Brief Articles

N-Deacetyl-*N*-aminoacylthiocolchicine Derivatives: Synthesis and Biological Evaluation on MDR-Positive and MDR-Negative Human Cancer Cell Lines

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A new series of *N*-deacetyl-*N*-(*N*-trifluoroacetylaminoacyl)thiocolchicine derivatives **9**−**15** have been synthesized starting from the corresponding *N*-deacetylthiocolchicine (**3**) and the *N*-trifluoroacetylamino acids **5**−**8** which were used as a racemic mixture. The trifluoroacetyl protecting group has been removed easily, giving the corresponding *N*-deacetyl-*N*-aminoacylthiocolchicines **16**−**22**. Optical pure compounds **9**−**22** were isolated from the diastereoisomeric mixture or were prepared starting from compound **3** and an optical pure amino acid derivative; the configuration of each compound was assigned unequivocally. The diastereoisomeric couples of amino acids synthesized were tested, and their antiproliferative activity on MDR-positive and MDR-negative human cancer cell lines was evaluated.

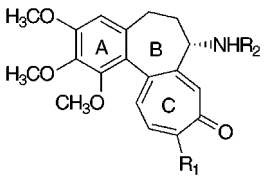
Introduction

Colchicine (**1**) (Chart 1), a major alkaloid extracted from the seed of *Colchicum autumnale* and *Gloriosa superba*, is a drug interfering with microtubule assembly both in vivo and in vitro, thereby causing cells to accumulate in mitotic arrest during the cell cycle.[1,2] Although the antitumor properties of many colchicinoids have been well-known for a long time, only one product, *N*-deacetyl-*N*-methylcolchicine (Colcemid), has been used for the treatment of Hodgkin's lymphoma and chronic granulocitic leukemia.[3] Its in vivo efficacy against melanoma[4] and prostatic cancer[5] has been established; its use in the treatment of neoplasms has been limited and sporadic. The lack of clinical interest in the colchicines seemingly arises from their extreme toxicity. Until now, the *Vinca rosea* alkaloids have been found superior in the treatment of advanced lymphomas and Hodgkin's disease. Furthermore, a major limitation in the treatment of cancer is the development of multidrug resistance (MDR); colchicine is known to be effluxed in cell lines expressing the classical MDR phenotype.[6]

Therefore, many attempts have been made to discover more effective and less toxic analogues of colchicine by modifying the substituents of its basic structure. Thiocolchicine (**2**)[7] (Chart 1), which has higher affinity for tubuline than colchicine along with a higher stability, was chosen as a prototype for the synthesis of new compounds.

In previous investigations[8] we noticed that a polar substituent on ring A of thiocolchicine drastically reduced the cytotoxicity on the MDR phenotype. The influence of the substituents on the amino group was unclear: unsubstituted derivatives were particularly active on sensitive MDR cell lines, whereas the simple methylation of the amino group produced a decrease of cytotoxicity.

Chart 1



1: $R_1 = OCH_3$; $R_2 = COCH_3$
2: $R_1 = SCH_3$; $R_2 = COCH_3$
3: $R_1 = SCH_3$; $R_2 = H$
4: $R_1 = SCH_3$; $R_2 = COCF_3$

To modulate toxicity and bioavailability, a large number of thiocolchicine analogues has been synthesized in which the acetamido group at C-7 has been varied considerably.[3,9−16] It is known that the binding of colchicinoids derivatives to tubulines depends on the configuration of the nonplanar biaryl system and compounds with an *aS* configuration bind readily to them. The bulkiness of the side chain at C-7 could also affect the configuration of the colchicinoids.

