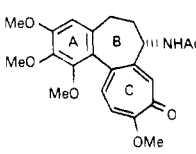
Synthesis and Biological Effects of Novel Thiocolchicines. 3. Evaluation of *N*-Acyldeacetylthiocolchicines, *N*-(Alkoxycarbonyl)deacetylthiocolchicines, and *O*-Ethyldemethylthiocolchicines. New Synthesis of Thiodemecolcine and Antileukemic Effects of 2-Demethyl- and 3-Demethylthiocolchicine[1]

Peter Kerekes,[†,§] Padam N. Sharma,[‡,§] Arnold Brossi,*[§] Colin F. Chignell,[∥] and Frank R. Quinn[⊥]

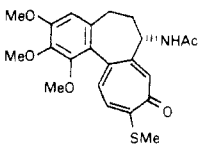
Section on Medicinal Chemistry, Laboratory of Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205, Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709, and Information Technology Branch, National Cancer Institute, Division of Cancer Treatment, National Institutes of Health, Bethesda, Maryland 20205. *Received January 7, 1985*

Novel and known analogues of thiocolchicine were evaluated in vitro in a tubulin binding assay and in vivo in mice for acute toxicity and in the P388 lymphocytic leukemia assay. This evaluation included *N*-acyldeacetylthiocolchicines, *N*-(alkoxycarbonyl)deacetylthiocolchicines, thiodemecolcine and its methyl carbamate, and *O*-ethyl ethers of demethylthiocolchicines. Selective ether cleavage of thiodemecolcine with concentrated sulfuric acid at 50 °C afforded the 2-demethyl congener, characterized as its *N,O*-diacetyl derivative. Several of the compounds showed high potency in the tubulin binding assay, matching the potency of colchicine. Several *N*-(alkoxycarbonyl)deacetylcolchicines (carbamates) exhibited strong binding affinity to tubulin but had only weak activities against the P388 tumor system, suggesting that other factors besides tubulin binding may be important for the biological effects. The compounds potent in the tubulin binding assay and in the P388 leukemia assay in mice were generally also toxic to mice in the acute toxicity test, showing thus a similar behavior of thiocolchicines to that observed earlier with colchicines. A considerable amount of data collected for 2-demethyl- and 3-demethylthiocolchicine suggests that the latter represents a broad-spectrum antitumor agent of considerable promise and possibly a less toxic substitute for colchicine.

Colchicine, a major alkaloid from *Colchicum autumnale*, has antimitotic properties but is too toxic to be of value as an antitumor drug. The synthesis of analogues with improved therapeutic properties, obtained by modifying the structure of colchicine and using a tubulin binding assay as a prescreen, has afforded compounds with improved properties, encouraging further studies.[1-3]



Colchicine



Thiocolchicine

Systematic variation of the substitution in the ring A and the acetamido function in ring B of colchicine, keeping the tropolonic ring C intact, has afforded several compounds that showed good tubulin binding affinity (in vitro activity). When tested in the P388 lymphocytic leukemia screen in mice (in vivo activity) and for acute toxicity in mice after intraperitoneal (ip) administration, it was found that compounds highly potent in vitro and in vivo were generally also highly toxic. Only the ethyl carbamate of deacetylcolchicine, found highly potent in the in vitro and in vivo assays, showed considerable lower acute toxicity and emerged as an interesting compound for further evaluation.

The object of this investigation was to discern whether similar changes in the thiocolchicine molecule,[4] a partially synthetic compound but considerably more stable toward acid hydrolysis than colchicine,[5] would afford similar data. Working with thiocolchicine, a compound developed and investigated intensively at the Roussel-Uclaf Laboratories in France during 1950–1960, had the additional advantage that we would benefit from chemical information collected on this compound.[3]

Chemistry. 2-Demethylthiocolchicine (1) and 3-demethylthiocolchicine (**2**), prepared by the published procedure,[6] afforded after *O*-ethylation with iodoethane in acetone in the presence of potassium carbonate the *O*-ethyl ethers **3** and **4**, respectively. Deacetylthiocolchicine (**5**), prepared from thiocolchicine by hydrolysis with 2 N HCl in methanol,[7] was reacted as the free base with benzoyl chloride or acyl anhydrides, similarly as reported in the deacetylcolchicine series,[2] affording the known *N*-benzoyl derivative **6**[7] and the *N*-acyl analogues **7–9**, respectively. The known *N*-formyl derivative **10**[7] was prepared from **5** by reaction with methyl formate. The carbamates **11–14**, including the known ethyl carbamate **11**,[7] were prepared from **5** by reaction with commercially available chloroformates in dichloromethane solution in the presence of triethylamine.[2,7] The inclusion of known compounds in our investigation was done purposely in order to check the identity of our materials with those made elsewhere and to include them in our biological screening. The carbamate **15** was obtained from 3-demethyldeacetylthiocolchicine, prepared from 3-demethylthiocolchicine by hydrolysis with 2 N HCl in methanol, and reaction of the crude base with ethyl chloroformate. Further hydrolysis of the carbamate

[†] Visiting Scientist from the Department of Chemistry, University of Debrecen, Hungary, who died on August 1, 1984, in Bethesda.

[‡] Visiting Fellow from the Central Drug Research Institute in Lucknow, India, now at the Department of Medicinal Chemistry, School of Pharmacy, University of Kansas, Lawrence, Kansas 66045.

