N-(2-Benzoylphenyl)-N-methylglycine Hydrochloride (14). A mixture of 10.5 g (0.05 mol) of 2-(methylamino)benzophenone (**17**), 6.5 g (0.053 mol) of ethyl chloroacetate, and 5.8 g (0.055 mol) of anhydrous sodium carbonate was heated in a steel bomb at 180 °C for 10 h. The reaction mixture was partitioned between methylene chloride and water, and the methylene chloride layer was dried (Na_2SO_4) and concentrated under reduced pressure to give 15.8 g of oil. The oil was purified by chromatography on 300 g of silica gel to yield 7.6 g (51%) of **20** as an oil.

A mixture of 6.5 g (0.022 mol) of **20** and 200 mL of 3 N hydrochloric acid was heated at reflux under a nitrogen atmosphere for 1.5 h. The solution was concentrated under reduced pressure and the residue was partitioned between benzene and 250 mL of a 5% sodium bicarbonate solution. The aqueous layer was made slightly acidic with concentrated hydrochloric acid and was extracted with benzene. The benzene layer was dried (Na_2SO_4) and concentrated under reduced pressure to give 4.3 g of yellow gum. The residue was dissolved in ethyl ether and filtered, and the filtrate was treated with ethereal hydrochloric acid. The solid that crystallized was collected by filtration and recrystallized from 2-propanol–ethyl ether to yield 1.0 g (15%) of **14** as a white solid, mp 177–178 °C, which rapidly loses hydrochloric acid and turns yellow in color when exposed to moisture. Anal. ($C_{15}H_{16}ClNO_3$) C, H, N.

N-(2-Benzoyl-5-methylphenyl)alanine Ethyl Ester (32). By use of the above general procedure, a mixture of 10.5 g (0.05 mol) of 2-amino-4-methylbenzophenone,[5] 9.9 g (0.055 mol) of ethyl 2-bromopropionate, and 5.3 g (0.05 mol) of anhydrous sodium carbonate gave 9.0 g (58%) of **32** as pale yellow needles, mp 76.5–78.5 °C (cyclohexane–benzene). Anal. ($C_{19}H_{21}NO_3$) C, H, N.

α,7-Dimethyl-2-oxo-4-phenyl-1H-quinazoline-1-acetic Acid Ethyl Ester (33). A mixture of 5.0 g (0.016 mol) of **32**, 9.0 g (0.1 mol) of ethyl carbamate, and 0.5 g of zinc chloride was heated under a nitrogen atmosphere at 190 °C for 4 h and then an additional 4.5 g (0.05 mol) of ethyl carbamate and 0.5 g of zinc chloride was added and heating was continued for 3 h. The reaction mixture was cooled, diluted with chloroform (250 mL), and filtered, and the filtrate was washed with water (2 × 500 mL). The filtrate was dried ($MgSO_4$) and concentrated under reduced pressure, and the residue was adsorbed onto a silica gel column (40 g). Elution with ethyl ether (500 mL) afforded, after evaporation of the solvent, a light yellow solid, which was recrystallized to yield 1.9 g (35%) of **33** as a white solid, mp 174–175 °C (hexane–tetrahydrofuran). Anal. ($C_{20}H_{20}N_2O_3$) C, H, N.

1,2-Dihydro-α,7-dimethyl-2-oxo-4-phenyl-1-quinazolineacetic Acid (4). A suspension of 1.6 g (0.005 mol) of **33** in 50 mL of 6% potassium hydroxide solution was heated at reflux for 45 min. The resulting clear solution was cooled, diluted with water (50 mL), washed with ethyl ether (50 mL), and acidified to pH 5 with dilute hydrochloric acid. The precipitate was collected by filtration and twice recrystallized from ethanol to yield 0.6 g (39%) of **4** as a white solid, mp 249–250 °C dec. Anal. ($C_{18}H_{16}N_2O_3$) C, H, N.

Acknowledgment. We thank Dr. Larry Jaques and Ashby Johnson, John Forehand, and Malcolm Stone for the spectral and analytical data, Larry Wagner for this technical assistance in conducting the pharmacological assays, and Dr. Warren Dannenburg, Jeffrey Bailey, and Linda Bixler for providing the prostaglandin synthetase inhibition data. Helpful discussions with Dr. William J. Welstead, Jr. are greatly appreciated.