On the basis of this information, we have synthesized a new series of tiocolchicines **9**−**15** and **16**−**22** functionalized at the nitrogen atom with amino acid residues. Only a few colchicinoids with a polar chain at C-7

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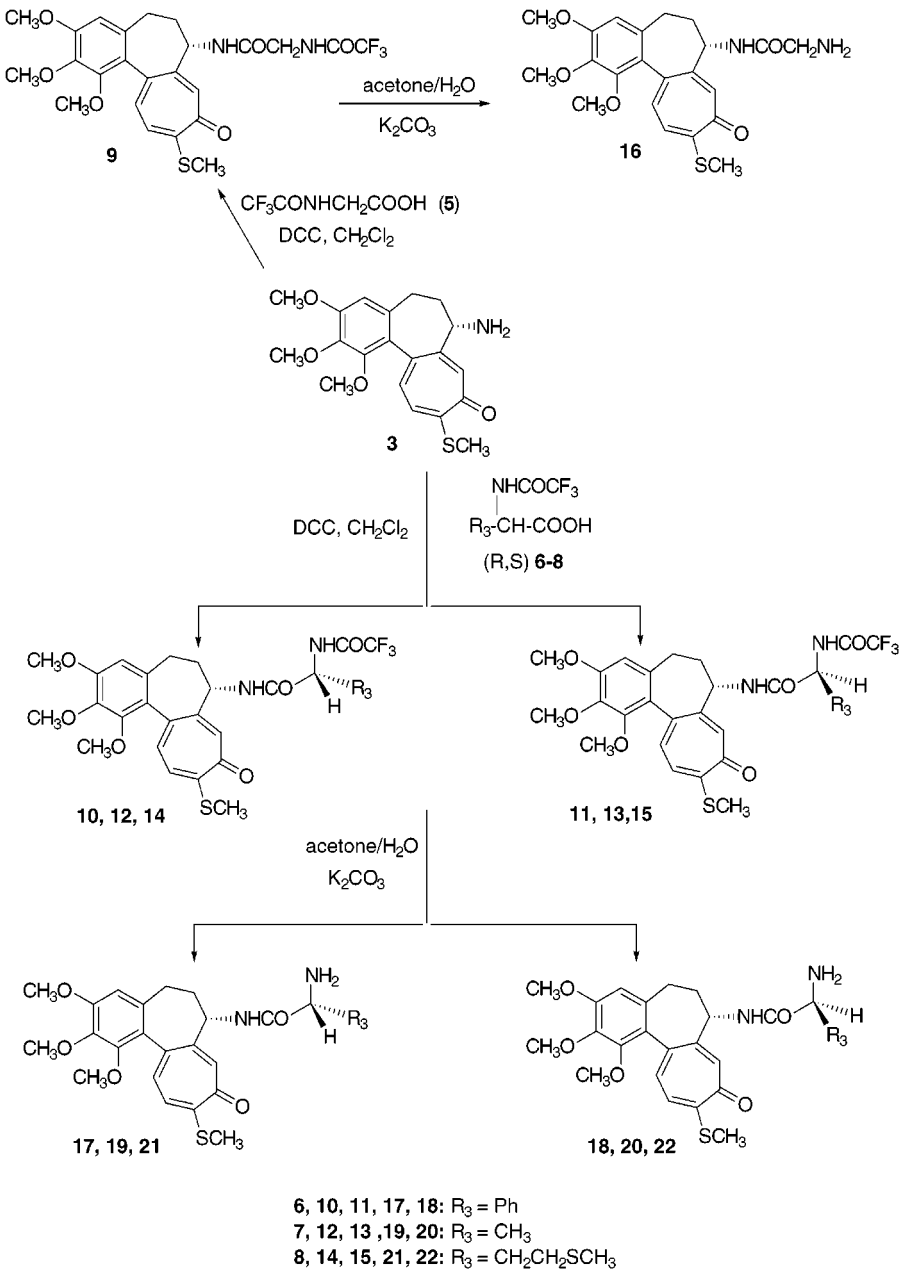
Scheme 1



have been prepared, and the side chains at C-7 do not contain a chiral center. The functionalization with an amino acidic residue offers the possibility to evaluate (i) the relationship between activity and the bulkiness of the group at C-α and/or (ii) the effect of the presence of a chiral center.

The choice of the trifluoroacetyl group as the *N*-protecting group in the amino acid moiety (compounds **9**−**15**) was made considering the activity enhancement in *N*-perfluoroacylthiocolchicines.[8] When racemic trifluoroacetylamino acids were used, couples of diastereoisomeric derivatives were obtained; the configuration of the single epimers was assigned unequivocally.

