Synthesis and Antineoplastic Activity of 5-Aryl-2,3-dihydropyrrolo[2,1-*b*]thiazole-6,7-dimethanol 6,7-Bis(isopropylcarbamates)

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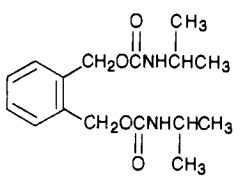
A series of 1-thia analogues of the pyrrolizine bis(carbamate) **9** (NSC-278214), namely 5-aryl-2,3-dihydropyrrolo-[2,1-*b*]thiazole-6,7-dimethanol 6,7-bis(isopropylcarbamates) (**7a–d**), were prepared by multistep syntheses from the proline analogue thiazolidine-2-carboxylic acid. The compounds were tested for growth inhibitory activity with the HL-60 human promyelocytic leukemia cell line. Three of the compounds had antileukemic activity equal to that of **9**, while a 4-chlorophenyl analogue was approximately 75% more potent. A simple aromatic derivative, 1,2-benzenedimethanol 1,2-bis(isopropylcarbamate) (**8**), had no activity in this system. Antitumor activity was also tested in a colony formation assay with HT-29 human colon carcinoma cells. Compounds **7a–d** reduced relative cell survival by over 3 logs at a concentration of 300 μM (2-h exposure), while a comparable inhibition was observed with 150 μM **9**. Hence compounds **7a–d** retain significant antineoplastic activity.

On the basis of the antitumor activity of a number of polyfunctional naturally occurring compounds including the mitomycins and some pyrrolizidine alkaloids, Anderson et al.[1-3] synthesized and showed that 5-substituted 2,3-dihydro-1*H*-pyrrolizine-6,7-dimethanol diesters exhibited antileukemic activity. It was also reported that bis(*N*-alkylcarbamate) derivatives of 5-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-pyrrolizine-6,7-dimethanol had potent antileukemic activity in the in vivo P388 assay. The most promising compound of this class was reported to be 5-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-pyrrolizine-6,7-dimethanol 6,7-bis(isopropylcarbamate) (**9**, NSC-278214), which had a broad spectrum of antitumor activity against murine tumors and human tumor xenografts. However, the latter compound suffers from having undesirable toxicity and solubility characteristics. In continuation of our interest in the chemistry of thiazolidine-2- and -4-carboxylic acids[4,5] we have synthesized and examined the antineoplastic activity of a series of 1-thia analogues of **9**, namely 5-aryl-2,3-dihydropyrrolo[2,1-*b*]thiazole-6,7-dimethanol 6,7-bis(isopropylcarbamates) (**7a–d**).



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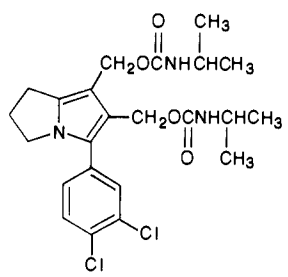

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Chemistry

Thiazolidine-2-carboxylic acid (**1**) was first reported by Fourneau et al.[6] However, no synthetic detail was given. We report an improved method for preparation of this important proline analogue. The amides (**2**, R = alkyl or aryl) were prepared by the reaction of **1** with the appropriate acid chloride in basic solutions (Scheme I). 3-Acetylthiazolidine-2-carboxylic acid (**2a**) was prepared upon the reaction of acetic anhydride with **1** in hot water (see Table I). The reaction of amides **2** with dimethyl acetylenedicarboxylate (DMAD) in the presence of acetic anhydride provides mesoionic oxazolone intermediates **3**, which according to Huisgen pyrrole synthesis[7] would undergo 1,3-dipolar cycloaddition to give unstable interme-

Table I. Percent Yields and Melting Points for **2a–e**

compd	% yield	mp, °C	formula[a]
2a	85	125–126	$C_6H_9NO_3S$
2b	94	109–110	$C_{11}H_{11}NO_3S$
2c	98	128–129	$C_{11}H_{10}FNO_3S$
2d	96	115–116	$C_{11}H_{10}ClNO_3S$
2e	97	134–135	$C_{11}H_9Cl_2NO_3S$

[a] All the compounds in this table gave satisfactory analysis for C, H, and N (±0.4%).