Registry No. **4**, 91409-57-3; **5**, 76477-50-4; **9**, 91409-58-4; **10**, 53491-43-3; **11**, 72504-22-4; **11**·HCl, 91409-59-5; **12**, 72504-38-2; **13**, 91409-61-9; **13**·HCl, 91409-60-8; **14**, 91409-63-1; **14**·HCl, 91409-62-0; **15**, 80099-62-3; **16**, 91409-64-2; **17**, 1859-76-3; **20**, 91409-65-3; **22**, 91409-66-4; **23**, 91409-67-5; **24**, 91409-69-7; **24**·HCl, 91409-68-6; **25**, 91409-70-0; **26**, 91409-71-1; **27**, 91409-72-2; **28**, 91409-78-8; **29**, 91409-73-3; **30**, 91423-94-8; **31**, 91409-74-4; **32**, 91409-75-5; **33**, 91409-76-6; ethyl chloroacetate, 105-39-5; 2-amino-4-methylbenzophenone, 4937-62-6; ethyl 2-bromopropionate, 535-11-5; ethyl carbamate, 51-79-6; 2-aminobenzophenone, 2835-77-0; ethyl 3-bromopropionate, 539-74-2; ethyl 3-bromo-2-methylpropionate, 59154-46-0; 3-aminobenzophenone, 2835-78-1; 4-aminobenzophenone, 1137-41-3; 2-benzoylphenol, 117-99-7; 2-phenoxyaniline, 2688-84-8; 2-amino-5-methylbenzophenone, 17852-28-7; 2-amino-5-methoxybenzophenone, 17549-79-0; 2-amino-5-chlorobenzophenone, 719-59-5; 2-amino-4′-methylbenzophenone, 36192-63-9; 2-amino-4′-methoxybenzophenone, 36192-61-7; 2-amino-4′-chlorobenzophenone, 2894-51-1; 2-amino-4′-bromobenzophenone, 1140-17-6; 2-amino-2′,4′-dichlorobenzophenone, 91409-77-7; 2-amino-4′-bromo-5-chlorobenzophenone, 60773-48-0; 2-amino-4′-bromo-5-chlorobenzophenone, 60773-48-0; prostaglandin synthetase, 9055-65-6.

Synthesis and Murine Antineoplastic Activity of Bis[(carbamoyloxy)methyl] Derivatives of Pyrrolo[2,1-*a*]isoquinoline[1]

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The synthesis of 4,5-dihydropyrrolo[2,1-*a*]isoquinolines is reported. A key intermediate in the synthesis of 8-methoxy-4,5-dihydropyrrolo[2,1-*a*]isoquinolines, 6-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (**6**), was prepared by using a regiospecific phenolic cyclization reaction. The P388 lymphocytic activity is reported for 1,2-bis(hydroxymethy)-5,6-dihydro-8-methoxy-3-methylpyrrolo[2,1-*a*]isoquinoline bis(isopropylcarbamate) (**11a**), 1,2-bis(hydroxymethyl)-5,6-dihydro-8-methoxy-3-methylpyrrolo[2,1-*a*]isoquinoline bis(cyclohexylcarbamate) (**11b**), 1,2-bis(hydroxymethyl)-5,6-dihydro-3-methylpyrrolo[2,1-*a*]isoquinoline bis(methylcarbamate) (**13a**), 1,2-bis(hydroxymethyl)-5,6-dihydro-3-methylpyrrolo[2,1-*a*]isoquinoline bis(ethylcarbamate) (**13b**), and 1,2-bis(hydroxymethyl)-5,6-dihydroxy-3-methylpyrrolo[2,1-*a*]isoquinoline bis(cyclohexylcarbamate) (**13c**); all of the compounds were active. Compound **11a** was tested in an expanded tumor panel and was shown to be active against B16 melanocarcinoma, $CD8F_1$ mammary, L1210 lymphoid leukemia, colon 38, and MX-1 human tumor breast xenograft systems.

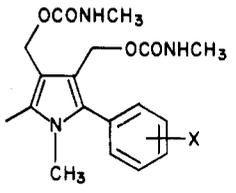
Recent reports on the significant antitumor activity of bis[(acyloxy)methyl]pyrrolizines and pyrroles have elicited a marked interest in this new class of agents. The rationale employed in the design of these compounds has, in part, been contingent upon the transmission of electronic effects from the phenyl ring to the pyrrole ring in these biaryl systems.[2] Accordingly, the biological activity would be modulated in proportion to the degree of electronic perturbation of the pyrrole.

(1) (a) Vinylogous Carbinolamine Tumor Inhibitors. 12. For part 11 in this series, see: Anderson, W. K.; Chang, C. -P.; McPherson, H. L., Jr. *J. Med. Chem.* **1983**, *26*, 1333. (b) Taken in part from the Ph.D. Dissertation of James S. New.