[§] National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

[∥] National Institute of Environmental Health Sciences.

[⊥] National Cancer Institute.

(1) For the preceding paper in this series, see: Brossi, A.; Sharma, P. N.; Atwell, L.; Jacobson, A. E.; Iorio, M. A.; Molinari, M.; Chignell, C. F. *J. Med. Chem.* **1983**, *26*, 1365.
(2) Rösner, M.; Capraro, H. G.; Jacobson, A. E.; Atwell, L.; Brossi, A.; Iorio, M. A.; Williams, T. H.; Sik, R. H.; Chignell, C. F. *J. Med. Chem.* **1981**, *24*, 257.
(3) Capraro, H. G.; Brossi, A. "The Alkaloids" Brossi, A., Ed.; Academic Press: New York, 1984; Vol. 23, pp 1–70.
(4) Velluz, L.; Muller, G. *Bull. Soc. Chim. Fr.* **1954**, 755.
(5) Velluz, L.; Muller, G. *Bull. Soc. Chim. Fr.* **1954**, 1072.
(6) Sharma, P. N.; Brossi, A. *Heterocycles* **1983**, *20*, 1587.
(7) Velluz, L.; Muller, G. *Bull. Soc. Chim. Fr.* **1955**, 194.

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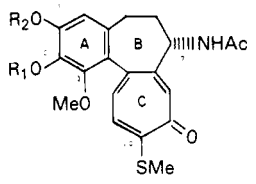
Table I. In Vivo P388 Mouse Leukemia Test Data, Tubulin Binding, and Toxicity of Thiocolchicines

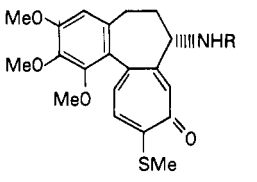
no.	compd	P388 dose,[a] mg/kg	T/C	MED[b] (T/C > 125)	tubulin binding,[c] % 25 μM	2.5 M	toxicity (LD)[d]
1	2-demethylthiocolchicine	10	148	2.5	73		68.1
2	3-demethylthiocolchicine	5	194	1.25	84		11.3
6	*N*-benzoyldeacetylthiocolchicine	2.5	155	<2.5	94	65	40
7	*N*-butyryldeacetylthiocolchicine	1.5	231	0.19	86		
8	*N*-(ethoxyacetyl)deacetylthiocolchicine	1.5	229	0.38	90	68	5.1
9	*N*-(trifluoroacetyl)deacetylthiocolchicine	0.62	148	0.32	98	81	0.9
10	*N*-(formyldeacetyl)thiocolchicine	0.75	171	<0.75	94	77	58.2
11	*N*-(ethoxycarbonyl)deacetylthiocolchicine	1.5	172	0.38	96	68	77.1
12	*N*-(phenoxycarbonyl)deacetylthiocolchicine	5	164	2.5	84		>50
13	*N*-(butoxycarbonyl)deacetylthiocolchicine	3	142	<3	94	81	81
14	*N*-[(vinyloxy)carbonyl]deacetylthiocolchicine	3	152	1.5	97	85	87.5
18	*N*-(trifluoroacetyl)thiodemecolcine	10	145	5.0	96	71	18.6
19	thiodemecolcine	100	131	50.0	79		>100
	colchicine	0.50	245	0.06	90	56	1.6
	thiocolchicine	0.18	193	0.05	96	76	1.0

[a] The dose levels are those that exhibit the highest T/C values (days test animals live/days control animals live × 100). [b] Minimum effective dose (milligrams/kilogram) that produced a T/C greater than the threshold value of 125. [c] Percentage by which the binding of [^{3}H]colchicine (2.5 M) to tubulin from rat brain is reduced in the presence of the thiocolchicine analogs (2.5 or 25 M). Each value is the average of triplicate determinations. For details, see ref 10. [d] Milligrams/kilogram after ip administration to mice.

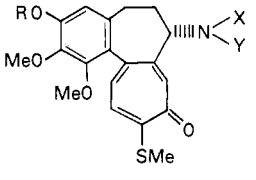
15 with 1 N HCl in methanol afforded the phenolic carbamate **16**. Known thiodemecolcine **19**[7] was prepared here differently and in analogy to the synthesis of demecolcine from colchicine:[8] The *N*-(trifluoroacetyl)deacetylthiocolchicine **9** afforded by methylation with iodomethane in acetone, in the presence of potassium carbonate, the thiodemecolcine analogue **18**, hydrolyzed to **19** by potassium carbonate in aqueous acetone. Treatment of thiodemecolcine (**19**) with concentrated sulfuric acid at 55–58 °C afforded the 2-demethyl analogue **20**, characterized by its crystalline *O*,*N*-diacetate **21**, obtained from **20** by acetylation with acetic anhydride in pyridine. The structures of **20** and **21** were assigned on the basis of the ^{1}H NMR spectrum of **21**, lacking the signal at δ 3.78 characteristic for the 2-OMe group.[6,9] Treatment of thiodemecolcine with methyl chloroformate finally afforded the carbamate **17** of the thiodemecolcine series.



