solvent was allowed to evaporate from the reaction flask. The residue was heated in an oil bath at 120 °C for 30 min, cooled, and treated with 150 mL of water to dissolve the residue. The solution was treated with 13.7 g (0.16 mol) of sodium bicarbonate and refrigerated 15 h. A solid separated that was collected and recrystallized from 60 mL of 2-propanol/water (2:1) to give 4.3 g (48%) of 8, mp 264–265 °C. Compunds 3–12 were prepared by this method.

1,3-Dihydro-4-isonicotinoyl-5-ethyl-2*H*-imidazol-2-one 1-Oxide (13, Table I). In 140 mL of acetic acid were dissolved 5.0 g (0.023 mol) of 1,3-dihydro-4-isonicotinoyl-5-ethyl-2*H*-imidazol-2-one (8) and 6.5 mL of 30% (0.057 mol) hydrogen peroxide. The solution was warmed on the steam bath for 30 min after which the solvent was evaporated to 50 mL and cooled. A solid separated that was collected and discarded. The filtrate was evaporated to dryness and the residue chromatographed over silica gel with chloroform/methanol (4:1) as eluent to give compound 13. The material was purified by recrystallization from ethanol to give 0.16 g (3%): mp 274–275 °C; MS, *m/e* 233 (M^+).

1-Ethylimidazo[7,6-*c*][1,7]-naphthyridine-3,5,10(2*H*)-trione (14). In 100 mL of methylene chloride were placed 5.0 g (0.051 mol) of 1,3-dihydro-4-ethyl-2*H*-imidazol-2-one and 20.4 g (0.15 mol) of anhydrous aluminum chloride followed by 7.60 g (0.051 mol) of quinolinic anhydride. The mixture was heated, and the methylene chloride was allowed to distill from the reaction mixture. The stirred residue was heated to 120 °C for 30 min, cooled, and quenched with 200 mL of water. On standing 15 h, a solid separated that was collected and recrystallized from absolute ethanol to give 0.4 g (2%) of a yellow solid: mp >300 °C; MS, *m/e* 243 (M^+). Anal. ($C_{12}H_9N_3O_3$) C, H, N.

Pharmacological Methods. Dogs of either sex, weighing 9–23 kg, were anesthetized with 35 mg/kg iv of sodium pentobarbital. The lungs were ventilated artificially with a Bird Mark 7 respirator following tracheal intubation. The left femoral vein was cannulated for the injection of drugs. The left femoral artery was cannulated, and the cannula was advanced into the thoracic aorta to measure systemic blood pressure. Blood pressure was recorded with a pressure transducer (Statham P23GC). The chest was opened at the left fifth intercostal space, and the pericardium was cut to expose the heart. A calibrated Walton–Brodie strain gauge arch was sutured to the left ventricle to record cardiac contractile force. Heart rate was recorded from the EKG (lead II) with a tachograph (Grass, 7D). The dogs were allowed to stabilize for at least 30 min following surgical preparation. Measurements of cardiac contractile force, heart rate, and blood pressure were made at 5- to 10-min intervals before and after drug administration.

Experimental compounds were given intravenously by injection. Sufficient time was allowed between doses for the variables to return completely to basal levels. Only one compound was administered to any one animal. Equieffective doses were obtained by extrapolation from dose–response curves.

All imidazolones were dissolved in normal saline or 1 N NaOH and normal saline, pH 12-13. Isoproterenol was dissolved in normal saline containing 0.01% ascorbic acid.

Registry No. 3, 100791-02-4; 4, 100791-03-5; 5, 100791-04-6; 6, 84490-13-1; 7, 82709-64-6; 8, 84490-12-0; 9, 100791-05-7; 10, 100791-06-8; 11, 100791-07-9; 12, 100791-08-0; 13, 100791-09-1; 14, 100791-10-4; 2-$C_5H_4NCO_2K$, 25108-36-5; 3-$C_5H_4NCO_2K$, 16518-17-5; 4-$C_5H_4NCO_2K$, 25108-37-6; 3-CsH_3N-2-$ClCO_2K$, 97510-86-6; 3-C_5H_3N-4-$ClCO_2K$, 100790-99-6; 2-CsH_4NCOCl, 29745-44-6; 3-C_5H_4NCOCl, 10400-19-8; 4-C_5H_4NCOCl, 14254-57-0; 3-C_5H_3N-2-ClCOCl, 49609-84-9; 3-C_5H_3N-4-ClCOCL, 100791-00-2; 1,3-dihydro-4-methyl-2*H*-imidazol-2-one, 1192-34-3; 1,3-dihydro-4-ethyl-2*H*-imidazol-2-one, 83962-06-5; 1,3-dihydro-4-propyl-2*H*-imidazol-2-one, 100791-01-3; quinolinic anhydride, 699-98-9.