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

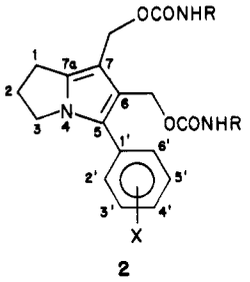
0022-2623/84/1827-1321$01.50/0

In one study we evaluated a series of 1,2-dimethyl-3,4-bis(hydroxymethyl)-5-phenylpyrrole bis(methylcarbamate) derivatives (1) in which X was varied.[3] We found that



1

antileukemic activity and host toxicity did not change to any considerable degree as a function of the electronic properties of X. The absence of any significant correlation between biological activity and the electronic parameters of the substituent in 1 can be interpreted in one of two ways: either the electronic effects are unimportant or they are weakly transmitted, causing only minor electronic perturbations in the pyrrole ring. The latter rationalization would appear tc be more tenable since we have observed a relationship between electronic effects and biological activity in the 5-phenylpyrrolizine series of compounds, 2.[1] In the 5-phenylpyrrolizine series electron-donating



2

substituents on the phenyl ring produced compounds that were generally more toxic than compounds with electron-withdrawing substituents, while the antitumor activities were comparable or slightly inferior in compounds with electron-donating substituents.

The degree of conjugation observed in any biaryl system is related to the ability of the system to adopt a coplanar conformation. Steric interactions oppose adoption of coplanar biaryl conformations. Two significant nonbonded interactions exist in the 5-phenylpyrrole derivatives 1 between the ortho hydrogens of the phenyl substituent and the hydrogens on the N-1 methyl group and the C-4 methylene. Examination of molecular models reveals that one of these nonbonded interactions is absent in the pyrrolizine compounds: the pyrrolizine C-3 methylene hydrogens lie outside the van der Waals radii of the ortho-hydrogen atoms of the C-5 phenyl ring. This is due to the angle distortion caused by the fusion of two five-membered rings (the angle defined by C-3/N-4/C-5 is approximately 144° as compared to 127° for the comparable angle in 1).[4] These interactions are summarized in Figure 1.

The 5-phenylpyrrolizine 2 (X = R = H) was studied on the MACCS system. The structure was run through an energy minimization program which involved four stages in the molecular mechanics. Each stage included a different force field and a separate computational approach. The dihedral angles calculated by the computer bond were

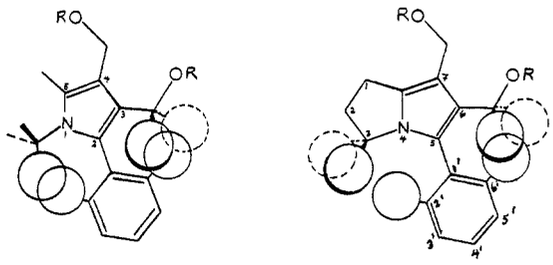
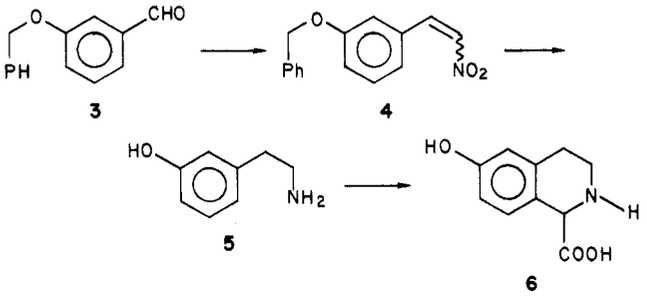


Figure 1. A comparison of nonbonded interactions in the pyrrole and series of compounds.

as follows: N4–C5–C1′–C2′ = 13.10°, N4–C5–C1′–C6′ = 165.05°, C6–C5–C1′–C2′ = 172.30°, and C6–C5–C1′–C6′ = 9.53°. These data support the notion that the phenyl and pyrrole rings in 2 are coplanar (or very nearly so).

This report describes the synthesis and antileukemic evaluation of initial members of a new set of analogues that possess tricyclic structures to limit the deviation from coplanarity of the phenyl and pyrrole rings. Specifically, derivatives of pyrrolo[2,1-*a*]isoquinoline were chosen for examination.