Chemistry

(−)-*N*-Deacetylthiocolchicine (**3**), which was prepared by an established procedure,[15] was the starting material for the preparation of a series of *N*-deacetyl-*N*-(*N*-trifluoroacetylaminoacyl)thiocolchicines **9**−**15** by condensation with *N*-protected amino acids. *N*-Trifluoroacetylglycine (**5**), (*R*,*S*)-*N*-trifluoroacetylphenylglycine (**6**), (*R*,*S*)-*N*-trifluoroacetylalanine (**7**), and (*R*,*S*)-*N*-trifluoroacetylmethionine (**8**) were prepared from the corresponding α-amino acids by protection at the nitrogen atom with the trifluoroacetyl group,[17] which can be removed in very mild conditions.

In a typical procedure, compound **3** was reacted with *N*-trifluoroacetylglycine (**5**) using dicyclohexylcarbodiimide (DCC) as condensing agent. This method resulted in a good yield of the expected compound **9** (90%).

The trifluoroacetyl group of compound **9** was selectively removed by hydrolysis with potassium carbonate in aqueous acetone at 60 °C, giving *N*-deacetyl-*N*-glycylthiocolchicine (**16**) (Scheme 1).

Condensation of **3** with α-amino acid derivatives **6**−**8** afforded the mixture of the corresponding diastereoisomeric *N*-acyl derivatives (**10**/**11**, **12**/**13**, **14**/**15** in a 1:1 ratio (crude reaction mixture, ^{1}H NMR). Compounds **10**

and **11** could not be separated by chromatography. Instead, a satisfactory separation was achieved in the case of both mixtures **12**/**13** and **14**/**15**.

Unseparated diastereoisomeric mixtures were subjected to hydrolysis in the same reaction conditions as reported above for compound **9** to yield the corresponding mixtures of the *N*-deacetyl-*N*-aminoacylthiocolchicine derivatives, epimeric at the amino acidic carbon (**17**/**18**; **19**/**20**; **21**/**22**) (Scheme 1). Only compounds **17** and **18** could be separated by column chromatography, whereas we failed to separate the pairs **19**/**20** and **21**/**22**. Pure compounds **19**−**22** were obtained by hydrolysis of the corresponding derivatives **12**−**15**, respectively, purified as above.

Correct assignment of the absolute configuration to the isolated epimeric compounds was impossible on the basis of spectroscopic data only. However, this was done unequivocally through the synthesis of pure compounds **10**, **12**, and **14** by condensation of **3** with (*R*)-*N*-trifluoroacetylamino acids **6**−**8**. Compound **11** was prepared in pure form by reacting **3** with (*S*)-*N*-trifluoroacetyl-*N*-phenylglycine (**6**). The hydrolysis of **10**, **12**, and **14** produced the corresponding amino acid derivatives **17**, **19**, and **21**.

Under the reported conditions, neither the condensation and the hydrolysis reaction affected the amino acidic stereocenter.

All compounds (**9**−**22**) were characterized by analytical (mp, $[\alpha]^{20}_D$, and MS) and spectroscopical (IR and ^{1}H NMR) data.

As shown, these compounds have a large negative optical rotation common to most colchicinoids, and this parameter is evidence of their *aS* biaryl configuration. Furthermore, also the ^{1}H NMR spectra are in fair agreement because the chemical shifts of H-11 (7.00−7.14 ppm) and H-12 (7.16−7.37 ppm), which are significant in providing information about the conformation, are in the same range of known *aS* compounds.[12,14]

It is worth noting that no thiocolchicine derivatives bearing a chiral chain at C-7 have previously been prepared in both configurations. Furthermore, the chirality of the chain does not affect the stability of the *aS* configuration which is maintained in both diastereoisomers.