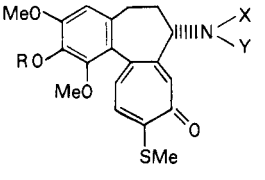
1. R_2 = Me, R_1 = H
2. R_1 = Me, R_2 = H
3. R_2 = Me, R_1 = Et
4. R_1 = Me, R_2 = Et



5. R = H
6. R = COPh
7. R = COC_3H_7
8. R = $COCH_2OEt$
9. R = $COCF_3$
10. R = CHO



11. R = Me, X = H, Y = CO_2Et
12. R = Me, X = H, Y = CO_2Ph
13. R = Me, X = H, Y = $CO_2{}^nBu$
14. R = Me, X = H, Y = $CO_2CH{=}CH_2$
15. R = Y = CO_2Et, X = H
16. R = X = H, Y = CO_2Et
17. R = X = Me, Y = CO_2Me



18. R = Y = Me, X = $COCF_3$
19. R = Y = Me, X = H
20. R = X = H, Y = Me
21. R = X = Ac, Y = Me

(8) Capraro, H. G.; Brossi, A. *Helv. Chim. Acta* **1979**, *62*, 965.
(9) Hufford, C. D.; Capraro, H. G.; Brossi, A. *Helv. Chim. Acta* **1980**, *63*, 50.

Biological Evaluation

1. Binding to Tubulin Protein. The binding of the thiocolchicine analogues to tubulin was determined by measuring their ability to displace [^{3}H]colchicine.[10] All compounds were initially tested at a 10-fold excess over the concentration of [^{3}H]colchicine. The results show that all the thiocolchicines were quite effective at competing with colchicine for its binding site on tubulin with *N*-(trifluoroacetyl)deacetylthiocolchicine being the most potent (Table I). The least active compound was 2-demethylthiocolchicine. This finding is in agreement with previous studies that have shown (**1**, **2**) that demethylation of ring A in colchicine always results in a decrease in tubulin binding. On the other hand, the ethyl ethers **3** (98% tubulin binding) and **4** (97% tubulin binding), obtained by *O*-ethylation of **1** and **2** respectively, available only in small quantities, showed potencies in the tubulin binding assay (TB) equal to that of colchicine when tested at 25 μM concentration. Several analogues that were equal to or better than colchicine were tested again at a 10-fold lower concentration. These studies showed that thiocolchicine and several of its analogues were even more potent than colchicine itself (Table I) with *N*-(trifluoroacetyl)deacetylthiocolchicine, *N*-(butoxycarbonyl)deacetylthiocolchicine, and *N*-(vinyloxy)deacetylthiocolchicine exhibiting the highest binding affinity. There appears to be little correlation between tubulin binding and murine antitumor activity, particularly among carbamates. Thus *N*-[(vinyloxy)carbonyl]deacetylthiocolchicine and its carbamate congeners **11**–**13**, while exhibiting a strong affinity for tubulin, had only weak activities against the P388 tumor system. This suggests factors other than tubulin binding may be important for biological activity.

2. P388 Assay. The compounds in this study were evaluated against P388 lymphocytic leukemia in mice according to protocols established by the Developmental Therapeutics Program, National Cancer Institute.[11] The tumors were implanted intraperitoneally. Drugs were administered ip on a QD1 × 9 regimen (nine injections). Antitumor activity is measured in terms of T/C (median

(10) Zweig, M. H.; Chignell, C. F. *Biochem. Pharmacol.* **1973**, *22*, 2142.
(11) National Cancer Institute, "In Vivo Cancer Models", NIH Publication No. 84-2635, Feb 1984.

survival time of treated animals/median survival time of untreated controls × 100). A compound is considered to demonstrate antitumor activity if duplicate tests give T/C values equal to or greater than 120%. A reproducible T/C equal to or greater than 175% is evidence of significant activity.

3. Toxicities. The toxicities of these compounds were examined by determining the LD50 of a compound after a single intraperitoneal injection. A group of 10 NIH general purpose mice (ca. 20 g) were used at each of several dose levels. Usually, at least five dose levels were used. The compound was introduced in an Emulphor EL-620 mixture.[12] One set of 10 mice, injected (ip) with the emulphor mixture alone, was used as a control for each compound. The LD50 and its 95% confidence interval were determined by probit analysis.[13] The surviving mice were counted 7 days after the single ip injection.

Structure–Activity Relationships. Thirteen analogues of thiocolchicine were tested for antitumor activity, acute toxicity, and tubulin binding affinity. It should be noted that antitumor testing was performed with use of a chronic treatment regimen (QD1 × 9), while toxicity was determined with a single acute dose. Chronic toxicity would be expected to be somewhat lower. The results are given in Table I. Test data results for colchicine and thiocolchicine are included for comparison. The T/C values given are the highest that were obtained and were shown to be reproducible. The minimum effective dose (MED) that produced a T/C equal to or greater than the threshold (T/C = 120), together with the highest T/C attained, provides a measure of the potency of the compound.

All of the 13 thiocolchicine analogues demonstrated reproducible antileukemic activity. The antitumor activity and the toxicity of the two compounds in which an A-ring methoxy group was demethylated proved to be highly position dependent, a finding that had been previously noted for the corresponding colchicine derivatives.[1] Demethylation at the 2-position (**1**) caused an appreciable decrease in both activity and toxicity, showing a maximum T/C of 148 and an LD50 of 68 mg/kg compared to 193 and 0.32 mg/kg for thiocholchicine. The 3-demethyl derivative (**2**), however, showed antitumor activity equal to that of thiocolchicine. It was, in addition, about 35-fold less toxic.