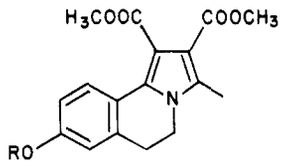
Chemistry. 4,5-Dihydropyrrolo[2,1-*a*]isoquinolines can be prepared conveniently by using 1,3-dipolar cycloaddition reactions with dimethyl acetylenedicarboxylate (DMAD) and mesoionic oxazolones derived from 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acids.[5] The regiospecific synthesis of 1,2,3,4-tetrahydro-6-hydroxyisoquinoline-1-carboxylic acid (6) was achieved with a biomimetic phenolic cyclization. The substrate for the phenolic cyclization, 2-(3-hydroxyphenyl)ethylamine (5), was prepared from 3-(benzyloxy)benzaldehyde (3, prepared from the corresponding phenol by treatment with benzyl chloride–potassium carbonate), which was converted to the crystalline nitrostyrene 4 by treatment with (nitromethyl)ammonium acetate.[6] Reduction of the nitrostyrene with lithium aluminum hydride followed by methanolic HCl cleavage of the benzyl ether gave 5. The phenolic cyclization proceeded in complete regiospecificity when a dilute ethanolic solution of 5 was treated with glyoxylic acid hydrate at 5 °C; the product, 6, crystallized from the exothermic reaction in near analytical purity.



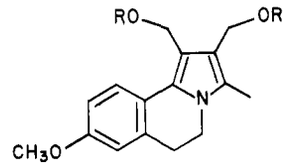
3 4 5 6

The amino acid 6 was smoothly converted to 7 by treatment with DMAD–acetic anhydride at 80 °C (higher reaction temperatures gave lower yields of less pure product). Hydrolysis of the acetate ester (sodium methoxide–methanol) followed by alkylation of the phenol 8 (iodomethane–potassium carbonate–acetone) gave 9. Lithium aluminum hydride reduction of 9 yielded 10, which was converted to 11a and 11b by treatment with isopropyl isocyanate and cyclohexyl isocyanate, respec-

(3) Anderson, W. K.; Halat, M. J. *J. Med. Chem.* **1979**, *22*, 977.
(4) The angles were determined from Dreiding models of the compounds in question. An X-ray structure of a different pyrrolizine showed a C-3/N-4/C-5 angle of 144°: Anderson, W. K.; Corey, P. F. *J. Org. Chem.* **1977**, *42*, 559.
(5) (a) Popp, F. D.; Soto, A. *J. Chem. Soc.* **1963**, 1760. (b) Padbury, J. J.; Lindwall, H. G. *J. Am. Chem. Soc.* **1945**, *67*, 1268. (c) Solomon, W. *J. Chem. Soc.* **1947**, 129.
(6) Howe, R.; Young, E. H. P.; Ainley, A. D. *J. Med. Chem.* **1969**, *12*, 998.

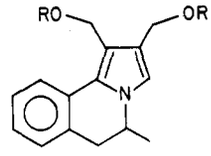


7, R = $COCH_3$
8, R = H
9, R = CH_3



10, R = H
11A, R = $CONHCH(CH_3)_2$
11B R = $CONHCONH\text{-}c\text{-}C_6H_{11}$

tively. Similarly, the diol **12** (prepared from the corresponding diester[5a]) was converted to **13a**, **13b**, and **13c** by treatment with the appropriate isocyanates.



12 R = H
13A R = $CONHCH_3$
13B R = $CONHC_2H_5$
13C R = $CONH\text{-}c\text{-}C_6H_{11}$

Biological Results and Discussion. The results of the evaluation of **11a**, **11b**, **13a**, **13b**, and **13c** for activity against murine P388 lymphocytic leukemia are summarized in Table I. These data are compared with data for related substances in the 2-phenylpyrrole series (1a, X = H; **1b**, X = 4′-OCH_3) and the 5-phenylpyrrolizine series (**2a**, X = H, R = CH_3; **2b**, X = 4′-OCH_3, R = CH_3).

A comparison of three pairs of bis(carbamates) (**1a** vs. **1b**, **2a** vs. **2b**, and **13c** vs. **11b**) reveals that effect of methoxy substitution is quantitatively different in each pair. There is no difference in the activities of the pyrroles **1a** and **1b**, while in the pyrrolizines **2a** and **2b** the methoxy compound appears to be more toxic (as judged by test animal weight change) and slightly more potent than its unsubstituted analogue **2a**. This tendency is less apparent in the tricyclic bis(cyclohexylcarbamate) derivatives **13c** and **11b**; however, the tricyclic compounds do appear to be more potent than **1** or **2**.

The tricyclic bis(2-propylcarbamate) **11a** was selected for further study in a tumor panel. The compound has shown a broad spectrum of activity against a wide range of tumors. Table II summarizes the data obtained for **11a** against five murine tumors and three human tumor xenografts.

The observation that potency, antileukemic activity, and host toxicity appear to be more responsive to changes in substitution in the pyrrolo[2,1-*a*]isoquinoline system than in the 2-phenylpyrrole system augurs well for our initial design hypothesis. Work already in progress will attempt to delineate structure–activity requirements of bis[(acyloxy)methyl] derivatives of pyrrolo[2,1-*a*]isoquinolines.