Antineoplastic Activity of 3′-(Chloroethyl)nitrosourea Analogues of 2′-Deoxyuridine and 2′-Deoxy-5-fluorouridine[1]

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The (chloroethyl)nitrosourea analgoues of 2′-deoxyuridine and 2′-deoxy-5-fluorouridine, 3′-[3-(2-chloroethyl)-3-nitrosoureido]-2′,3′-dideoxyuridine (3′-CdUNU, 7) and 3′-[3-(2-chloroethyl)-3-nitrosoureido]-2′,3′-dideoxy-5-fluorouridine (3′-CFdUNU, 8), have been synthesized by treatment of the corresponding 3′-amino nucleosides with chloroethyl isocyanate, followed by nitrosation of the resulting ureas. Nucleoside nitrosoureas 7 and 8 exhibited marked anticancer activity against L1210 leukemia in tumor-bearing mice. At an optimum dosage level of 40 mg/kg, 7 and 8 produced 90% and 60% "cures" (>60-day survivors), respectively. The structure–activity relationships are discussed.

It has been shown by Wheeler et al.[4] that alteration of the carrier portion of nitrosoureas affects their physical and chemical properties and by Schein et al.[5,6] that bone marrow toxicity is reduced in nitrosourea derivatives containing a glucose carrier. Several nitrosourea analogues of thymidine have been synthesized earlier in our laboratory,[7] and the synthesis of other nucleoside nitrosoureas has also been reported.[8-10] Among these compounds, not only 3′-[3-(2-chloroethyl)-3-nitrosoureido]-3′-deoxythymidine (3′-CTNU) is threefold more potent than 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in the inhibition of L1210 leukemia cells in culture[7] but also more strikingly pyrimidine deoxyribonucleosides (thymidine, 2′-deoxyuridine, 2′-deoxycytidine) specifically prevent the inhibitory effects of this nitrosourea analogue of thymidine.[11]

(1) This paper has been presented in part; see: Lin, T. S.; Prusoff, W. H. In "Abstracts of Papers", 188th National Meeting of the American Chemical Society, Philadelphia, Pa, Aug 26–31, 1984; American Chemical Society: Washington, DC, 1984; Abstr CARB 2.
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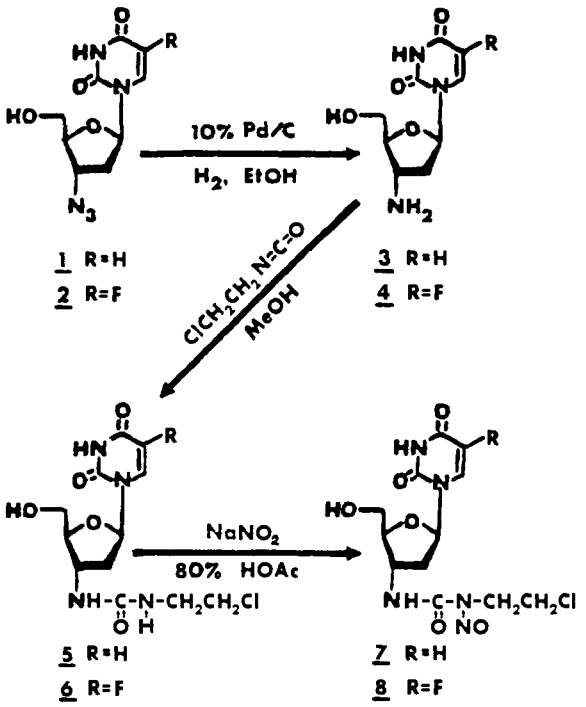
Scheme I



Furthermore, 3′-CTNU has produced "cures" (>60-days survivors) when administered i.p. as a single injection at the optimum dosage level of 40 mg/kg to mice bearing either L1210 or P388 leukemia.[12] On the basis of these findings we recently have synthesized the 3′-(chloroethyl)nitrosourea analogues of 2′-deoxyuridine and 2′-deoxy-5-fluorouridine, **7** (3′-CdUNU), and **8** (3′-CFdUNU), as potential anticancer agents, as well as for structure–activity relationship studies.