Table II. Percent Yields and Melting Points for Compounds **5–7**

compd	% yield	mp, °C	formula[a]
5a	62[b]	124–125	$C_{11}H_{13}NO_4S$
5b	82	137–138	$C_{16}H_{15}NO_4S$
5c	92	173–174	$C_{16}H_{14}FNO_4S$
5d	74	162–163	$C_{16}H_{14}ClNO_4S$
5e	80	151–152	$C_{16}H_{13}Cl_2NO_4S$
6a	78	100–110	$C_9H_{13}NO_2S$
6b	65	108–109	$C_{14}H_{15}NO_2S$
6c	97	96–97	$C_{14}H_{14}FNO_2S$
6d	88	127–128	$C_{14}H_{14}ClNO_2S$
6e	87	142–413	$C_{14}H_{13}Cl_2NO_2S$
7a	92	76–78	$C_{22}H_{29}N_3O_4S$
7b	95	87–89	$C_{22}H_{28}FN_3O_4S$
7c	80	83–84	$C_{22}H_{28}ClN_3O_4S$
7d	90	64–66	$C_{22}H_{27}Cl_2N_3O_4S$

[a] All compounds in this table gave satisfactory analysis for C, H, and N (±0.4%). [b] Prepared also by method B, yield 51%, mp 124–125 °C.

Table III. Inhibition of Growth of HL-60 Human Leukemia Cells[a]

compd	IC50, μM	potency[b]
9	1.5	1.00
7a	1.5	1.00
7b	1.6	0.94
7c	0.85	1.76
7d	1.9	0.79
8	>300	<0.005

[a] HL-60 cells were suspended in RPMI 1640 medium containing 10% heat inactivated fetal bovine serum (GIBCO) at 10^5/mL. Cell concentrations were determined with a Coulter Counter, and the IC50 was defined as the concentration of drug required to reduce cell number on day 7 by 50%. Values are results from three experiments. [b] Potency relative to **9**.

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(1) Anderson, W. K.; Corey, P. F. *J. Med. Chem.* **1977**, *20*, 812.

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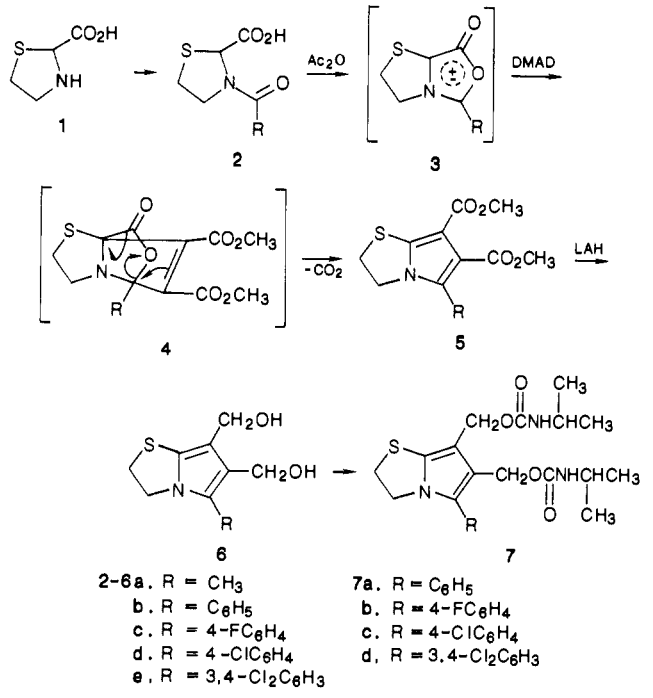
Scheme I



diates 4, which rapidly eliminate carbon dioxide to give diesters 5. Lithium aluminium hydride reduction of diesters 5 gave the corresponding diols 6, which afford the antileukemic carbamates 7 upon the reaction with isopropyl isocyanate. Compounds 5–7 are reported in Table II.