Biological Results

The thiocolchicine derivatives **9**−**15** and **16**−**22**, as diastereoisomeric mixtures (1:1), and pure diastereoisomeric compounds **19** and **20** were tested in vitro for their antitumor activity and their impact on the cell cycle using two human breast cancer cell lines (MDR-negative MDA-MB 231 and MDR-positive MCF-7 ADRr), comparing their activity with colchicine (**1**), thiocolchicine (**2**), *N*-deacetylthiocolchicine (**3**), and *N*-trifluoroacetyl derivative **4** by the method previously described.[8] Results were expressed as percentages of control, and the concentration of test agent which results in 50% inhibition of growth (IC_{50}) was calculated. (Table 1)

Pure diastereoisomers **19** and **20** and their 1:1 mixture were tested for their ability to inhibit cell growth. As shown in Table 1, the IC_{50} values between the single diastereoisomers **19** and **20** were similar in both cell lines whereas a significant difference in the antiproliferative activity was observed between the 1:1 mixture and single stereoisomers.

Table 1. Tumor Cell Growth Inhibition by Thiocolchicine Analogues after 72 h of Treatment

	IC_{50} (nM)[a]	
compd	MDA-MB 231	MCF-7 ADRr
1	1.8	12 000
2	0.6	400
3	2.2	4 200
4	1.3	35
9	6	12 000
10/11	24	1 500
12/13	12	2 100
14/15	37	10 000
16	7.6	10 000
17/18	3	1 700
19	150	12 000
20	90	10 000
19/20	19	800
21/22	35	20 000

[a] For IC_{50} determination, all experiments were performed in triplicate samples replicated three times. Data obtained were inserted in a nonlinear model of the sigmoid representing the theoretical dose−response curve (Statistica 5.0 package). Pharmacological methods are reported.[8]

The increase in antiproliferative activity of the mixture is probably due to an alteration in the kinetics of tubulin−colchicinoids interaction or in the binding capacity of colchicinoids, with the increase in the number of binding sites on tubuline.

On the basis of these preliminary data, we decided to test the effect of diastereoisomeric mixtures on the proliferation of MDA-MB 231 and MCF-7 ADRr cells.

It is to be noted that the activity of these thiocolchicine derivatives is very different on the two cancer cell lines; these results are not surprising considering that colchicine is known to be effluxed in cells expressing the classical MDR phenotype[6] and that many colchicinoids are known to show different activities in MDR-positive and MDR-negative cell lines.[5]

Thiocolchicine (**2**) was the most active agent on the MDA-MB 231 cells in terms of growth inhibition (IC_{50} = 0.6 nM), followed in decreasing order by *N*-trifluoroacetyl derivative **4**, colchicine (**1**), *N*-deacetyl derivative **3**, and epimers **17/18** (IC_{50} = 1.3, 1.8, 2.2, and 3 nM, respectively) (Table 1).

On MCF-7 ADRr cells *N*-trifluoroacetylaminothiocolchicine (**4**) was the most active compound (IC_{50} = 35 nM) followed in decreasing order by thiocolchicine (**2**), epimers **19/20**, **10/11**, **17/18** (IC_{50} = 400, 800, 1500, and 1700 nM, respectively) (Table 1). Interestingly the IC_{50} value of *N*-trifluoroacetylaminothiocolchicine (**4**) is about 11-fold lower than that of **2**.

From the present results it appears quite evident that, at least in vitro, the functionalization of the amino group at C-7 with polar substituents, such as amino acids, modifies the antiproliferative activity on both sensitive and resistant cell lines.

The following conclusions on the structural changes that might influence antiproliferative activity can be drawn:

(i) The methionine derivatives (**14/15**, **21/22**), which are characterized by a long chain, were less active in both cell lines; these data confirmed literature results observed for amide colchicinoids with a long chain at C-7.[14]

(ii) The α-phenylglycinyl (**10/11**, **17/18**) and alanyl derivatives (**12/13**, **19/20**) have a moderate effect on the MCF-7 ADRr cells; from these results it can be deduced that the presence of a substituent on C-α has a positive effect, enhancing the inhibition of the proliferation with respect to the α-unsubstituted ones (compounds **9** and **16**).

(iii) The introduction of a trifluoroacetyl group (compound **4**) increases activity in both breast cancer cell lines, but considering our results we can conclude that when the trifluoroacetyl group is linked to the nitrogen atom of an amino acid residue this does not increase the activity (compounds **9**−**15**).

The main mechanism of drug resistance for colchicine is related to expression, native or acquired, of the MDR phenotype.[6] Therefore, the discovery of new colchicine analogues with specific activity in MDR-positive cells is of potential clinical interest.

