Synthesis and Biological Activities of Chloroethylurea, Methylurea, and Nitrosourea Analogues of N-Deacetylmethylthiocolchicine[1]

Tai-Shun Lin,* George T. Shiau, William H. Prusoff,

Department of Pharmacology and Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510

and Robert E. Harmon

Department of Chemistry, Western Michigan University, Kalamazoo, Michigan 49001. *Received May 2, 1980*

A series of urea and nitrosourea analogues of N-deacetylmethylthiocolchicine (1) has been synthesized, and their antineoplastic and antiviral activities were evaluated. The objective for combination of two active moieties, such as thiocolchicine and nitrosourea, into one molecule was the generation of compounds with potential improved biological and pharmacological properties. The ED_{50} for **2**, **3**, **4**, and **5** was 1.6, 1.2, 3.3, and 1.8 × 10^{-8} M for L1210 cells and 3.0, 2.7, 2.9, and 2.6 × 10^{-8} M for S-180 cells, respectively. The synthesis and cytotoxic and antiviral properties of these compounds are discussed.

Colchicine and many of its derivatives are powerful mitotic poisons, antiinflammatories, and inhibitors of tumor growth.[2,3] The biological and biochemical effects of colchicine and related compounds have been reviewed recently.[4] The cytotoxic effect may be related to the formation of a colchicine–tubulin complex which prevents microtubule polymerization.[5]

Thiocolchicines, in which sulfur replaces oxygen in the troponoid methoxy group of a colchicine derivative, were first prepared by Velluz and Muller[6–9] in 1954. Recently, a series of new thiocolchicines was prepared with groups larger than methyl on the sulfur atom.[10] A hydrochloride salt of N-deacetylmethylthiocolchicine (1) inhibited the binding of colchicine to microtubule protein and with nearly equivalent antimitotic and antiinflammatory activities.[3] Compound 1 also had about the same activity against mouse leukemia as colchicine.[10] The crystal and molecular structure of N-deacetylmethylthiocolchicine hydrochloride dihydrate was recently determined by X-ray diffraction.[11]

Important aspects of the mechanism of action, pharmacology, and clinical utility of the nitrosoureas as antineoplastic agents have been recently reviewed.[12] It has been suggested that therapeutic effectiveness might be maximized in drugs possessing high alkylating and low carbamoylating activities.[13] Recently, a new class of nitrosourea compounds, the chloroethyl- and methylnitrosourea analogues of thymidine, has been synthesized,[14] and some of these inhibited the replication of L1210 cells in culture more effectively than 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). The present report describes the combination into a single molecule of two active moieties,

Scheme I

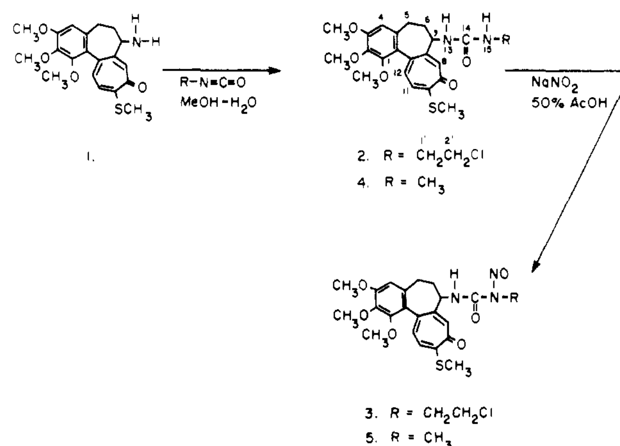

Table I. Effect of Chloroethylurea, Methylurea, and Nitrosourea Analogues of N-Deacetylmethylthiocolchicine on the Growth of L1210 Cells, Sarcoma 180 Cells, and Herpes Simplex Virus Type 1 in Vitro

compd	L1210 ED_{50} × 10^8 M	S-180 ED_{50} × 10^8 M	HSV-1 % inhibn
1	1.9[a]		81 (30)[b]
2	1.6	3.0	72 (20)
3	1.2	2.7	76 (1)
4	3.3	2.9	85 (175)
5	1.8	2.6	86 (10)
EtS[c]	11.4		
BCNU[d]	400		
IdUrd[e]			99.3 (50)