Chemistry

The (chloroethyl)nitrosourea analogues of 2′-deoxyuridine and 2′-deoxy-5-fluorouridine (**7**, **8**) have been synthesized by treatment of the respective 3′-aminonucleosides **3** and **4** with chloroethyl isocyanate in MeOH, followed by nitrosation of the resulting ureas **5** and **6** with $NaNO_2$ in 80% HOAc. Compounds **7** (3′-CdUNU) and **8** (3′-CFdUNU) were isolated and purified by silica gel column chromatography. The structures of ureas **5** and **6**, and nitrosoureas **7** and **8** were identified and characterized by NMR spectroscopy data and elemental analysis. the N_1 H and N_3 H in the (chloroethyl)urea moiety of **5** and **6** were split by the neighboring 3′-H in the ribo sugar ring and the methylene protons in the chloroethyl group and appeared as doublets at δ 6.60 and 6.55 and triplets at δ 6.15 and 6.13, respectively. In the nitrosoureas **7** and **8**, however, the N_1 H doublet shifted downfield at δ 9.12 and 9.13 while the N_3 H signal disappeared, indicating that the N_3 H has been replaced by a nitroso group. In compounds **6** and **8**, the 6-H in the pyrimidine ring was split by the 5-fluoro atom and appeared as a doublet at δ 8.26 and 8.31 with a J_{HF} values of 7.26 and 7.23 Hz, respectively. The starting 3′-aminonucleosides **3** and **4** were prepared by catalytic hydrogenation from the corresponding 3′-azidonucleosides 1 and **2**, which were synthesized by the methodology previously developed in our laboratory.[13,14]

Table I. Comparison of Several Nitrosourea Nucleosides with BCNU Relative to Their Half-Life, Alkylating Activity, Carbamoylating Activity, and Cytotoxicity against L1210 Cells in Vitro

compd	half-life,[a] min	alkylating[b] act.	carbomoylating[c] act.	ED_{50},[d] μM
BCNU	50.0	1.00	1.00	4.0
3′-CTNU	37.3	2.00	0.85	1.5
7 (3′-CdUNU)	27.3	1.65	0.85	12.5
8 (3′-CFdUNU)	36.1	1.78	0.77	2.5

[a] Half-life determined by incubation at 37 °C in PBS (pH 7.4). [b] Alkylating activity of all compounds is compared to BCNU; for BCNU, $\Delta A_{540}/120 = 0.42$. At least three determinations were made for each compound, and the standard deviation was less than 10% in all cases. [c] Carbamoylating activity of all compounds is compared to BCNU; for 3′-CTNU, 78% of 5′-AdThd was present as products other than unreacted 5′-AdThd. Three determinations were made for each compound with the standard deviation less than 10% of the mean in all cases. [d] ED_{50} values were estimated from dose–response curves complied from at least three separate experiments and represent the drug concentration needed to inhibit cell growth by 50%.

The synthetic sequence to these nucleoside ureas and nitrosoureas is outlined in Scheme I.