Biological Results

The effect of the synthesized compounds on the growth of HL-60 human leukemia cells in suspension culture is shown in Table III. Compounds **7a**, **7b**, and **7d** were approximately equitoxic to the HL-60 cells as compared to **9**. Compound **7c** exhibited an increased potency of roughly 75%. To examine the in vitro antileukemic activity of the simplest six-membered aromatic congener of **9** as compared to new antileukemic agents reported in this paper, 1,2-benzenedimethanol 1,2-bis(isopropylcarbamate) (**8**) was synthesized by the reaction of isopropyl isocyanate with 1,2-benzenedimethanol and tested. The inactivity of this compound in our test system suggests that the active compounds probably do not act as simple alkylating agents through their carbamoyl moieties. The presence of the biaryl system may be important for the biological activity of these compounds.[3]

Compounds **7a–d** also were cytotoxic to HT-29 human colon carcinoma cells (Figure 1). Approximately a 4 log cell kill resulted from a 2-h treatment at a concentration of 300 μM **7a–d**; a similar inhibition was observed with 150 μM **9**.

The synthesis and antitumor activity evaluation of 5-aryl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6,7-dimethanol 6,7-bis-

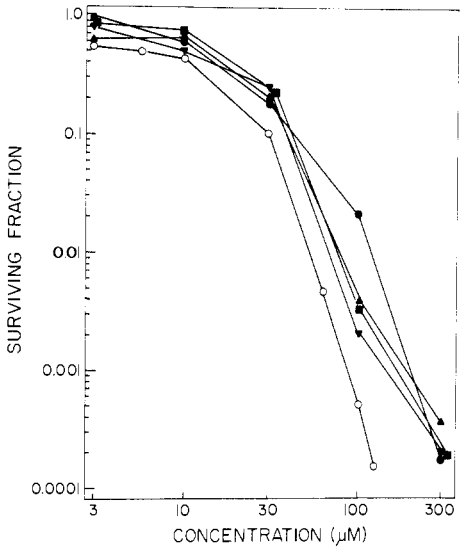


Figure 1. For each experiment, 200–20 000 HT-29 cells were plated in triplicate in 60-mm petri dishes and allowed to attach to the substrate overnight. The indicated concentrations of drugs were added and incubated for 2 h at 37 °C. The drug-containing medium was removed, and the cells were washed with phosphate-buffered saline and resuspended in fresh medium. After an additional 10 days, colonies were stained and counted manually. Surviving fraction is expressed relative to that of control cells; cloning efficiency of control cells was 57% ± 7%. Compounds tested were (○) **9**, (●) **7a**, (■) **7b**, (▲) **7c**, (▼) **7d**.

(carbamate)analogues of compounds **7** are in progress.

Experimental Section

Melting points (uncorrected) were taken with a Fisher-Johns apparatus. NMR spectra were determined for deuteriochloroform solutions containing 1% tetramethylsilane, unless otherwise specified, with a Varian M-360 spectrophotometer. Analyses were done by Schwarzkopf Microanalytical Laboratory.

Thiazolidine-2-carboxylic Acid (1). The following method gave excellent results: To a stirring solution of glyoxylic acid monohydrate (4.6 g, 0.05 mol) (or its equivalent of 50% aqueous solution), 2-aminoethanethiol hydrochloride (5.68 g, 0.05 mol) in ethanol (30 mL) and pyridine (8 mL) were added. After being stirred for 2 h, the crystalline mass was filtered and dried. It was recrystallized from 70% EtOH, giving white prisms (6.12 g, 92%): mp 181–182 °C dec (lit.[6] mp 181 °C dec); NMR (CF_3COOH) δ 3.32 (m, 2 H, CH_2), 3.98 (m, 2 H, CH_2), 5.65 (s, 1 H, CH). Anal. ($C_4H_7NO_2S$) C, H, N.

3-Acetylthiazolidine-2-carboxylic Acid (2a). To a suspension of acid 1 (2.66 g, 0.02 mol) in water (8 mL) was added acetic anhydride (8 mL) gradually, and the mixture was warmed at 90 °C while being stirred. After a clear solution was obtained, it was heated for 1 h. Evaporation under reduced pressure gave an oil to which methanol (10 mL) and ether (20 mL) were added, and the mixture was refrigerated for 24 h to give white crystals (2.62 g, 75%): mp 125–126 °C; NMR (CF_3COOH) δ 2.28 (s, 3 H, CH_3), 3.28 (m, 2 H, CH_2), 3.91 (m, 2 H, CH_2), 5.61 (s, 1 H, CH). Anal. ($C_6H_9NO_3S$) C, H, N.