Experimental Section

Melting points (uncorrected) were determined in open capillaries with a Thomas-Hoover Unimelt apparatus. IR spectra were determined for potassium bromide pellets, unless otherwise specified, with a Perkin-Elmer Model 727B spectrophotometer. UV spectra were determined for 95% ethanol solutions with a Carey Model 118C spectrophotometer. NMR spectra were determined for chloroform-*d* solutions, unless otherwise specified, containing approximately 1% of tetramethylsilane as an internal standard with a Varian T60A spectrometer. Microanalyses were performed by Atlantic Microlabs, Inc., Atlanta, GA.

3-(Benzyloxy)-β-nitrostyrene (4). A stirred solution of 3-(benzyloxy)benzaldehyde (**3**; 27 g, 0.13 mol) and ammonium acetate (10.8 g, 0.14 mol) in glacial acetic acid (108 mL) was treated with nitromethane (15.2 g, 0.25 mol) and heated to reflux (110 °C) for 3 h. The solution, which changed from brown to dark green-black, was cooled and diluted with cold water (3 vol). The dark green precipitate was collected and crystallized from chloroform–hexane (1:1) to yield yellow crystals (30.2 g, 85%) (**caution: product is a severe skin irritant**): mp 89 °C (lit.[6] mp 89 °C); IR 3160, 3140, 3050, 2950, 1640, 1580, 1520, 1360, 1280, 1230, 1160, 1100, 1030, 940–890, 780–670 cm^{-1}; NMR δ 5.06 (s, 2 H), 6.86–7.60 (m, 10 H), 7.89 (d, *J* = 12 Hz, 1 H).

2-(3-Hydroxyphenyl)ethylamine (5). A solution of **4** (8.8 g, 0.035 mol) in dichloromethane (40 mL) was added dropwise to a stirred suspension of lithium aluminum hydride (3.1 g, 0.081 mol) in anhydrous ether (130 mL) at room temperature. The mixture was stirred for 30 min after the addition was completed and treated with slow, dropwise additions of water (4.2 mL), 15% sodium hydroxide (4.2 mL), and water (12.6 mL). The mixture was filtered through a sintered glass disk (porosity m), and the filtrate was dried (sodium sulfate) and concentrated in vacuo to give a brown syrup (7.8 g, 98%), which could be purified by distillation [bp 210–215 °C (0.5 torr)] or by crystallization of the white hydrochloride salt (mp 180.5–183 °C) from absolute ethanol–anhydrous ether (2:1). Some decomposition of the brown pot material occurred during the distillation, but this method was superior to crystallization of the salt and *success in subsequent steps depends on purification at this stage*. The reaction product, 2-[3-(benzyloxy)phenyl]ethylamine, was a light yellow syrup: IR ($CHCl_3$) 3180, 3050, 2960, 1490, 1440, 1380, 1260, 1160, 940–860, 760–680 cm^{-1}; NMR δ 1.33 (s, 2 H, br), 2.53–3.20 (m, 4 H), 5.06 (s, 2 H), 6.63–6.96 (m, 3 H), 7.00–7.53 (m, 6 H).

A solution of 2-[3-(benzyloxy)phenyl]ethylamine in a 1:1 mixture of methanol/hydrochloric acid (3 vol) was heated (3 h) at reflux. The reddish-brown solution was cooled and extracted with dichloromethane (2 × 1 vol), and the aqueous phase was made alkaline with sodium carbonate, evaporated to dryness in vacuo, and dried overnight under high vacuum (P_2O_5). The resultant salts were exhaustively extracted with hot ethyl acetate, and the ethyl acetate solution was filtered, further concentrated, and chilled to yield tan crystals (48%). Seed crystals are usually necessary to induce crystallization. Recrystallization of the product from boiling benzene produced **5** as tan needles: mp 72–75 °C (lit.[7] mp 103–104 °C); IR 3600–1800, 1590, 1480, 1430, 1295, 1160, 880 (w), 790 (w) cm^{-1}; NMR (D_2O–DSS) δ 2.74–3.6 (m, 4 H), 3.60–3.80 (s, 2 H, overlapping m), 6.69–7.69 (m, 4 H).