Biological Activity and Structure–Activity Relationships

The ED_{50} values of nitrosoureas **7**, **8**, 3′-CTNU, and BCNU against L1210 leukemia cells in culture were determined, and the results are listed in Table I. The chemical reactivities and the cytotoxicity of these nitrosoureas, 3′-CTNU, and BCNU were also compared. The half-life of each compound was determined by incubation in phosphate-buffered saline (pH 7.4) at 37 °C, followed by reversed-phase chromatographic analysis by the methodology of Brubaker and Prusoff.[15] A plot of log [nitrosourea] vs. time was linear in each case, indicating pseudo-first-order kinetics. The half-life (27.3 min) of **7** is about 25% less than that of the other two nucleoside nitrosoureas (**8**, 3′-CTNU), and although the difference is statistically significant, it is not very large. The site of structural variation (5-position of the pyrimidine ring) is isolated from inductive effects of resonance interaction with the site (3′-(chloroethyl)nitrosourea moiety) involved in the rate-determining step of the decomposition, and one would expect these three nucleoside nitrosoureas to exhibit similar half-lives. The alkylating activities of these nitrosoureas were measured by the extent of alkylation of 4-(4-nitrobenzyl)pyridine that occurs within 120 min at 37 °C and pH 6.4. The difference in alkylating ability of 3′-CTNU, **7**, and **8** is not considered to be significant. Alkylating activity is dependent in part upon the rate of decomposition of the nitrosoureas, since decompostion is a prerequisite for the formation of the alkylating species. There is no apparent correlation between the alkylating activities and the ED_{50} values. For example, compound **7**, which is the least cytotoxic, has an alkylating activity 65% greater than that of BCNU. It is possible that alkylating activity measured at pH 6 does not necessarily correspond to the degree of alkylation that occurs at physiological pH. Carbamoylating activity was determined by measurement of the extent of carbamoylation of 5′-amino-5′-deoxythymidine (5′-AdThd) upon incubation with the nitrosourea at 37 °C for 6 h in phosphate-buffered saline.[16] The carbamoylating activities fall within a relatively narrow range from 0.77 to 1.0 (Table I). There is

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Table II. Comparison of the ED_{50} Values of Several 3′-Amino Analogues of Pyrimidine Deoxyribonucleosides and Their Corresponding Nitrosourea Derivatives on the Replication of L1210 Cells in Vitro

compd	ED_{50},[a] μM
3′-CTNU	1.5
3′-AdThd	0.8
3′-CdUNU (7)	12.5
3′-AdUrd	18.0[b]
3′-CFdUNU (8)	2.5
3′-AFdUrd	15.0[b]

[a] The ED_{50} values were estimated from dose–response curves complied from at least two independent experiments and represent the drug concentration required to inhibit replication of L1210 by 50%. [b] Data from Lin et al.[13,14]

no correlation of carbamoylating activity with the ED_{50} values; the most active (3′-CTNU) and the least active compound (7) exhibit identical carbamoylating activity.

All of the compounds tested were cytotoxic as shown in Table I. There is no clear relationship between half-life, alkylating activity, or carbamoylating activity and the ED_{50} values. The cytotoxicity is clearly not related to the formation of the corresponding 3′-aminonucleoside analogues formed during decomposition (Table II). The ED_{50} for the 3′-aminodeoxyribonucleosides derived from compounds 7 and 8 are significantly greater than that of the nitrosourea analogues from which they could be derived from. The reverse relationship would have been expected if the activity were due to the aminonucleoside formed as a by-product of decomposition of the nitrosoureanucleosides.

Additional evidence for the active component not being the aminonucleosides are the findings that when 3′-AdUrd or 3′-AFdUrd were administered to mice bearing the L1210 leukemia no increase in survival of the treated mice relative to the untreated mice was found.[13,14]

However nitrosoureas 7 and 8 exhibited marked anticancer activity against L1210 leukemia in tumor-bearing mice, when given by a single intraperitoneal injection, 24 h after tumor inoculation. At an optimum dosage level of 40 mg/kg, 7 and 8 produced 90% and 60% "cures" (>60-days survivors), respectively. The findings are shown in Table III.

Although compound 7 has a relatively low alkylation activity and a relatively high ED_{50} value in vitro compared to that of the other two nucleoside nitrosoureas (3′-CTNU, 8), it has equally good anticancer activity in vivo. Clearly, the anticancer activity is a complex function of these and other variables.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. Thin-layer chromatography was performed on EM silica gel 60 F_{254} sheets (0.02 mm). The UV spectra were recorded on a Beckman-25 spectrophotometer, and the NMR spectra were taken on a Bruker WM-500 spectrophotometer at 500 MHz with Me_4Si as internal reference. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, 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.