[a] Reference 9; converted to molarity by multiplication by the factor $10^{-3}/373$. [b] Concentration of testing solution in μM. [c] Reference 16; EtS is N-deacetyl-N-(methylcarbamoyl)ethylthiocolchicine, a compound in which ethyl replaces the methyl group in the troponoid methylthio moiety of 4. [d] Reference 13; BCNU is 1,3-bis(2-chloroethyl)-1-nitrosourea. [e] IdUrd is 5-iodo-2′-deoxyuridine.

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thiocolchicine and either chloroethyl- or methylnitrosourea, and evaluation of its cytotoxic and antiviral properties.

Chemistry. *N*-Deacetyl-*N*-[(2-chloroethyl)carbamoyl]methylthiocolchicine (**2**) was prepared from *N*-deacetylmethylthiocolchicine[10] (**1**) by reaction with an excess amount of 2-chloroethyl isocyanate in methanol–water at 0 °C. Analysis by thin-layer chromatography (TLC) showed 60% conversion to product in 1 h with no increase in the desired material with longer reaction time or more reagent. The product **2** was purified by column chromatography on silica gel and isolated as an amorphous powder in 52% yield. In contrast to **2**, *N*-deacetyl-*N*-(methylcarbamoyl)methylthiocolchicine (**4**) was prepared in nearly quantitative yield. It was synthesized by a reaction of **1** with excess methyl isocyanate in methanol–water. An analysis by TLC showed that a complete conversion of **1** to **4** was accomplished within 5 min. The urea analogues **2** and **4** were then nitrosated with sodium nitrite in dilute acetic acid solution to afford the desired nitrosourea analogues of *N*-deacetylmethylthiocolchicine, **3** and **5**. No isomeric contamination was detected in the nitrosation. The nitrosation of urea to yield nitrosourea isomers has been well discussed.[15] The exclusive formation of **3** and **5** was probably attributed to steric hindrance due to the bulky colchicine moiety. The structures of compounds **2–5** were determined by elemental analysis, TLC, and spectroscopic analysis (Scheme I).

Biological Activity. The compounds synthesized were tested for cytotoxic and antiviral activities in cell culture. The testing methods used in this study have been described previously.[16] The preliminary results are shown in Table I. For comparison, the effective doses of BCNU,[14] 1,3-bis(2-chloroethyl)-1-nitrosourea, and that of *N*-deacetyl-*N*-(methylcarbamoyl)ethylthiocolchicine[17] are also included in Table I. All compounds synthesized in this study inhibited the growth of both murine leukemia L1210 cells and of murine sarcoma 180 cells in vitro to about the same extent as the starting material, *N*-deacetylmethylthiocolchicine (**1**), but about 100 times more than BCNU. This indicated that a modification of **1** to nitrosourea analogues did not alter the original biological activity due to **1**. On the other hand, the modified analogues may in certain situations have the advantage of also possessing the biological function of the nitrosourea moiety. The ED_{50} for the nitrosourea analogues **2**, **3**, **4**, and **5** were 1.6, 1.2, 3.3, and 1.8×10^{-8} M for L1210 cells and 3.0, 2.7, 2.9, and 2.6×10^{-8} M for S-180 cells, respectively.

The compounds were also tested for antiviral activity against herpes simplex virus type 1 in Vero cells. The established antiviral agent 5-iodo-2′-deoxyuridine was used as the positive control. The results are shown in the last column of Table I. The antiviral activities are in the range of 72 to 86% inhibition (less than 0.85 log). Thus, none of these agents is significantly inhibitory relative to 5-iodo-2′-deoxyuridine (2.2 log decrease in plaque-forming units).