3-Benzoylthiazolidine-2-carboxylic Acid (2b). To an ice-cold stirring solution of 1 (1.33 g, 0.01 mol) in 2 N NaOH (6 mL) were added benzoyl chloride (1.54 g, 0.011 mol) and 2 N NaOH (6 mL) in five equal and alternate portions over 15 min. The solution was acidified with HCl. A gum was obtained, which was extracted with AcOEt, washed with H_2O, and dried over $MgCl_2$. Evaporation of the solvent gave a solid. It was recrystallized from AcOEt–hexane, giving white crystals (2.3 g, 97%): mp 109–110 °C; NMR δ 3.31 (m, 2 H, CH_3), 3.88 (m, 2 H, CH_2), 5.39 (s, 1 H, CH), 7.89 (s, 5 H, C_6H_5), 10.52 (s, 1 H, COOH). Anal. ($C_{11}H_{11}NO_3S$) C, H, N.

Compounds **2c–e** were prepared similarly (see Table I).

Dimethyl 2,3-Dihydro-5-methylpyrrolo[2,1-*b*]thiazole-6,7-dicarboxylate (5a). A. From Acid 2a. A mixture of acid **2a** (0.7 g, 4 mmol), DMAD (2 mL), and acetic anhydride (4 mL)

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(6) Fourneau, J. P.; Efimowsky, O.; Gaignlault, J.-C.; Jaquier, R.; Le Ridant, C. *C. R. Seances Acad. Sci., Ser. C* **1971**, *272*, 1515.
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was heated in an oil bath at 120 °C for 1 h. Flash evaporation of the reaction product gave a solid. It was recrystallized from ether to give pure diester **5a** (635 mg, 62%): mp 124–125 °C; NMR δ 2.55 (s, CH_3, 3 H), 3.85 (m, CH_2, 2 H), 4.01 (s, OCH_3, 3 H), 4.03 (s, OCH_3, 3 H), 4.22 (m, CH_2, 2 H). Anal. ($C_{11}H_{13}NO_4S$) C, H, N.

Compounds **5b–e** were prepared similarly. They were recrystallized from MeOH (see Table II).

B. From 1. A mixture of 1 (2.66 g, 0.02 mol), DMAD (4 mL), and acetic anhydride (8 mL) were heated (120 °C for 1 h), and the product was purified as described in method A giving pure diester **5a** (2.64 g, 51%), mp 124–125 °C.

5-(4-Fluorophenyl)-2,3-dihydropyrrolo[2,1-*b*]thiazole-6,7-dimethanol (6c). Diester **5c** (1.342 g, 4 mmol) was dissolved in CH_2Cl_2 (20 mL) and dropwise added to a molar solution of LAH in ether (10 mL), while the solution was being stirred at room temperature. The reaction mixture was then refluxed for 1 h. It was then cooled to ice-bath temperature, and ether saturated with water (10 mL) and finally water (1 mL) were added. The mixture was filtered, and the precipitate was washed twice with hot CH_2Cl_2 and finally with hot acetone. Evaporation of the combined solvents gave a white solid, which was recrystallized from CH_2Cl_2 to give a white crystalline powder of **6c** (1.152 g, 97%): mp 96–97 °C; NMR δ 3.45 (br, 2 H, OH), 3.72 (m, 2 H, NCH_2), 4.11 (m, 2 H, SCH_2), 4.40 (d, J = 6 Hz, 2 H, CH_2OH), 4.57 (d, J = 6 Hz, 2 H, CH_2OH), 7.45 (m, 4 H, C_6H_4). Anal. ($C_{14}H_{14}FNO_2S$) C, H, N.

All other compounds **6** were prepared similarly and recrystallized from CH_2Cl_2 (see Table II).