Previous studies reported that the *N*-trifluoroacetylaminothiocolchicine **4** is much more cytotoxic toward drug-resistant cell lines than wild-type cell lines.[3,8] Our results confirmed the potent antiproliferative activity of *N*-trifluoroacetyl derivative **4** on MCF-7 ADRr. Interestingly, also the new couple of epimers **19/20** has a good activity on MDR-positive cells.

In conclusion, colchicine derivatives **4** and **19/20** seem to have in vitro antitumor properties sufficient to justify further development as new antitumor agents. Studies in vivo are now in progress in our laboratory to demonstrate the antiproliferative activity of these derivatives in animal models, along with their toxic effects.

Experimental Section

Chemistry. Melting points were determined using a Büchi 510 (capillary) apparatus. IR spectra were recorded on a JASCO IR Report 100 spectrophotometer. NMR spectra were obtained with Bruker AC 200 and Varian Gemini 200 instruments. TLC: ready-to-use silica gel plates. Column chromatography: silica gel [Kieselgel 60-70 230 ASTM (Merck)] with the eluant indicated.

General Procedure for the Preparation of *N*-Deacetyl-*N*-(*N*-trifluoroacetylaminoacyl)thiocolchicine 9−15. *N*-Deacetylthiocolchicine **3** (400 mg, 1.07 mmol) and the *N*-trifluoroacetylamino acid **5**−**8** (1.07 mmol) were dissolved at room temperature in dichloromethane (6 mL) under a nitrogen atmosphere, with stirring. DCC (1.07 mmol) was added. After 2 h the suspension was cooled at 0 °C, and the precipitated urea was filtered. The crude reaction mixture was chromatographed on silica gel column, eluting with dichloromethane/methanol (1:0 to 0:1). Compounds **9,12**−**15** were isolated as pure solids after recrystallization from dichloromethane/ethyl ether. A mixture of diastereoisomers **10** and **11**, not separable by column chromatography, was isolated starting from racemic *N*-trifluorophenylalanine (**6**).

***N*-Deacetyl-*N*-(*N*-trifluoroacetylglicyl)thiocolchicine (9):** yield 90%; mp 162 °C (dec); $[\alpha]^{20}_D$ −241 (*c* 0.84, $CHCl_3$); IR (Nujol) 3400−3100 (NH), 1720 ($COCF_3$), 1660 (CONH), 1600 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.09−2.60 (m, 4 H, H-5,6), 2.46 (s, 3 H, SCH_3), 3.65 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 3.86−4.22 (m, 2 H, CH_2), 4.62−4.82 (m, 1 H, H-7), 6.56 (s, 1 H, H-4), 7.14 (d, *J* = 10.8 Hz, 1 H, H-11), 7.37 (d, *J* = 10.8 Hz, 1 H, H-12), 7.51 (s, 1 H, H-8), 7.51−7.54 (m, 1 H, $NHCOCF_3$), 8.34 (d, *J* = 7.7 Hz, 1 H, NH); EIMS *m/e* 526 (M^+), 498 (100), 465, 328, 313, 297, 126.

***N*-Deacetyl-*N*-(*N*-trifluoroacetyl-(*R*)-phenylglycyl)thiocolchicine (10) and *N*-Deacetyl-*N*-(*N*-trifluoroacetyl-(*S*)-phenylglycyl)thiocolchicine (11):** yield 65%; IR (Nujol) 3400−3100 (NH), 1720 ($COCF_3$), 1660 (CONH), 1600 (CO, tropolone) cm^{-1}; EIMS *m/e* 602 (M^+), 574, 329, 282, 203 (100). **10**: mp 187−188 °C (dec); $[\alpha]^{20}_D$ −204 (*c* 0.48, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.96−2.49 (m, 4 H, H-5,6), 2.44 (s, 3 H, SCH_3), 3.68 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 4.49−4.55 (m, 1 H, H-7), 5.39 (d, *J* = 5.9, 1 H, CHPh), 6.49 (s, 1 H, H-4), 7.10 (d, *J* = 10.6 Hz, 1 H, H-11), 7.08−7.41 (m, 7 H, H-12, H-8 and ArH), 7.84 (d, *J* = 5.9 Hz, 1 H, $NHCOCF_3$), 7.95 (d, *J* = 7.3 Hz, 1 H, NH). **11**: mp 173−176 °C (dec); $[\alpha]^{20}_D$ −75 (*c* 0.48, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.80−2.60 (m, 4 H, H-5,6), 2.42 (s, 3 H, SCH_3), 3.71 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.96 (s, 3 H, OCH_3), 4.60−4.73 (m, 1 H, H-7), 5.42 (d, *J* = 6.8, 1 H, CHPh), 6.54 (s, 1 H, H-4), 6.76 (s, 1 H, H-8), 7.04 (d, *J* = 10.4 Hz, 1 H, H-11), 7.22−7.34 (m, 7 H, NH and H-12 and ArH), 7.67 (d, *J* = 6.8 Hz, 1 H, $NHCOCF_3$).