Although thiocolchicine is a slightly less active antitumor agent than colchicine, modification at the 7-position provided two analogues (**7** and **8**) whose activity and potency are equivalent to that of colchicine itself. In addition, the ethoxyacetyl analogue **8** proved to be less toxic than either colchicine or thiocolchicine. Introduction of a trifluoroacetamido group at position 7 (**9**) substantially decreased the activity without a corresponding decrease in its toxicity, which is of the same order of magnitude as that of thiocolchicine. A similar effect had been previously noted for the corresponding colchicine derivative.[1] The *N*-formyl derivative **10** proved to be a potent compound with an antitumor activity not substantially different from that of thiocolchicine. Its toxicity (58.2 mg/kg) was, however, considerably less than that of the parent.

The introduction of a carbamate ester function at position 7 resulted in the known carbamate **11** and the three analogues **12–14** in which both antitumor activity and potency were diminished. Substitution of the benzamido group at position 7 (**6**) resulted in a similar loss of antitumor activity. These four analogues would be expected to be substantially more lipophilic than either colchicine or thiocolchicine and this increased lipophilicity may influence transport in in vivo systems.[14] The *N*-methyl-*N*-trifluoroacetyl analogue **18** was somewhat less potent than congener **9**, although its overall antileukemic activity was about the same. Thiodemecolcine (**19**) had moderate antitumor activity and proved to be the least toxic compound of the series.

Table II. Spectrum of Antitumor Activity of 3-Demethylthiocolchicine in in Vivo Systems[a,b]

compd	P388	3B131	L1210	3MBG5	3M531
3-demethylthiocolchicine (**2**)	++	++	+	++	++
colchicine	++	++	+		+
trimethylcolchicinic acid (TMCA)	+		++		+
4-formylcolchicine	++	+	+	+	+
N-(ethoxycarbonyl)deacetylcolchicine	++	++	++		++
thiocolchicine	+	NT	+	NT	+

[a] 3B131 is intraperitoneally implanted B16 melanoma. L1210 is intraperitoneally implanted L1210 leukemia. 3MBG5 is the subrenal capsule mammary carcinoma MX-1 xenograft. M531 is sarcoma M5076. A single plus (+) denotes reproducible minimal activity; double plus (++) denotes reproducible significant activity. (b) NT indicates that no testing was performed in that system. [b] See ref 11.

In a previous study on colchicine derivatives,[1] it was observed that a direct relationship between potency in the P388 system and tubulin binding could not be detected. The present study further confirms this finding. Several of the analogues in this series (**9–18**) were less active than either colchicine or thiocolchicine and yet showed tubulin binding affinity equal to or greater than that of either parent.

Table II gives the broad-spectrum antitumor activity of one of the most promising members of this series, 3-demethylthiocolchicine (**2**). The activities of colchicine and trimethylcolchicinic acid, both of which have been used clinically, are included for comparison. Of particular interest is the significant activity of this compound against the three usually refractory tumors, B16 melanoma, the subrenal capsule mammary carcinoma MX-1 xenograft, and sarcoma M5076.

Experimental Section

Melting points (uncorrected) were determined with a Fisher-Johns apparatus. The analytical samples were dried in vacuo over P_2O_5 at 90–100 °C for 24 h. Microanalyses were performed by the Section on Microanalytical Services and Instrumentation of this Laboratory. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter with the solvents and concentrations specified. IR spectra were recorded on a Beckman IR 4230 spectrometer. Nuclear magnetic resonance spectra were determined by using a JEOL JNM-FX 100 and Varian XL 300 spectrometer with Me_4Si as the internal reference (s = singlet, d = doublet, t = triplet, m = multiplet). Electron-ionization mass spectra (EIMS) were obtained with V. G. Micromass 7070F mass spectrometer (70 eV, source temperature 210 °C), and chemical-ionization mass spectra (CIMS) were determined with a Finnigan 1015D spectrometer with a Model 6000 data collection system. Thin-layer chromatography plates were purchased from Analtech, Inc., Newark, DE, and silica gel 60 (0.040–0.063 mm) EM Reagents was used for column chromatography. The solvent system used for TLC analysis was $CHCl_3$–MeOH (9:1), and the materials were developed with iodine vapors.

2-Ethyl-2-demethylthiocolchicine (3). A mixture of 2-demethylthiocolchicine (**1**; 160 mg, 0.4 mmol), iodoethane (0.3 mg, 2 mmol), anhydrous K_2CO_3 (0.14 g, 1 mmol), and acetone (5 mL)

(12) Emulphor EL-620 is a polyoxyethylated vegetable oil obtained from the GAF Corporation, Linden, NJ.

(13) Finney, D. J. "Probit Analysis", 2nd ed.; Cambridge University Press: Cambridge, England, 1964.