The alkylating activities of compound **3** and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) were determined and compared by the procedures of Wheeler et al.[18,19] At least three determinations were made for each compound, and the standard error was less than 10% of the mean in all cases. The results indicated that the ratio of the alkylating activity of **3** to that of BCNU was 1.4 to 1.0. This may indicate that the rate of generating the chloroethyl carbonium ion from the degradation of **3** was somewhat faster than the rate of generating the same carbonium ions from the degradation of BCNU. Wheeler et al.[18] have reported after studying a series of 1-(2-haloethyl)-1-nitrosoureas that the alkylating activity, which may reflect in an inverse manner the chemical half-life of the nitrosourea, is an important factor in yielding a desirable therapeutic index.

Experimental Section

Melting points were measured with a Thomas-Hoover Unimelt apparatus and are not corrected. TLC was performed on plastic films coated with silica gel Merck F-254 (EM Laboratories, Inc., Elmsford, NY) with 1:4 (v/v) ethanol–ethyl acetate (solvent A) or with ethyl acetate. Elementary analyses were performed by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values. The UV spectra were recorded on a Beckman Model 25 spectrophotometer. The ^{1}H NMR spectra were obtained on a Bruker 270 HX spectrometer at 270 MHz at a concentration of 2 mM in chloroform-*d* with Me_4Si as the internal reference. Chemical shifts (δ) are reported in parts per million and coupling constants (J) in hertz.

***N*-Deacetyl-*N*-[(2-chloroethyl)carbamoyl]methylthiocolchicine (2).** To a solution of *N*-deacetylmethylthiocolchicine[10] (**1**; 0.95 g, 2.5 mmol) in 30 mL of $MeOH-H_2O$ (2:1) at 0 °C was added slowly 2-chloroethyl isocyanate (0.43 g, 4 mmol). The reaction was followed with TLC on silica gel eluted with solvent A. The reaction was quick but incomplete. An excess 2-chloroethyl isocyanate was added. After stirring for 2 h, the reaction mixture was concentrated and chromatographed on a column of silica gel and eluted with solvent A. The fractions were examined by TLC and similar fractions were combined. Thus, two main fractions were collected. The first fraction was evaporated to afford a yellow solid. This material was recrystallized from ethyl acetate to give pure **2** (0.63 g, 52%): mp 150 °C dec; TLC R_f 0.80 (solvent A), 0.34 (EtOAc); UV λ_{max} (EtOH) 380, 290, 255 nm ($\epsilon \times 10^{-4}$ 1.89, 1.17, and 2.13); NMR δ 7.71 (s, 1 H, H-8), 7.39 (d, 1 H, $J_{11,12}$ = 10.1 Hz, H-12), 7.15 (d, 1 H, H-11), 6.79 (d, 1 H, $J_{7,13}$ = 7.9 Hz, NH-13), 6.55 (s, 1 H, H-4), 6.01 (t, 1 H, $J_{15,1'}$ = 7.5 Hz, NH-15), 4.69 (m, 1 H, H-7), 3.95, 3.91, 3.68 (3 s, 3 H each, OCH_3 of C-2, C-3, and C-1),[9] 3.51 (t, 2 H, $J_{1',2'}$ = 7.0, CH_2-2′), 3.3 (m, 2 H, CH_2-1), 2,47 (s, 3 H, SCH_3), 2.47, 2.36 (br m, 2 H each, CH_2-6 and CH_2-5 partially overlapped with SCH_3). Anal. ($C_{23}H_{27}ClN_2O_5S$) C, H, N; C: calcd, 57.67; found, 56.94. The second fraction was evaporated to afford a pale yellow foam of unreacted starting material **1** (0.33 g, 35%): TLC R_f 0.18 (solvent A), 0.04 (EtOAc).