***N*-Deacetyl-*N*-(*N*-trifluoroacetyl-(*R*)-alanyl)thiocolchicine (12) and *N*-Deacetyl-*N*-(*N*-trifluoroacetyl-(*S*)-alanyl)thiocolchicine (13):** yield 71%; IR (Nujol) 3350−3100 (NH), 1720 ($COCF_3$), 1660 (CONH), 1600 (CO, tropolone) cm^{-1}; EIMS *m/e* 540 (M^+), 512, 328 (100), 313, 297, 266, 140. **12**: mp 167−169 °C (dec); $[\alpha]^{20}_D$ −115 (*c* 0.64, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.50 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.98−2.57 (m, 4 H, H-5,6), 2.43 (s, 3 H, SCH_3), 3.69 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.96 (s, 3 H, OCH_3), 4.65−4.74 (m, 2 H, H-7 and C*H*CH_3), 6.47 (s, 1 H, H-4), 7.10 (d, *J* = 10.6 Hz, 1 H, H-11), 7.35 (d, *J* = 10.6 Hz, 1 H, H-12), 7.38 (s, 1 H, H-8), 7.54 (d, *J* = 6.5 Hz, 1 H, NH), 8.39 (d, *J* = 7.7 Hz, 1 H, NH). **13**: mp 166 °C (dec); $[\alpha]^{20}_D$ −100 (*c* 0.54, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.44 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.85−2.57 (m, 4 H, H-5,6), 2.46 (s, 3 H, SCH_3), 3.69 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 4.68−4.78 (m, 2 H, H-7 and C*H*CH_3), 6.55 (s, 1 H, H-4), 7.13 (d, *J* = 10.6 Hz, 1 H, H-11), 7.28 (d, *J* = 8.2, $NHCOCF_3$), 7.37 (d, *J* = 10.6 Hz, 1 H, H-12), 7.51 (s, 1 H, H-8), 8.13 (d, *J* = 7.3 Hz, 1 H, NH).

***N*-Deacetyl-*N*-(*N*-trifluoroacetyl-(*R*)-methionyl)thiocolchicine (14) and *N*-Deacetyl-*N*-(*N*-trifluoroacetyl-(*S*)-methionyl)thiocolchicine (15):** yield 81%; IR (Nujol) 3400−3100 (NH), 1710 ($COCF_3$), 1660 (CONH), 1600 (CO, tropolone) cm^{-1}. **14**: mp 178−179 °C (dec); $[\alpha]^{20}_D$ −122 (*c* 0.18, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.95−2.56 (m, 8 H, H-5,6 and CH_2CH_2-SCH_3), 2.11 (s, 3 H, SCH_3), 2.44 (s, 3 H, SCH_3), 3.68 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.96 (s, 3 H, OCH_3), 4.68−4.83 (m, 2 H, H-7 and C*H*CH_2), 6.55 (s, 1 H, H-4), 7.10 (d, *J* = 10.6 Hz, 1 H, H-11), 7.34 (d, *J* = 10.6 Hz, 1 H, H-12), 7.38 (s, 1 H, H-8), 7.49 (d, *J* = 6.6 Hz, 1 H, NH), 7.94 (d, *J* = 7.6 Hz, 1 H, NH). **15**: mp 178−180 °C (dec); $[\alpha]^{20}_D$ −121 (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.90−2.55 (m, 8 H, H-5,6 and $CH_2CH_2SCH_3$), 2.07 (s, 3 H, SCH_3), 2.46 (s, 3 H, SCH_3), 3.68 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 4.72−4.83 (m, 2 H, H-7 and C*H*CH_2), 6.55 (s, 1 H, H-4), 7.09 (d, *J* = 10.6 Hz, 1 H, H-11), 7.26 (d, *J* = 8.3 Hz, 1 H, NH), 7.34 (d, *J* = 10.6 Hz, 1 H, H-12), 7.38 (s, 1 H, H-8), 7.69 (d, *J* = 7.6 Hz, 1 H, NH).