(14) Quinn, F. R.; Beisler, J. S. *J. Med. Chem.* **1981**, *24*, 251.

was refluxed for 20 h. Usual workup and crystallization from acetone–ether yielded **3** (102 mg, 59.3%): mp 188–190 °C; $[\alpha]^{19}_D$ −212.3° (*c* 0.48, $CHCl_3$); IR ($CHCl_3$) 3445 (NH), 3300, 1670 (C=O), 1600 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (t, 3 H, OCH_2CH_3), 4.10 (m, 2 H, OCH_2CH_3); EIMS, *m/e* 429 (M^+). Anal. ($C_{23}H_{27}NO_5S \cdot C_3H_6O$) C, H, N.

3-Ethyl-3-demethylthiocolchicine (4) (with iodomethane) was prepared from 3-demethylthiocolchicine (**2**; 160 mg, 0.6 mmol) as above (time of reaction 3 days) after crystallization from acetone (150 mg, 87.2%): mp 250–252 °C; $[\alpha]^{19}_D$ −206.3° (*c* 0.52, $CHCl_3$) cm^{-1}; IR ($CHCl_3$) 3355 (NH), 3320, 1672 (C=O), 1605; NMR ($CdCl_3$) δ 1.46 (t, 3 H, OCH_2CH_3), 4.08 (m, 2 H, OCH_2CH_3); EIMS, *m/e* 429 (M^+). Anal. ($C_{23}H_{27}NO_5S \cdot C_3H_6O$) C, H, N.

Preparation of *N*-Acyl- and *N*-(Alkoxycarbonyl)deacetylthiocolchicine Derivatives (6–8 and 11–14). These compounds were prepared from deacetylthiocolchicine (**5**) with the appropriate acid chlorides, anhydride, or chloroformates in the presence of pyridine or triethylamine. A typical procedure is as follows for the preparation of **6**.

***N*-Benzoyldeacetylthiocolchicine (6).** To an ice-cold stirred solution of deacetylthiocolchicine (**5**; 559 mg, 1.49 mmol) in dry CH_2Cl_2 (2 mL) and pyridine (0.12 mL, 122 mg, 1.59 mmol) was added dropwise benzoyl chloride (0.174 mL, 209 mg, 1.49 mmol). The reaction mixture was stirred at room temperature until TLC showed the absence of **5** (1 h) and then decomposed by the addition of H_2O (2 mL). The organic layer was washed with 0.5 N HCl (3 × 2 mL), 10% aqueous $NaHCO_3$ solution (3 × 2 mL), and H_2O (2 × 2 mL), dried (Na_2SO_4), and evaporated to afford a solid residue, which was crystallized from a mixture of CH_2Cl_2/Et_2O to give pure yellowish **6** as a solid (450 mg, 63%): mp 280 °C; $[\alpha]^{25}_D$ −110° (*c* 0.14, $CHCl_3$); EIMS , *m/e* 477 (M^+) (lit.[9] mp 283–285 °C; $[\alpha]_D$ −86° (*c* 0.5, $CHCl_3$). Anal. ($C_{27}H_{27}NO_5S$) C, H, N, S.

***N*-Butyryldeacetylthiocolchicine (7) (with butyryl chloride)**: crystallized from a mixture of CH_2Cl_2–Et_2O to afford a pure yellowish solid (55%): mp 205 °C; $[\alpha]^{25}_D$ −225° (*c* 0.25, $CHCl_3$); EIMS, *m/e* 493 (M^+). Anal. ($C_{24}H_{29}NO_5S \cdot {}^1/_4H_2O$) C, H, N, S.

***N*-(Ethoxyacetyl)deacetylthiocolchicine (8) (with ethoxyacetic anhydride)**: crystallized from a mixture of acetone–Et_2O to give yellow crystals: mp 163–165 °C; $[\alpha]^{26}_D$ −204.7° (*c* 0.57, $CHCl_3$); EIMS, *m/e* 459 (M^+). Anal. ($C_{24}H_{29}NO_6S$) C, H, N, S.

***N*-(Ethoxycarbonyl)deacetylthiocolchicine (11) (with ethyl chloroformate)**: crystallized from a mixture of CH_2Cl_2–Et_2O to afford a pure yellowish solid (77%): mp 197 °C; $[\alpha]^{25}_D$ −246° (*c* 0.5, $CHCl_3$); EIMS, *m/e* 445 (M^+) (lit.[9] mp 194–195 °; $[\alpha]_D$ −241° (*c* 0.5, $CHCl_3$). Anal. ($C_{23}H_{27}NO_6S$) C, H, N, S.

***N*-(Phenoxycarbonyl)deacetylthiocolchicine (12) (with phenyl chloroformate)**: crystallized from a mixture of CH_2Cl_2–Et_2O to afford a pure yellowish solid (61%): mp 152 °C; $[\alpha]^{25}_D$ −219° (*c* 0.3, $CHCl_3$); CIMS, *m/e* 494 ($M^+ + 1$). Anal. ($C_{27}H_{27}NO_6S$) C, H, N, S.

***N*-(Butoxycarbonyl)deacetylthiocolchicine (13) (with Butyl Chloroformate).** Crude **13** was purified by column chromatography (SiO_2, elution with $CHCl_3$–MeOH, 97:3) and crystallized from acetone–Et_2O to yield yellow crystalline **13** (50%): mp 180–182 °C; $[\alpha]^{21}_D$ −217.2° (*c* 0.58, $CHCl_3$); CIMS, *m/e* 474 ($M^+ + 1$). Anal. ($C_{25}H_{31}NO_6S$) C, H, N, S.