***N*-Deacetyl-*N*-[*N*-(2-chloroethyl)-*N*-nitrosocarbamoyl]methylthiocolchicine (3).** To an ice-cooled solution of **2** (0.50 g, 1.04 mmol) in 30 mL of 50% aqueous acetic acid was added sodium nitrite (0.15 g, 2.2 mmol). The reaction mixture was stirred at 0 °C for 1 h and neutralized with sodium bicarbonate solution and then extracted with chloroform (3 × 50 mL). The chloroform layer was concentrated and chromatographed on silica gel with elution by ethyl acetate. The major fraction was evaporated to dryness and rubbed with ethanol to afford a pale yellow powder of **3** (0.36 g, 68%): mp 174 °C dec; TLC R_f 0.92 (solvent A), 0.78 (EtOAc); UV λ_{max} (EtOH) 380, 285, 255 nm ($\epsilon \times 10^{-4}$ 2.04, 1.72, 2.67); NMR δ 7.57 (d, 1 H, $J_{7,13}$ = 7.5 Hz, NH-13), 7.32 (d, 1 H, $J_{11,12}$ = 10.6 Hz, H-12), 7.30 (s, 1 H, H-8), 7.07 (d, 1 H, H-11), 6.59 (s, 1 H, H-4), 4.76 (m, 1 H, H-7), 4.13 (m, 1 H, J_{gem} = 13.6 Hz, $J_{1',2'}$ = 7.1 Hz, H-1′a), 4.02 (m, 1 H, $J_{1',2'}$ = 7.1 Hz, H-1′b, overlapped with OCH_3), 3.96, 3.93, 3.70 (3 s, 3 H each, OCH_3 of C-2, C-3, and C-1), 3.40 (t, 2 H, CH_2-2′), 2.60, 2.51 (m, 2 H each, CH_2-6 and CH_2-5), 2.44 (s, 3 H, SCH_3). Anal. ($C_{23}H_{26}ClN_3O_6S$) C, H, N.

***N*-Deacetyl-*N*-(methylcarbamoyl)methylthiocolchicine (4).** Methyl isocyanate (0.20 g, 3.5 mmol) was added to a solution of **1** (0.50 g, 1.3 mmol) in 20 mL of $MeOH-H_2O$ (2:1) at 0 °C. The reaction was completed in 5 min as shown by TLC. The reaction mixture was evaporated to dryness and extracted with $CHCl_3$–

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H_2O. The chloroform layer was evaporated to afford a pale yellow powder. This was recrystallized from ether–ethyl acetate to give product 4 (0.55 g, 96%): mp 165 °C dec; TLC R_f 0.58 (solvent A), 0.06 (EtOAc); UV λ_{max} (EtOH) 380, 290, 255 nm ($\epsilon \times 10^{-4}$ 1.92, 1.20, 2.13); NMR δ 7.67 (s, 1 H, H-8), 7.38 (d, 1 H, $J_{11,12}$ = 10.1 Hz, H-12), 7.14 (d, 1 H, H-11), 6.79 (d, 1 H, $J_{7,13}$ = 7.1, NH-13), 5.52 (br s, 1 H, NH-15), 4.66 (m, 1 H, H-7), 3.95, 3.91, 3.68 (3 s, 3 H each, OCH_3 of C-2, C-3, and C-1), 2.70 (s, 3 H, NCH_3), 2.46 (s, 3 H, SCH_3), 2.49, 2.33 (br m, 2 H each, CH_2-6 and CH_2-5). Anal. ($C_{22}H_{26}N_2O_5S$) C, H, N; C: calcd, 61.37; found, 59.88.