General Procedure for the Preparation of *N*-Deacetyl-*N*-aminoacyl-thiocolchicine 16−22. A mixture of *N*-trifluoroacetyl derivative **9**−**15** (0.65 mmol) and K_2CO_3 (119 mg, 1.2 mmol) was stirred in water (2.5 mL) and acetone (2.5 mL) at 60 °C. After 5 h the reaction mixture was cooled and a saturated solution of NaCl (2 mL) was added. The organic layer was extracted with chloroform (3 × 10 mL) and dried over anhydrous Na_2SO_4. The crude reaction mixture was chromatographated on silica gel column eluting with dichloromethane/methanol (1:0 to 0:1). Compounds **16**−**18** were isolated as pure solids after recrystallization from dichloromethane/ethyl ether. A mixture of diastereoisomers **19**/**20** and **21**/**22**, not separable by column chromatography, was isolated starting from a mixture of the corresponding diastereoisomers **12**/**13** and **14**/**15**.

***N*-Deacetyl-*N*-glycylthiocolchicine (16):** yield 90%; mp 138 °C; $[\alpha]^{20}_D$ −228 (*c* 1.13, $CHCl_3$); IR (Nujol) 3400−3100 (NH), 1660 (CONH), 1600 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70 (s, 2 H, NH_2), 1.80−2.62 (m, 4 H, H-5,6), 2.43 (s, 3 H, SCH_3), 3.34 (s, 2 H, CH_2), 3.66 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 4.60−4.72 (m, 1 H, H-7), 6.54 (s, 1 H, H-4), 7.04 (d, *J* = 10.3 Hz, 1 H, H-11), 7.16 (s, 1 H, H-8), 7.29 (d, *J* = 10.3 Hz, 1 H, H-12), 7.90 (d, *J* = 7.3 Hz, 1 H, NH); EIMS *m/e* 430 (M^+), 402, 397, 338, 328, 48 (100).

***N*-Deacetyl-*N*-(*R*)-phenylglycylthiocolchicine (17) and *N*-Deacetyl-*N*-(*S*)-phenylglycinylthiocolchicine (18):** yield 55%. IR (Nujol) 3500−3100 (NH), 1660 (CONH), 1600 (CO, tropolone) cm^{-1}; EIMS *m/e* 506 (M^+), 358, 330, 315, 106 (100). **17**: mp 189−190 °C (dec); $[\alpha]^{20}_D$ −253 (*c* 0.26, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.88 (s, 2 H, NH_2), 2.22−2.52 (m, 4 H, H-5,6), 2.42 (s, 3 H, SCH_3), 3.62 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 4.48 (s, 1 H, CHPh),4.58−4.64 (m, 1 H, H-7), 6.52 (s, 1 H, H-4), 7.03 (d, J = 10.5 Hz, 1 H, H-11), 7.23−7.38 (m, 7 H, H-12, H-8 and ArH), 7.91 (d, J = 7.4 Hz, 1 H, NH). **18**: mp 192−194 °C (dec); $[\alpha]^{20}_D$ −350 (*c* 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.82 (s, 2 H, NH_2), 2.17−2.56 (m, 4 H, H-5,6), 2.44 (s, 3 H, SCH_3), 3.60 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 4.51 (s, 1 H, CHPh), 4.58−4.66 (m, 1 H, H-7), 6.51 (s, 1 H, H-4), 7.00 (d, J = 10.8 Hz, 1 H, H-11), 7.15−7.38 (m, 7 H, H-12, H-8 and ArH), 7.86 (d, J = 7.3 Hz, 1 H, NH).