3′-[3-(2-Chloroethyl)ureido]-2′,3′-dideoxyuridine (5). 2-Chloroethyl isocyanate (1.51 g, 14.0 mmol) was added slowly to a stirring solution of 3′-amino-2′,3′-dideoxyuridine[14] (3; 2.70 g, 11.8 mmol) in 100 mL of MeOH at 0 °C. Upon completion of the addition the reaction mixture was kept in refrigerator overnight. The insoluble material was removed by filtration. The filtrate was concentrated to a small volume and then chromatographed on a silica gel column ($CHCl_3$–EtOH, 4:1) to yield 1.74 g (44%) of product: mp, 155–157 °C dec; R_f 0.57 ($CHCl_3$–EtOH, 4:1) UV (MeOH) λ_{max} 260 nm (ε 9940); NMR (Me_2SO-d_6) δ 2.07–2.14 (m, 1 H, 2′-H_A), 2.14–2.26 (m, 1 H, 2′-H_B), 3.32 (t, 2 H, CCH_2Cl), 3.50–3.70 (m, 4 H, 5′-H and NCH_2C), 3.73 (m, 1 H, 3′-H), 4.10–4.21 (m, 1 H, 4′-H), 5.06 (br s, 1 H, 5′-OH, D_2O exchangeable), 5.63 (d, 1 H, 5-H), 6.11 (dd, 1 H, 1′-H, J = 6.33 and 6.28 Hz), 6.15 (t, 1 H, CONHC, D_2O exchangeable), 6.60 (d, 1 H, 3′-NHCON, D_2O exchangeable), 7.88 (d, 1 H, 6-H), 11.3 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{12}H_{17}N_4ClO_5$) C, H, N.

3′-[3-(2-Chloroethyl)ureido]-2′,3′-dideoxy-5-fluorouridine (6). 2-Chloroethyl isocyanate (1.39 g, 13.3 mmol) was added dropwise to a solution of 3′-amino-2′,3′-dideoxy-5-fluorouridine[14] (4; 2.70 g, 11.1 mmol) in MeOH (20 mL) at 0 °C. The reaction mixture was stirred at the same temperature for an additional 1 h until the reaction was complete (followed by TLC). The solution was then concentrated and chromatographed on a silica gel column ($CHCl_3$–MeOH, 4:1). The fraction containing pure 6 were combined. The solvents were evaporated to dryness to give a glassy residue that was crystallized from ether to produce 2.20 g (57%) of white solid: mp 145 °C dec; R_f 0.23 ($CHCl_3$–MeOH, 4:1); UV (MeOH) λ_{max} 270 nm (ε 8174); NMR (Me_2SO-d_6) δ 2.09–2.16 (m, 1 H, 2′-H_A), 2.17–2.28 (m, 1 H, 2′-H_B), 3.33 (t, 2 H, C-CH_2Cl), 3.51–3.59 (m, 4 H, 5′-H and NCH_2C), 3.73 (m, 1 H, 3′-H), 4.14–4.17 (m, 1 H, 4′-H), 5.19 (br s, 1 H, 5′-OH, D_2O exchangeable), 6.07 (dd, 1 H, 1′-H, J = 5.89 and 4.76 Hz), 6.13 (t, 1 H, CONHC, D_2O exchangeable), 6.55 (d, 1 H, 3′-NHCON, D_2O exchangeable), 8.26 (d, 1 H, 6-H, J_{HF} = 7.26 Hz), 9.78 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{12}H_{16}N_4ClFO_5$) C, H, N.

3′-[3-(2-Chloroethyl)-3-nitrosoureido]-2′,3′-dideoxyuridine (7). Sodium nitrite (0.62 g, 8.95 mmol) was added slowly to a stirred solution of urea 5 (1.49 g, 4.48 mmol) in 40 mL of 80% aqueous HOAc at 0 °C. The reaction mixture was stirred at the same temperature for another 4 h and then kept in the refrigerator overnight. The solution was treated with AG 50W-X8 (H^+) resin (6 g) to remove Na^+. The resin-containing mixture was stirred for 30 min and then filtered. The filtrate was concentrated to a glassy syrup and chromatographed on a silica gel column ($CHCl_3$–EtOH, 6:1). The fraction containing 7 were combined, and the solvents were removed in vacuo below 35 °C to give a glassy residue. The residue was dissolved in 20 mL of MeOH and then added slowly to a mixture of ether (250 mL) and hexane (150 mL) with stirring. The resulting solid precipitate was collected by filtration and dried immediately at room temperature under vacuum to afford 1.24 g (77%) of product. Compound 7 began to effervesce around 70 °C and decomposed at 165 °C: R_f 0.80