***N*-[(Vinyloxy)carbonyl]deacetylthiocolchicine (14) (with Vinyl Chloroformate).** Crude **14** was purified by column chromatography (silica gel, elution with $CHCl_3$–MeOH, 97:3) and crystallized from acetone–Et_2O to give yellow crystalline **14** (52.6%): mp 213–215 °C; $[\alpha]^{19}_D$ −259.8° (*c* 0.47, $CHCl_3$); CIMS, *m/e* 402 ($M^+ + 1$). Anal. ($C_{23}H_{25}NO_6S$) C, H, N.

***N*-Formyldeacetylthiocolchicine (10).** To a solution of deacetylthiocolchicine (**5**; 1.5 g, 4 mmol) in dry DMF (3 mL) was added dropwise ethyl formate (12 mL) and the reaction mixture was refluxed for 90 h. A yellow solid separated out during the reaction. The solvents were evaporated to give a brown gummy residue, which was dissolved in $CHCl_3$ (80 mL), washed with 5% HCl (1 × 30 mL) and H_2O (2 × 30 mL), dried (Na_2SO_4), and evaporated. The residue was crystallized from acetone to afford pure yellow solid **10** (1.07 g, 66.5%): mp 255–256 °C dec; $[\alpha]^{19}_D$ −259.8° (*c* 0.47, $CHCl_3$); CIMS, *m/e* 402 ($M^+ + 1$) (lit.[9] mp 258–260 °C; $[\alpha]_D$ −275° (*c* 0.5, $CHCl_3$)). Anal. ($C_{21}H_{23}NO_5S$) C, H, N, S.

Synthesis of Thiodemecolcine (19). 1. *N*-(Trifluoroacetyl)deacetylthiocolchicine (9). To an ice-cold stirred heterogeneous mixture of *N*-deacetylthiocolchicine (**5**; 4.11 g, 11 mmol) and Na_2CO_3 (11.66 g, 110 mmol) in 400 mL of dry ether, trifluoroacetic anhydride (23.1 g, 15.53 mL, 110 mmol). The orange solution was stirred at room temperature for 5 h, during which time a yellow solid separated out. The solid was filtered off and the ether solution evaporated to give a brown oily residue. The solid and the oily residue were dissolved in a mixture of $CHCl_3$–H_2O [200 mL (1:1)] and the aqueous layer was extracted with $CHCl_3$ (2 × 3o mL). The combined organic layer was washed with brine (3 × 30 mL), dried (Na_2SO_4), and evaporated. The yellowish-brown residue solidified on trituration with Et_2O. The crude product (4.72 g, 95%) was recrystallized from acetone to yield yellow crystalline **9** (4.1 g, 79.5%): mp 184–195 °C; $[\alpha]^{21}_D$ −117.7° (*c* 1.0, $CHCl_3$); IR ($CHCL_3$) 3440, (NH) 3250, 1768 (C=O), 1605 cm^{-1}; NMR ($CDCl_3$) δ 2.40 (s, 3 H, SMe), 3.64, 3.88, and 3.92 (3 s, 9 H, 3 × OMe), 4.72 (m, 1 H, C_7H), 6.52 (s, 1 H, Ar H), 7.16 (d, 1 H, *J* = 8 Hz, Ar H), 7.36 (d, 1 H, *J* =8 Hz, Ar H), 7.41 (s, 1 H, Ar H); EIMS, *m/e* 469 (M^+). Anal. ($C_{22}H_{22}F_3NO_5S$) C, H, N.

2. *N*-(Trifluoroacetyl)thiodemecolcine (18). A heterogeneous mixture of **9** (3.29 g, 7 mmol), acetone (40 mL), K_2CO_3 (4.7 g, 34 mmol), and iodomethane (5 mL) was stirred at room temperature for 10 days. The reaction mixture was then dissolved in $CHCl_3$ (120 mL), water added (30 mL), the organic layer separated, and the aqueous layer extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were washed with water (3 × 30 mL), dried (Na_2SO_4), and evaporated. The residue was crystallized from Et_2O to afford yellow crystalline **18** (3.03 g, 89.6%): mp 193–194 °C; $[\alpha]^{19}_D$ −241° (*c* 0.78, $CHCl_3$); IR ($CHCl_3$) 1681 (C=O), 1600 (Ar) cm^{-1}; NMR ($CDCl_3$) δ 2.40 (s, 3 H, SMe), 3.36 (s, 3 H, NMe), 3.68, 3.88, and 3.92 (3 s, 9 H, 3 OMe), 4.84 (m, 1 H, C_7H), 6.52 and 6.84 (2 s, 2 H, Ar H), 7.0 and 7.28 (2 d, 2 H, *J* = 8 Hz, 2 Ar H); EIMS, *m/e* 483 (M^+). Anal. ($C_{23}H_{24}F_3NO_5S$) C, H, N.

