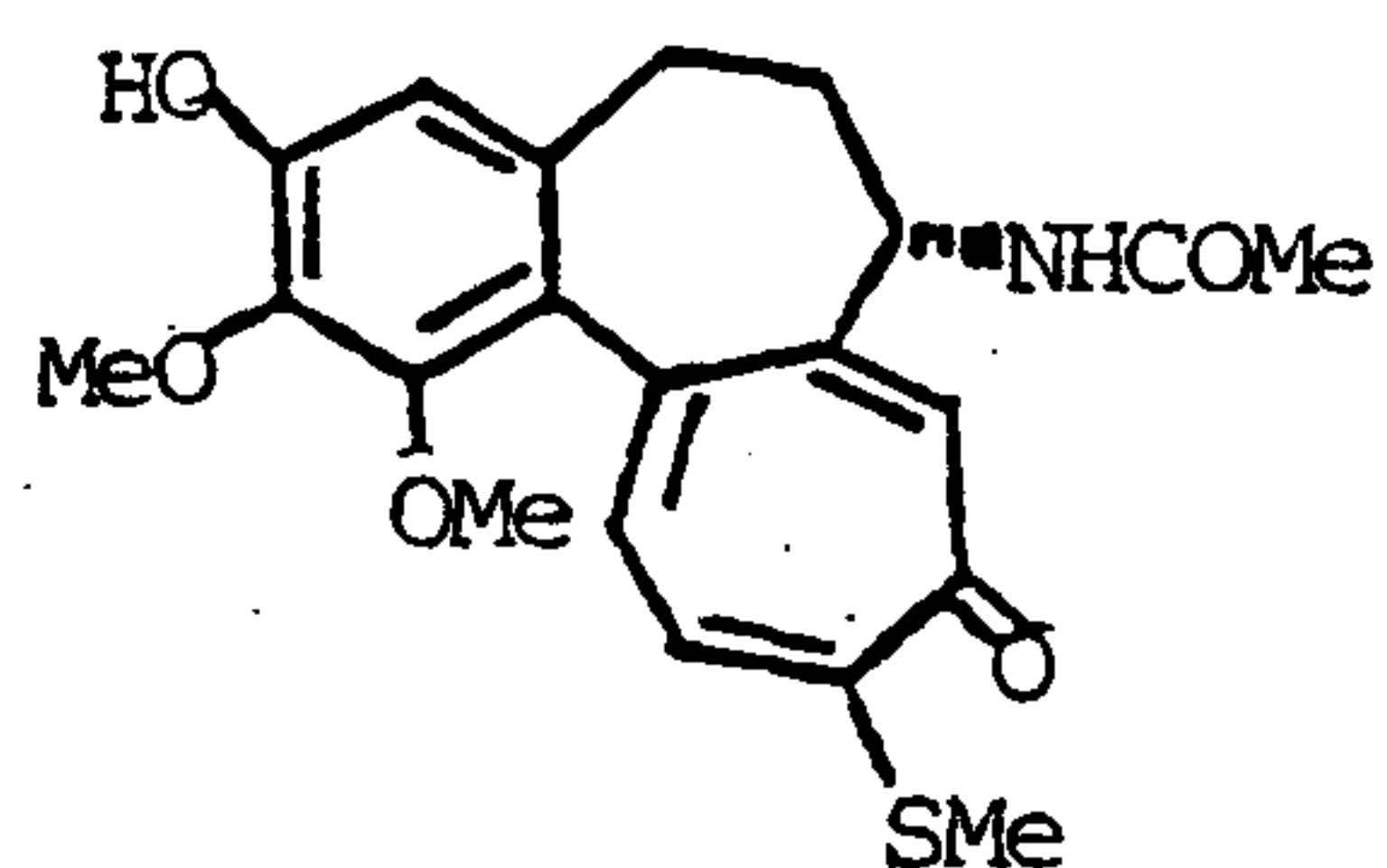
compound IV



compound V

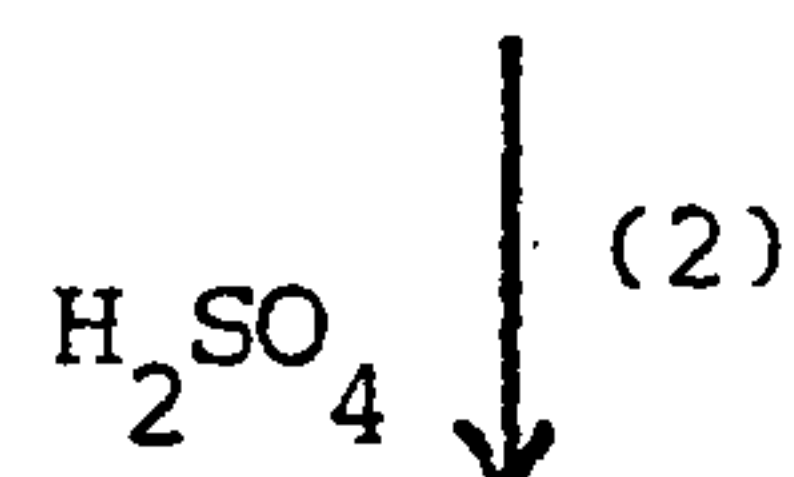