Table III. Effects of (Chloroethyl)nitrosourea Analogues of 2′-Deoxyuridine and 2′-Deoxy-5-fluorouridine on the Survival and Weight of Mice Bearing the L1210 Leukemia

compd	dosage,[a] mg/kg	av. days survival	T/C × 100[b]	wt at onset, g	wt at day 30, g	percent change in wt	percent and time of max wt loss
	none	8.4					
7 (3′-CdUNU)	20	20.4	243 (6/11)	18.8	22.0	+9	none
	40	31	369 (10/11)	19.0	22.1	+5	2%; days 6–8
	80	8.3	99 (1/5)	19.5	20.8	+13	21%; day 11
	none	8.4					
8 (3′-CFdUNU)	20	18.7	223 (3/10)	26.1	22.0	+4	none
	40	14	167 (6/10)	19.5	22.1	+5	3%; days 5–8
	80	9	107 (0/5)	20.5	21.3	+4	14%; day 9

[a] Drugs were administered by a single intraperitoneal injection, 24 h after tumor implantation. [b] T/C × 100 represents the ratio of the survival time of treated to control animals × 100 in the dying animals. The average survival time of the untreated tumor-bearing control animals was 8.4 days. The values in the parentheses represent the number of mice that survived >60 days relative to the number of mice treated.

($CHCl_3$–EtOH, 6:1); UV (EtOH) λ_{max} 260 nm; NMR (Me_2SO-d_6) δ 2.26–2.34 (m, 1 H, 2′-H_A), 2.38–2.48 (m, 1 H, 2′-H_B), 3.59–3.64 (m, 4 H, 5′-H and CCH_2Cl), 4.02 (m, 1 H, 4′-H), 4.11 [m, 2 H, $N(NO)CH_2C$], 4.52–4.60 (m, 1 H, 3′-H), 5.00–5.18 (br s, 1 H, 5′-OH, D_2O exchangeable), 5.66 (d, 1 H, 5-H), 6.23 (dd, 1 H, 1′-H, J = 6.41 and 6.50 Hz), 7.91 (d, 1 H, 6-H), 9.12 [d, 1 H, 3′-NHCON(NO), D_2O exchangeable], 11.3 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{12}H_{16}N_5ClO_6$) C, H, N.

3′-[3-(2-Chloroethyl)-3-nitrosoureido]-2′,3′-dideoxy-5-fluorouridine (8). To a solution of **6** (1.20 g, 3.40 mmol) in 10 mL of 80% aqueous HOAc was added sodium nitrite (0.48 g, 7.00 mmol) slowly at 0 °C. The reaction mixture was stirred at the same temperature for 4 h and then treated with AG 50W-X8 (H^+) resin (3.2 g) to remove Na^+. The resin was separated by filtration, and the filtrate was evaporated in vacuo to give a glassy residue. Compound **8** was isolated and purified by silica gel column chromatography using $CHCl_3$–EtOH (3:1) as eluting solvent system. The fractions containing pure **8** were combined, concentrated, and redissolved in a minimum amount of EtOH. The solution was then added slowly to a stirred mixture of ether–hexane (1:2). The resulting precipitate was collected by filtration and dried at room temperature under vacuum, to afford 0.5 g (34%). Compound **8** started to effervesce around 94 °C and decomposed at 162 °C: R_f 4.9 ($CHCl_3$–EtOH, 3:1); UV (MeOH) λ_{max} 2.69 nm (ε 10435); NMR (Me_2SO-d_6) δ 2.28–2.38 (m, 1 H, 2′-H_A), 2.41–2.48 (m, 1 H, 2′-H_B), 3.59–3.69 (m, 4 H, 5′-H and CCH_2Cl), 4.12 [t, 2 H, $N(NO)CH_2C$], 4.54–4.62 (m, 1 H, 3′-H), 4.14–4.17 (m, 1 H, 4′-H) 5.27 (br s, 1 H, 5′-OH, D_2O exchangeable), 6.22 (dd, 1 H, 1′-H, J = 4.70 and 4.92 Hz), 8.31 (d, 1 H, 6-H, J_{HF} = 7.23 Hz), 9.13 [d, 1 H, 3′-NHCON(NO), D_2O exchangeable), 11.8 (s, 1 H, 3′-NH, D_2O exchangeable). Anal. ($C_{12}H_{15}N_5ClFO_6$) C, H, N.