***N*-Deacetyl-*N*-(*R*)-alanylthiocolchicine (19) and *N*-Deacetyl-*N*-(*S*)-alanylthiocolchicine (20):** yield 85%; IR (Nujol) 3600−3100 (NH), 1660 (CONH), 1600 (CO, tropolone) cm^{-1}; EIMS *m/e* 444 (M^+), 411, 401, 358, 330, 44 (100). **19**: mp 179−181 °C (dec); $[\alpha]^{20}_D$ −238 (*c* 0.42, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.33 (d, J = 7.1 Hz, 3 H, CH_3), 1.80 (s, 2 H, NH_2), 1.80−2.62 (m, 4 H, H-5,6), 2.43 (s, 3 H, SCH_3), 3.67 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 3.42−3.55 (m, 1 H, C*H*CH_3), 4.50−4.70 (m, 1 H, H-7), 6.54 (s, 1 H, H-4), 7.04 (d, J = 10.3 Hz, 1 H, H-11), 7.15 (s, 1 H, H-8), 7.29 (d, J = 10.3 Hz, 1 H, H-12), 8.01 (d, J = 8.2 Hz, 1 H, NH). **20**: mp 216−218 °C (dec); $[\alpha]^{20}_D$ −244 (*c* 0.09, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.30 (d, J = 7.0 Hz, 3 H, CH_3), 2.00 (s, 2 H, NH_2), 1.65−2.65 (m, 4 H, H-5,6), 2.43 (s, 3 H, SCH_3), 3.65 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 3.46−3.59 (m, 1 H, C*H*CH_3), 4.52−4.72 (m, 1 H, H-7), 6.54 (s, 1 H, H-4), 7.04 (d, J = 10.3 Hz, 1 H, H-11), 7.16 (s, 1 H, H-8), 7.29 (d, J = 10.3 Hz, 1 H, H-12), 8.00 (d, J = 8.2 Hz, 1 H, NH).

***N*-Deacetyl-*N*-(*R*)-methionylthiocolchicine (21) and *N*-Deacetyl-*N*-(*S*)-methionylthiocolchicine (22):** yield 97%. IR (Nujol) 3500−3100 (NH), 1660 (CONH), 1600 (CO, tropolone) cm^{-1}. Anal. Calcd for $C_{25}H_{32}N_2O_5S_2$: C, 59.49; H, 6.39; N, 5.55. Found: C, 59.62; H, 6.49; N, 5.39. **21**: mp 115−117 °C (dec); $[\alpha]^{20}_D$ −222° (*c* 0.41, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.62 (s, 2 H, NH_2), 1.60−2.65 (m, 8 H, H-5,6 and $CH_2CH_2SCH_3$), 2.08 (s, 3 H, SCH_3), 2.43 (s, 3 H, SCH_3), 3.45−3.54 (m, 1 H, C*H*CH_2), 3.67 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 4.56−4.69 (m, 1 H, H-7), 6.54 (s, 1 H, H-4), 7.03 (d, J = 10.8 Hz, 1 H, H-11), 7.17 (s, 1 H, H-8), 7.27 (d, J = 10.8 Hz, 1 H, H-12), 8.00 (d, J = 8.2 Hz, 1 H, NH). **22:** mp 165−168 °C (dec); $[\alpha]^{20}_D$ −230° (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.62 (s, 2 H, NH_2), 2.00−2.85 (m, 8 H, H-5,6 and $CH_2CH_2SCH_3$), 2.08 (s, 3 H, SCH_3), 2.42 (s, 3 H, SCH_3), 3.62−3.69 (m, 1 H, C*H*CH_2), 3.65 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 4.55−4.75 (m, 1 H, H-7), 6.55 (s, 1 H, H-4), 7.04 (d, J = 10.8 Hz, 1 H, H-11), 7.21 (s, 1 H, H-8), 7.32 (d, J = 10.8 Hz, 1 H, H-12), 8.25 (d, J = 8.2 Hz, 1 H, NH).

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