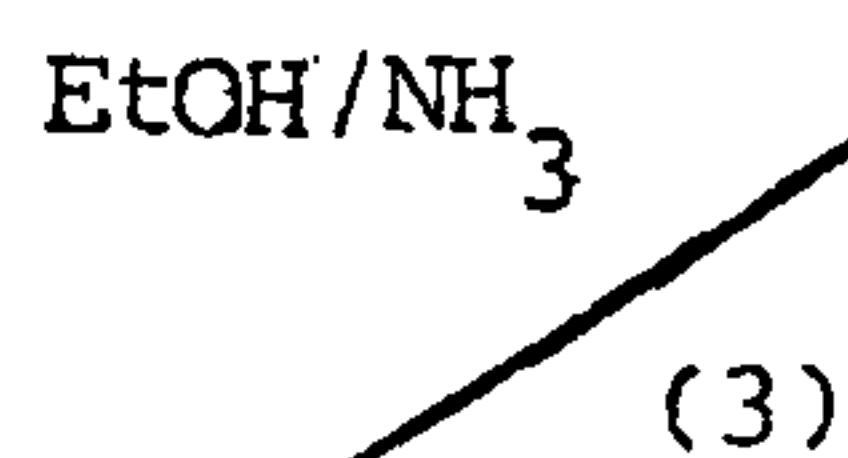


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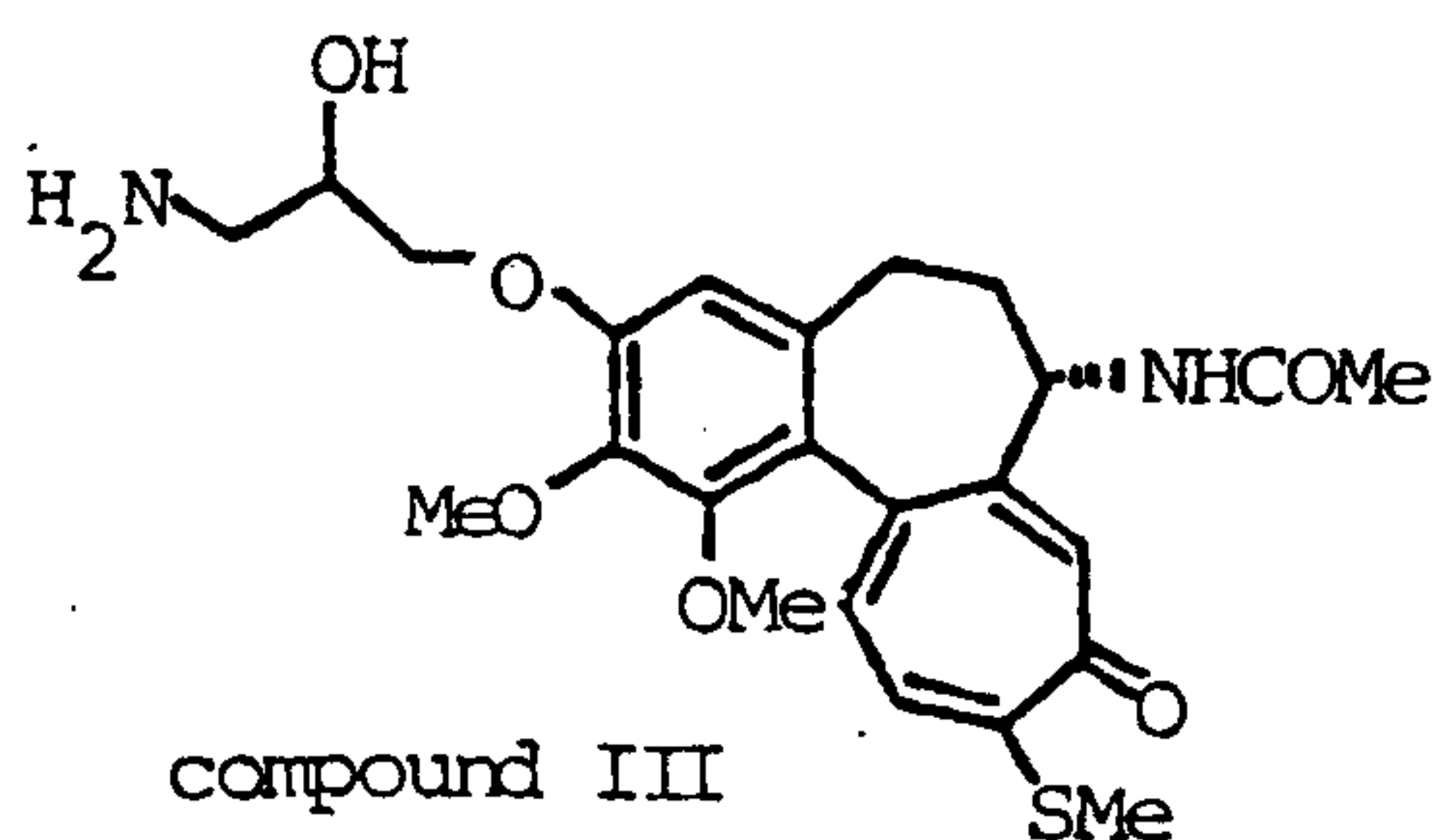