6-Hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic Acid (6). A stirred solution of **5** (7.9 g, 0.057 mol) in absolute ethanol (200 mL) was treated dropwise with a solution of glyoxylic acid hydrate (4.74 g, 0.057 mol) in ethanol (20 mL) at 5 °C. A fine white precipitate formed shortly after the last addition, but stirring was continued for 1 h with gradual warming to room temperature. The mixture was filtered to give a light yellow solid, which was crystallized from water–ethanol (1:1) to yield **6** as white crystals (8.15 g, 74%): mp 175–176 °C dec; IR 3000–2800, 2650–2100, 1900–1780, 1660–1400 (br),1400–900, 890 cm^{-1}; NMR (D_2O–DSS) δ 4.23–2.93 (m, 5 H, CH_2, CH_2, CH), 4.56 (s, N H), 6.80–7.06 (m, 2 H), 7.69 (d, 1 H), 8.32 (s, 1 H). Anal. ($C_{10}H_{11}NO_3$) C, H, N.

Dimethyl 8-Acetoxy-5,6-dihydro-3-methylpyrrolo[2,1-*a*]-isoquinoline-1,2-dicarboxylate (7). A mixture of **6** (0.93 g, 0.0048 mol), dimethyl acetylenedicarboxylate (0.75 g, 0.0053 mol), and acetic anhydride was slowly heated to 55 °C where carbon dioxide evolution was most intense. The solution was further heated to 70 °C until gas evolution had ceased; the dark solution was concentrated in vacuo, and the solid residue was crystallized from methanol to yield white crystals (1.28 g, 75%): mp 149–151 °C; IR 3010 (w), 2950 (w), 1760, 1720 (sh), 1700, 1490, 1440, 1370, 1300, 1210 cm^{-1}; NMR δ 2.26 (s, 3 H), 2.46 (s, 3 H), 2.93 (t, *J* = 6 Hz, 2 H), 3.80 (s, 3 H), 3.89 (s, 3 H), 3.73–4.06 (t, 2 H, overlapping 2 s), 6.80–7.06 (m, 2 H), 7.56–7.76 (m, 1 H). Anal. ($C_{19}H_{19}NO_6$) C, H, N.

Dimethyl 5,6-Dihydro-8-hydroxy-3-methylpyrrolo[2,1-*a*]isoquinoline-1,2-dicarboxylate (8). A mixture of **7** (0.20 g,

(7) Hahn, G.; Stiehl, K. *Chem. Ber.* **1938**, *71*, 2154.
(8) Anderson, W. K.; Chang, C.-P.; Corey, P. F.; Halat, M. J.; Jones, A. N.; McPherson, H. L., Jr.; New, J. S.; Rick, A. C. *Cancer Treat. Rep.* **1982**, *66*, 91.
(9) Anderson, W. K. *Cancer Res.* **1982**, *42*, 2168.

Table I. Activity of Mono-, Bi-, and Tricyclic Compounds against Murine P388 Lymphocytic Leukemia[a,b]

compd	% T/C (and test animal wt change in grams) at dose,[c,d] mg/kg						
	200	100	50	25	12.5	6.25	3.13
1a		toxic	90 (−4.6)	145 (−3.9)	130 (−3.3)	(135 (−2.5)	
1b		toxic	152 (−4.4)	145 (−3.4)	138 (−3.2)	135 (−2.4)	
2a	60 (−3.5)	124 (−1.8)	124 (−2.1)				
2b	toxic	125 (−4.0)	131 (−4.0)	132 (−3.3)	132 (−3.2)		
11a	toxic	toxic	toxic	153 (−5.6)	153 (−3.0)	136 (−2.1)	142 (−1.1)
11b	toxic	92 (−6.0)	151 (−6.5)	137 (−6.7)	134 (−4.4)	137 (−3.3)	
13a	toxic	toxic	79 (−5.0)	169 (−4.8)	153 (−4.4)	138 (−1.0)	138 (−0.3)
13b[f]	toxic	toxic	toxic	toxic	185 (−6.0)	141 (−1.2)	142 (−1.3)
13c	toxic	toxic	101 (−6.4)[e]	182 (−4.6)	178 (−4.3)		

[a] Determined under the auspices of the National Cancer Institute. For general screening procedures and data interpretation, see: Geran, R. I.; Greenberg, N. H.; McDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*(2), 1. [b] Ascitic fluid containing approximately 6×10^6 cells was inoculated intraperitoneally into male CDF_1 mice. The results of the tests are expressed as % T/C, which is the percent of the median survival time of the test animals compared to control animals, and unless otherwise specified, results are evaluated 30 days after tumor inoculation. The weight difference between the test and control animals is given in parentheses. [c] The compounds were administered as suspensions in distilled water containing Tween 80 unless otherwise specified and are given by intraperitoneal injection; a total of nine daily doses are given, beginning 24 h after tumor inoculation. [d] A dose is labeled as "toxic" if fewer than five of six test animals fail to survive for 5 days after tumor inoculation. [e] Only 5/6 animals survived beyond day five of the test. [f] A dose of 1.56 mg/kg gave 125% T/C (−1.0 g test animal weight change).