5-(3,4-Dichlorophenyl)-2,3-dihydropyrrolo[2,1-*b*]thiazole-6,7-methanol 6,7-Bis(isopropylcarbamate) (7d). A stirring mixture of diol **6d** (330 mg, 1 mmol), triethylamine (2 drops), and isopropyl isocyanate (0.5 mL, 10 equiv) in CH_2Cl_2 (10 mL) was refluxed for 24 h under nitrogen. The solvent was evaporated under reduced pressure. To the residue was added hexane (20 mL), and the precipitate was filtered. It was dissolved in CH_2Cl_2 (5 mL) and filtered. To the filtrate was added hexane to produce turbidity. Refrigeration gave pure **7d** (450 mg, 90%): mp 64–66 °C; NMR δ 1.15 (d, J = 5 Hz, 12 H, CH_3), 2.85–3.24 (m, 2 H, CH), 3.76 (m, 2 H, NCH_2), 4.21 (m, 2 H, SCH_2), 4.85 (s, 2 H, CH_2), 5.11 (s, 2 H, CH_2), 7.98–8.23 (m, 3 H, C_6H_3). Anal. ($C_{22}H_{27}Cl_2N_3O_4S$) C, H, N.

All other compounds **7** were prepared similarly (see Table II).

1,2-Benzenedimethanol 1,2-Bis(isopropylcarbamate) (8). A solution of 1,2-benzenedimethanol (500 mg, 3.6 mmol) and isopropyl isocyanate (1 mL, 10 equiv) in CH_2Cl_2 (15 mL) containing trimethylamine (5 drops) was refluxed for 24 h under nitrogen. After cooling, ether (5 mL) and hexane (20 mL) were added, and the fine needles were filtered and recrystallized from ether–hexane as silky fine needles (1 g, 90%): mp 150–151 °C; NMR (CF_3COOH) δ 1.11 (d, J = 6 Hz, 12 H, CH_3), 3.02 (m, 2 H, CH), 5.21 (s, 4 H, CH_2), 7.91 (m, 4 H, C_6H_4). Anal. ($C_{16}H_{24}N_2O_4$) C, H, N.

Biological Studies. HL-60[8] human promyelocytic leukemia cells were obtained from Dr. R. Gallo. Cells were suspended in RPMI 1640 medium containing 10% heat inactivated fetal bovine serum (GIBCO) and incubated in a humidified 10% CO_2 atmosphere at 37 °C. For each experiment, cells were resuspended at 10^5/mL, and the indicated concentration of drug was added. Cell concentrations were determined with a Coulter Counter on day 7, and the IC50 was defined as the concentration of drug required to reduce cell number by 50%.

HT-29 cells[9] were provided by Dr. L. Augenlicht. Monolayer cultures were grown in RPMI 1640 with 10% fetal bovine serum, as described above. For each experiment, 200–20 000 cells were plated in 60-mm petri dishes and allowed to attach to the substrate overnight. The indicated concentrations of drugs were added and incubated for 2 h at 37 °C. The drug-containing medium was removed, and the cells were washed with phosphate-buffered saline and resuspended in fresh medium. After an additional 10 days, colonies were stained and counted manually. Cloning efficiency of control cells was 57% ± 7%.

Compound **9** was obtained from the Drug Synthesis & Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. Drugs were dissolved in a small volume of DMSO and added to the cell cultures such that the maximum concentration of DMSO in the culture was 0.2%; this concentration of DMSO did not affect cell growth.

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Registry No. 1, 16310-13-7; **2a**, 51131-84-1; **2b**, 114199-22-3; **2c**, 114199-23-4; **2d**, 114199-24-5; **2e**, 114199-25-6; **5a**, 114199-26-7; **5b**, 114199-27-8; **5c**, 114199-28-9; **5d**, 114199-29-0; **5e**, 114199-30-3; **6a**, 114199-31-4; **6b**, 114199-32-5; **6c**, 114199-33-6; **6d**, 114199-34-7; 6e, 114199-35-8; **7a**, 114199-36-9; **7b**, 114199-37-0; 7c, 114199-38-1; 7d, 114199-39-2; **8**, 114199-40-5; DMAD, 762-42-5; *p*-FC_6H_4COCl, 403-43-0; *p*-ClC_6H_4COCl, 122-01-0; 3,4-$(Cl)_2C_6H_3COCl$, 3024-72-4; glyoxylic acid, 298-12-4; 2-aminoethanethiol hydrochloride, 156-57-0; benzoyl chloride, 98-88-4; isopropyl isocyanate, 1795-48-8; 1,2-benzenedimethanol, 612-14-6.

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