***N*-Deacetyl-*N*-(*N*-methyl-*N*-nitrosocarbamoyl)methylthiocolchicine (5).** Sodium nitrite (0.20 g, 2.9 mmol) was added slowly to a solution of 4 (0.48 g, 1.1 mmol) in 40 mL of 50% aqueous acetic acid at 0 °C. The reaction mixture was neutralized with sodium bicarbonate solution and extracted with chloroform (3 × 50 mL). The chloroform layer was washed with water, dried over anhydrous magnesium sulfate, and then evaporated to dryness. This was dissolved in a minimum amount of ethyl acetate to yield pale yellow rectangular prisms of 5 (0.43 g, 85%) on standing at room temperature overnight: mp 177 °C dec; TLC R_f 0.88 (solvent A), 0.75 (EtOAc); UV λ_{max} (EtOH) 380, 285, 253 nm ($\epsilon \times 10^{-4}$ 1.95, 1.23, 2.58); NMR δ 7.56 (d, 1 H, $J_{7,13}$ = 6.6 Hz, NH-13), 7.32 (d, 1 H, $J_{11,12}$ = 7.1 Hz, H-12), 7.31 (s, 1 H, H-8), 7.07 (d, 1 H, H-11), 6.58 (s, 1 H, H-4), 4.75 (m, 1 H, H-7), 3.96, 3.92, 3.70 (3 s, 3 H each, OCH_3 of C-2, C-3, and C-1), 3.10 (s, 3 H, NCH_3), 2.61, 2.50, 2.36, 2.03 (4 m, 1 H each, CH_2-6 and CH_2-5), 2.44 (s, 3 H, SCH_3). Anal. ($C_{22}H_{25}N_3O_6S$) C, H, N.

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Crystal Structures of Calcium Channel Antagonists: 2,6-Dimethyl-3,5-dicarbomethoxy-4-[2-nitro-, 3-cyano-, 4-(dimethylamino)-, and 2,3,4,5,6-pentafluorophenyl]-1,4-dihydropyridine

A. M. Triggle, E. Shefter, and D. J. Triggle*

Department of Pharmaceutics and Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260. *Received April 10, 1980*

The crystal structures of 2,6-dimethyl-3,5-dicarbomethoxy-4-(2-nitrophenyl)-1,4-dihydropyridine (Nifedipine) and the 3-cyano-, 4-(dimethylamino)- and 2,3,4,5,6-pentafluorophenyl derivatives were determined. The 1,4-dihydropyridine ring in all four compounds has a boat-type conformation with varying degrees of puckering at the C4 position. Increasing distortion from planarity at this position shows a limited correlation with decreasing biological activity, determined as the ability to inhibit the Ca^{2+}-dependent muscarinic-induced mechanical responses of guinea pig ileal longitudinal smooth muscle.

The regulation of intracellular Ca^{2+} concentration is of fundamental significance to a host of cellular processes, including excitation–contraction and stimulus–secretion coupling.[1-3] Entry of extracellular Ca^{2+} can occur through a variety of mechanisms, including the use of Ca^{2+} channels. These channels can be defined in terms of ion selectivity, electrophysiological properties, and through the use of antagonists.[2,4] Amongst these antagonists are 1,4-dihydropyridines, including Nifedipine [2,6-dimethyl-3,5-dicarbomethoxy-4-(2′-nitrophenyl)-1,4-dihydropyridine], which is one of the most potent of the Ca^{2+} antagonists and is a powerful negative inotropic and smooth-muscle relaxant species.[2,4-6] Many 1,4-dihydropyridines related to Nifedipine have been synthesized, but few attempts have been made to generate a quantitative structure–activity relationship.[2,7,8] In this paper we report the solid-state structure of Nifedipine and three related compounds as a contribution toward the definition of a structure–activity relationship for this important class of compounds.

Experimental Section

Crystals suitable for single-crystal diffraction studies of these compounds were grown from methanol. The crystallographic parameters measured for these crystals are listed in Table I. X-ray diffraction intensity data were collected by the stationary counter–stationary crystal technique using Ni-filtered Cu Kα radiation and balanced Ni–Co filters. The data collection extended to 100 or 110° in 2θ. The measured intensities were corrected for α_1–α_2 splitting, absorption, and Lorentz-polarization effects. The number of reflections measured for each compound and the number whose intensities were significantly above background (noted as observed reflections) are given in Table I.

The structures were solved by "direct methods" using the program MULTAN.[9] They were refined by difference electron-density maps and by lease-squares analysis using a block diaganol approximation to the normal equations. The hydrogens were included in the final stages of least-squares refinement. The nonhydrogen atoms were refined with anisotropic thermal pa-

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