Table II. Antineoplastic Activity of **11a**

tumor[a]	optimum % T/C[b] (dose, mg/kg)	schedule[c]
B16 melanocarcinoma	180 (12.5)	Q01DX09, day = 1
$CD8F_1$ mammary	−27* (250)	Q01DX01, day = 21
L1210 lymphoid leukemia	152 (25)	Q01DX09, day = 1
Lewis lung carcinoma	inactive (3.12–50)	Q01DX09, day = 1
colon 38	48* (100)	Q07DX02, day = 2
CX-1 colon xenograft	inactive (300–600)	Q04DX04, day = 1
MX-1 breast xenograft	−100* (600)	Q04DX03, day = 1
LX-1 lung xenograft	55* (150)	Q04DX03, day = 1

[a] See ref 8 for discussion of tumor panel protocols and ref 9 for the xenograft protocols. [b] The compound, given as a suspension in water plus Tween 80, was administered intraperitoneally for all but the xenograft assays where the compound was given by subcutaneous injection. The % T/C values marked by an asterik (*) represent tumor weight reduction relative to controls; a negative value indicates a reduction in the weight of the initially implanted tumor (i.e., percentage of the mean tumor test weight change/mean test tumor initial weight). [c] The first three elements of the schedule code indicate the frequency of dosage (e.g., Q01 = dosed daily; Q07 = dosed every 7 days). The next four elements indicate the total number of doses given (e.g., DX09 = nine doses; DX01 = one dose). The "day =" statement indicates when the first dose was given; the tumor was implanted on day zero.

0.5 mmol) and sodium methoxide (0.033 g, 0.62 mmol) in methanol (30 mL) was stirred and heated (60 °C) for 12 h. The mixture was concentrated in vacuo and the light yellow salt was dissolved in water, acidified with 5% HCl, and extracted with dichloromethane (3 × 50 mL). The organic phase was dried (Na_2SO_4) and concentrated to a light yellow syrup, which was crystallized from cold ethyl acetate to yield **8** as white crystals (0.15 g, 98%): mp 149–150 °C; IR 3500–3150, 1720, 1690 (sh), 1490, 1440, 1390, 1200, 1180, 890 cm^{-1}; NMR δ 2.43 (s, 3 H), 2.86 (t, J = 6 Hz, 2 H), 3.80 (s, 3 H), 3.89 (s, 3 H), 3.36–4.00 (t, 2 H, overlapping 2 s), 6.53–6.83 (m, 2 H), 7.06–7.63 (m, 2 H). Anal. ($C_{17}H_{17}NO_5$) C, H, N.