3. Thiodemecolcine (19). A suspension of **18** (2.42 g, 5 mmol) and K_2CO_3 (1 g) in acetone (20 mL) and water (20 mL) was heated at 60–65 °C (bath temperature) for 24 h. The dark orange solution was diluted with brine (50 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with brine (1 × 30 mL), dried (Na_2SO_4), and evaporated. The residue (brown gum) was triturated with Et_2O (15 mL) to give a yellow solid (1.8 g, 92.8%). The crude product was recrystallized from MeOH to yield yellow crystalline solid **19** (1.62 g, 83.5%): mp 218–220 °C; $[\alpha]^{29}_D$ −163.9° (*c* 0.67, $CHCl_3$); IR ($CHCl_3$) 1600 cm^{-1}; NMR ($CDCl_3$) δ 2.2 (s, 3 H, NMe), 2.41 (s, 3 H, SMe), 3.59, 3.88, and 3.90 (3 s, 9 H, 3 × OMe); CIMS, *m/e* 388 ($M^+ + 1$) (lit.[9] mp 222 °C $[\alpha]_D$ −164° (*c* 0.5, $CHCl_3$)). Anal. ($C_{21}H_{25}NO_4S$) C, H, N. S.

***N*-(Methoxycarbonyl)thiodemecolcine (17) (with methyl chloroformate)**: prepared from **19** (387 mg, 1 mmol) by the standard procedure. The crude product was crystallized from acetone/Et_2O to afford yellow crystalline solid **17** (357 mg, 80.2%): mp 161–163 °C; $[\alpha]^{28}_D$ −312.5° (*c* 0.55, $CHCl_3$); IR ($CHCl_3$) 1685 (C=O), 1600 cm^{-1}; NMR ($CDCl_3$) δ 2.40 (s, 3 H, SMe), 3.16, 3.62, 3.88, and 3.92 (4 s, 12 H, 4 OMe), 3.51 (s, 3 H, NMe), 4.64 (m, 1 H, C_7H), 6.52 (s, 1 H, Ar H), 6.92–7.28 (m, 3 H, 3 Ar H); EIMS, *m/e* 445 (M^+). Anal. ($C_{23}H_{27}NO_6S$) C, H, N, S.

2-Demethylthiodemecolcine (20) and Diacetyl Derivative 21. Thiodemecolcine (**19**; 387 mg, 1 mmol) was dissolved in concentrated H_2SO_4 (3 mL), and the solution was heated for 2 h at 55–58 °C (bath temperature) and stirred at room temperature overnight. The deep red solution was diluted poured on ice and the pH of the solution adjusted to 5 with 10% aqueous $NaHCO_3$ solution. The reaction mixture was extracted with $CHCl_3$ (4 × 20 mL), washed with brine (1 × 15 mL), dried (Na_2SO_4), and evaporated. The crude product was purified by column chromatography (SiO_2, elution with $CHCl_3$–MeOH, 95:5) to give 0.26 g of **20** as a yellow oil (EIMS, *m/e* 373 (M^+). For further characterization **20** was transformed by usual acetylation to the *N*,*O*-diacetyl derivative (**21**) to afford after crystallization from acetone–Et_2O yellow crystalline solid **21** (138 mg, 30.1%): mp 229–231 °C; $[\alpha]^{19}_D$ −279.4° (*c* 0.54, $CHCl_3$); IR ($CHCl_3$) 1762 (OAc), 1640 (C=O), 1602 cm^{-1}; NMR ($CDCl_3$) δ 2.08 and 2.36 (2 s, 6 H, OAc, NAc), 2.40 (s, 3 H, SMe), 3.25 (s, 3 H, NMe), 3.60 (s, 3 H, 1-OMe), 3.84 (s, 3 H, 3-OMe), 4.94 (m, 1 H, C_7H), 6.56 (s, 1 H,

Ar H), 6.88–7.32 (m, 3 H, Ar H); CIMS, *m/e* 458 ($M^+ + 1$). Anal. ($C_{24}H_{27}NO_6S$) C, H, N.

3,*N*-Bis(ethoxycarbonyl)-3-demethyldeacetylthiocolchicine (15). 3-Demethylthiocolchicine (**2**; 2.01 g, 5 mmol) was refluxed for 16 h in a mixture of 50 mL of methanol and 100 mL of 2 N HCl. Methanol was evaporated under reduced pressure and the residue was made alkaline with concentrated NH_4OH and extracted with $CHCl_3$–MeOH (3:1) (3 × 100 mL). The combined organic layer was washed with brine (2 × 10 mL), dried (Na_2SO_4), and evaporated to yield 1.96 g of 3-demethyldeacetylthiocolchicine as an orange foam (1.37 g, 3.8 mmol), which was treated with ethyl chloroformate by using the standard method. Crude **15** was purified by column chromatography (SiO_2, elution with $CHCl_3$–MeOH, 98:2) to give a yellow solid (1.01 g, 57.6%). A sample was crystallized from EtOAc–Et_2O to yield yellow crystalline **15**: mp 219–221 °C; $[\alpha]^{24}_D$ −130.3° (*c* 0.59, $CHCl_3$); IR ($CHCl_3$) 3460 (NH), 1766 (carbamate), 1721 (C=O), 1615 cm^{-1}; NMR ($CDCl_3$) δ 1.17 (t, 3 H, OCH_2CH_3), 1.4 (t, 3 H, OCH_2CH_3), 2.43 (s, 3 H, SMe), 3.64 (s, 3 H, 1-OMe), 3.96 (s, 3 H,2-OMe), 4.00 (m, 2 H OCH_2CH_3), 4.35 (m, 2 H, OCH_2CH_3), 4.40 (m, 1 H, C_7H), 5.21 (d, 1 H, *J* = 7 Hz, NH) 6.77 (s, 1 H, Ar H), 7.04 (d, 1 H, *J* = 10 Hz, Ar H), 7.29 (d, 1 H, *J* = 10 Hz, Ar H), 7.3 (s, 1 H, Ar H); CIMS, *m/e* 504 ($M^+ + 1$). Anal. ($C_{24}H_{27}NO_6S$) C, H, N, S.