compound I

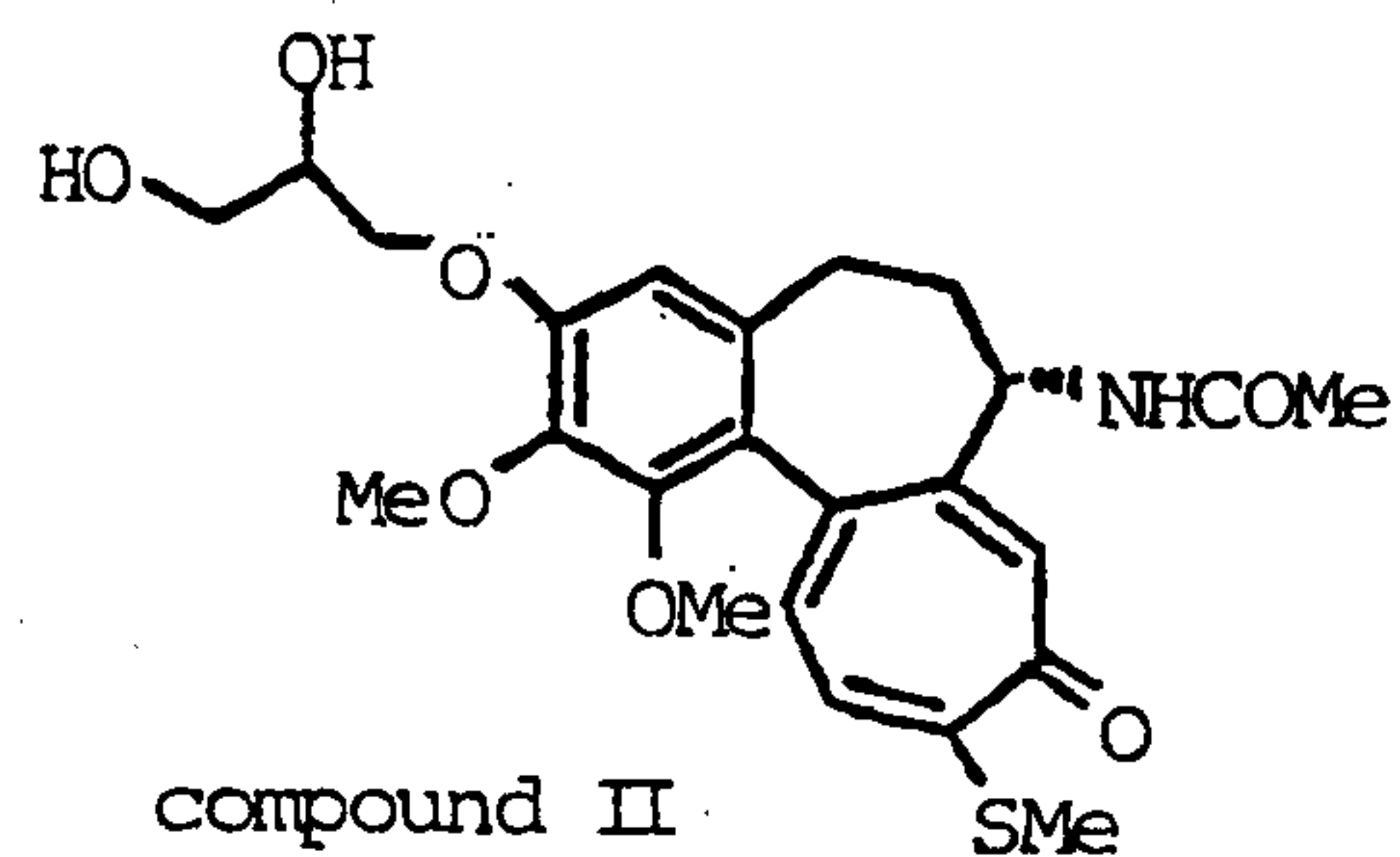
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compound III



compound II

25 3. The use of the compounds of claim 1 for the preparation of medicaments for use as muscle relaxants, antiinflammatories, antispastics or antigouts.

4. Pharmaceutical compositions containing a compound of claim 1 as active ingredient, in admixture with
30 pharmaceutically acceptable carriers and excipients.

5. Pharmaceutical compositions according to claim 4 in

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the form of capsules, tablets, granulates, suppositories, creams, injectable solutions, ointments and gels.