Assays for the Determination of Alkylation and Carbamoylating Activity. Alkylation activity was determined by the method of Wheeler et al.[4] and carbamoylation activity by the method of Brubaker et al.[13] Determinations of half-life of the compounds were performed as follows: a 100 μM stock solution of the nitrosourea in absolute ethanol was prepared and the concentration measured by UV spectroscopy. A 100-μL portion of the stock solution was pipetted into a 3-mL glass vial containing 1900 μL of phosphate-buffered saline (pH 7.4) maintained at 37 °C in a water bath. Immediately following addition of the nitrosourea the solution was vortexed for 5 s. Aliquots (15 μL) were removed at t = 0, 1, 2, 3, 4, 5, 8 and 24 h and analyzed by reversed-phase liquid chromatography.[15] The plot of log [nitrosourea] vs. time for each nitrosourea was linear, indicating pseudo-first-order kinetics in each case.

Biological Test Procedures. Mouse L1210 cells were maintained as suspension cultures in Fischer's medium supplemented with 10% horse serum at 37 °C in a humidified atmosphere of 5% CO_2–95% air. Under these conditions the generation time for L1210 cells is approximately 12 h. The test compound at the given concentration was added to L1210 cells (2×10^4 cells/mL), which were in their exponential phase of growth. The increase in cell number of the drug-free culture (control), as well as that of the cultures supplemented with the test compound, was determined after 24, 48, and 72 h of growth.

Transplantation of L1210 ascites cells was carried out by withdrawing peritoneal fluid from donor CDF_1 female mice bearing 7-day growths. The suspension was centrifuged for 2 min (1600g), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was determined with a Coulter particle counter, and the cell population was adjusted to 10^6 cells/mL. A 0.1-mL portion of the resulting cell suspension (containing approximately 10^5 cells) was injected intraperitoneally into each animal. The drug was administered by a single intraperitoneal injection, beginning 24 h after tumor implantation. The test compound was injected as a solution in isotonic saline. The drug was administered intraperitoneally in a volume of 0.25 mL. For any one experiment, animals were distributed into group of five or six mice of comparable weight and maintained throughout the course of the experiment of a suitable diet and water ad libitum. Controls given injections of comparable volume of vehicle (saline) were included in each experiment. Mice were weighed during the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity.

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Synthesis and Biological Evaluation of Cyclic Analogues of *l*-Carnitine as Potential Agents in the Treatment of Myocardial Ischemia[1]

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A series of cyclic rigid analogues of *l*-carnitine has been synthesized and examined for activity as substrates for the carnitine–acylcarnitine translocase, the enzyme that mediates transport of fatty acids into the mitochondria. Synthetic steps to seven of these analogues are described in this paper. Bioassay of these compounds is conducted in a preparation of isolated heart mitochondria that have been previously loaded with [^{14}C]-*l*-carnitine. Efflux of radioactivity from the mitochondria is then monitored in the presence of the compound being evaluated in order to assess the amount of enzyme activity initiated. The palmityl ester of *l*-*N*,*N*-dimethyl-*trans*-2-carboxy-4-hydroxypyrrolidinium chloride elicited a 13.63 and 63.07% efflux of [^{14}C]-*l*-carnitine at concentrations of 3 and 50 mM, respectively. This represents the first instance in which a nonnaturally occurring analogue of *l*-carnitine has been shown to undergo transport via this mitochondrial translocase, suggesting the possibility that cyclic carnitine analogues may find utility as agents in the treatment of myocardial ischemia.

It is well established that carnitine acts as a carrier molecule in the transport of fatty acids into the mitochondria and that this process is mediated by a mitochondrial enzyme known at the carnitine/acylcarnitine translocase.[2,3] This enzyme, acting in conjunction with two identical carnitine acyltransferases (CAT_1 and CAT_2) on opposite sides of the inner mitochondrial membrane,

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