***N*-(Ethoxycarbonyl)-3-demethyldeacetylthiocolchicine (16).** A solution of **15** (806 mg, 1.6 mmol) in methanol (25 mL) and 1 N HCl (25 mL) was refluxed for 24 h. Methanol was removed in vacuum and the residue extracted with $CHCl_3$ (3 × 30 mL), washed with brine (2 × 10 mL), dried (Na_2SO_4), and evaporated. The crude product was purified by column chromatography (SiO_2, elution with $CHCl_3$–MeOH, 98:2) to afford a yellow foam, which was crystallized from EtOAc–Et_2O to yield yellow crystalline solid **16** (460 mg, 66.7%): mp 230–231 °C; $[\alpha]^{24}_D$ −242.3° (*c* 0.65, $CHCl_3$); IR ($CHCl_3$) 3540 (OH), 3460 (NH), 1720 (C=O), 1620 cm^{-1}; NMR ($CDCl_3$) δ 1.19 (t, 3 H, OCH_2CH_3), 2.45 (s, 3 H, SMe), 3.64 (s, 3 H, 1-OMe), 4.03 (s, 3 H, 2-OMe), 4.05 (m, 2 H, OCH_2CH_3), 4.44 (m, 1 H, C_7H), 5.24 (d, 1 H, *J* = 7 Hz, NH), 5.97 (s, 1 H, OH), 6.22 (s, 1 H, Ar H), 7.06 (d, 1 H, *J* = 10 Hz, Ar H), 7.28 (d, 1 H, *J* = 10 Hz, Ar H), 7.34 (s, 1 H, Ar H); CIMS, *m/e432* ($M^+ + 1$). Anal. ($C_{22}H_{25}NO_6S$) C, H, N, S.

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Synthesis and Biological Evaluation of Phosphonamidate Peptide Inhibitors of Enkephalinase and Angiotensin-Converting Enzyme

R. L. Elliott,[†] N. Marks,[‡] M. J. Berg,[‡] and P. S. Portoghese*[†]

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455, and Rockland Research Institute, Wards Island, New York 10035. *Received November 7, 1984*

The effectiveness of phosphonamidate peptide analogues as inhibitors of rat kidney or human brain metalloendopeptidase (enkephalinase, E.C. 3.4.24.11) and angiotensin-converting enzyme (ACE, 3.4.15.1) has been explored with a series of enkephalin analogues in which the scissile Gly^3–Phe^4 amide bond has been replaced with a phosphonamidate moiety. These compounds exhibited good inhibitory potency against enkephalinase with several of the analogues having K_i values in the submicromolar range as contrasted to micromolar or higher toward ACE. Within a series of [(*N*-acylamino)methyl]phosphonamidates there was a dramatic decrease in inhibitory activity against enkephalinase as the *N*-acyl moiety was substituted with larger, more hydrophobic acyl groups. Likewise, the inhibitory activity of the [(*N*-acylamino)methyl]phosphonamidates against ACE was attenuated by larger phenylalkyl acyl functionalities, although not to the same degree as against enkephalinase. However, phosphonamidate pentapeptide analogues of (Leu)enkephalin and (D-Ala^2,D-Leu^5)enkephalin showed good inhibitory potency against both enzymes. Interestingly, these two (Leu)enkephalin phosphonamidate analogues were completely inactive in the electrically stimulated guinea pig ileum and mouse vas deferens preparations. Conformational factors that may be involved in this inactivity are discussed.

The discovery of the enkephalins[1] initiated intensive research into the pharmacological aspects and physiological significance of these neuropeptides. Much of this research has focused on the neurochemical role and metabolic fate of the enkephalins and has led to the discovery and characterization of a membrane-bound zinc endopeptidase (enkephalinase) involved in the in vivo degradation of the enkephalins.[2-5] Extensive studies on the substrate specificity of this enzyme have been complemented by other studies investigating the effectiveness of various inhibitors against this enzyme. These enkephalinase inhibitors have been of great value as pharmacological tools and may have therapeutic application.

[†] University of Minnesota.
[‡] Rockland Research Institute.

(1) Hughes, J.; Smith, T. W.; Kosterlitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R. *Nature* **1975**, *258*, 577–579.
(2) Malfroy, B.; Swerts, J. P.; Guyon, A.; Roques, B. P.; Schwartz, J. C. *Nature* **1978**, *276*, 523–526.
(3) De La Baume, S.; Yi, C. C.; Schwartz, J. C.; Chaillet, P.; Marcais-Collado, H.; Costentin, J. *Neuroscience (Oxford)* **1983**, *8* (1), 143–151.
(4) Benuck, M.; Berg, M. J.; Marks, N. *Life Sci.* **1981**, *28*, 2643–2650.
(5) Gorenstein, C.; Snyder, S. H. *Proc. R. Soc. London, B* **1980**, *210*, 123–132.

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