Dimethyl 5,6-Dihydro-8-methoxy-3-methylpyrrolo[2,1-*a*]isoquinoline-1,2-dicarboxylate (9). A solution of **8** (20.0 g, 0.064 mol), iodomethane (43.69 g, 0.32 mol), and potassium carbonate (43.5 g, 0.32 mol) in anhydrous acetone was heated (41 °C) and stirred for 12 h. The mixture was filtered, and the salts were dissolved in water and extracted with dichloromethane (2 × 200 mL). The filtrate was poured into water to produce a white solid, which was extracted into dichloromethane and combined with the organic wash above. The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give a colorless gum, which was crystallized from methanol to yield **9** as white crystals (19.6 g, 93%): mp 149–150 °C; IR 3000–2900, 1700, 1600, 1440, 1300, 1200 (br), 860 cm^{-1}; UV λ_{max} (ϵ) 207 (31 500), 296.5 (19 800); NMR δ 2.43 (s, 3 H), 2.89 (t, J = 6 Hz, 2 H), 3.80 (s, 6 H), 3.89 (s, 3 H), 3.60–4.00 (t, 2 H, overlapping 2 s), 6.56–6.89 (m, 2 H), 7.26–7.73 (m, 1 H). Anal. ($C_{18}H_{19}NO_5$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-8-methoxy-3-methylpyrrolo[2,1-*a*]isoquinoline (10). A solution of **9** (3.0 g, 9.1 mmol) in dry dichloromethane 25 mL) was slowly added dropwise to a stirring suspension of lithium aluminum hydride (0.82 g, 0.021 mol) in anhydrous ether at room temperature. Workup was begun 30 min beyond the last addition in a manner identical with that used in the reduction of **4**. Concentration of the filtrate in vacuo yielded a light yellow oil, which afforded white crystals from a chilled 5:1 mixture of dichloromethane–petroleum ether (2.02 g, 82%): mp 115–117 °C dec; IR 3600–3000, 2900, 1500, 1240, 970 cm^{-1}; NMR δ 2.20 (s, 3 H), 2.69–3.20 (m, 4 H, s overlapping t), 3.73 (s, 3 H, overlapping t), 3.89–3.60 (t, 2 H, overlapping s), 4.89 (s, 2 H, br), 4.49 (s, 2 H, br), 6.66–6.86 (m 2 H), 7.60 (d, J = 8 Hz, 1 H). Anal. ($C_{16}H_{19}NO_3$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-8-methoxy-3-methylpyrrolo[2,1-*a*]isoquinoline Bis(isopropylcarbamate) (11a). A solution of **10** (2.6 g, 9.5 mmol) and diazabicyclooctane (0.2 g) in freshly dried dioxane (75 mL) was treated portionwise with freshly prepared isopropyl isocyanate (1.61 g, 0.019 mol), and the mixture was heated (60 °C) for 7 h in a sealed reaction vessel that was purged with dry nitrogen. The light yellow solution was concentrated to produce a solid that was chromatographed (RP-2 silica gel–ethyl acetate) to yield an off-white residue. This solid was recrystallized from ethyl acetate to give white crystals (2.86 g, 68% before chromatography): mp 179.5–184 °C dec with oil bath preheated to 175 °C; IR 3350, 2950, 2860 (w), 1680, 1540, 1260, 1240, 1040 cm^{-1}; NMR δ 1.13 (d, J = 6 Hz, 12 H), 2.29 (s, 3 H), 2.96 (t, J = 6 Hz, 2 H), 3.53–4.20 (m, 7 H), 5.29 (s, 2 H), 6.63–7.00 (m, 2 H), 7.56 (d, J = 8 Hz, 1 H). Anal. ($C_{24}H_{33}N_3O_5$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-8-methoxy-3-methylpyrrolo[2,1-*a*]isoquinoline Bis(cyclohexylcarbamate) (11b). A stirred solution of **10** (5.0 g, 0.018 mol) and diazabicyclooctane (0.2 g) in freshly dried dioxane (60 mL) was treated portionwise with freshly prepared cyclohexyl isocyanate (5.6 g, 0.045 mol) over a 2-h period. The reaction vessel was purged with nitrogen, sealed, and heated (60 °C) for 48 h; the mixture was concentrated in vacuo to yield a yellow foam. Chromatography (RP-2 silica gel 60–ethyl acetate) on a column 13 in. × 1 in. led to significant decomposition of the product, but the early fractions did yield a solid that was recrystallized from ethyl acetate to give **11b** as white crystals (6.7 g, 71% before chromatography): mp 191–194 °C dec; IR 3350, 2950, 2850 (w), 1680, 1540, 1310, 1250, 1230, 1040 cm^{-1}; NMR δ 0.49–2.00 (m, 20 H), 2.03 (s, 3 H), 2.89 (t, J = 6 Hz, 2 H), 3.06–4.00 (m, 7 H, with sharp s at 3.73), 4.33–4.80 (2 overlapping s, 2 H, br), 5.03 (s, 2 H), 5.23 (s, 2 H), 6.53–6.89 (m, 2 H), 7.46 (d, J = 8 Hz, 1 H). Anal. ($C_{30}H_{41}N_3O_5 \cdot 0.3CH_3CO_2CH_2CH_3$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-3-methylpyrrolo-[2,1-*a*]isoquinoline (12). A solution of dimethyl 5,6-dihydro-

3-methylpyrrolo[2,1-*a*]isoquinoline-1,2-dicarboxylate[5] (23.4 g, 0.078 mol) in dry dichloromethane was added dropwise over a period of 1 h to a stirred suspension of lithium aluminum hydride (6.84 g, 0.18 mol) in anhydrous ether (210 mL) at room temperature. The mixture was stirred 1 h beyond completion of the addition; workup followed the procedure described under the reduction of **4**. The mixture was concentrated in vacuo to give a solid that was crystallized from tetrahydrofuran–petroleum ether (2:1) to yield **12** as white crystals (16.4 g, 87%): mp 140–142 °C; IR 3600–3000, 2950, 2900, 1600, 1530, 1480, 1340, 930 cm^{-1}; NMR δ 2.26 (s, 3 H), 2.69–2.93 (s, 2 H, br overlapping t), 2.96 (t, J = 6 Hz, 2 H), 4.93 (t, J = 6 Hz, 2 H), 4.6 (s, 2 H), 4.83 (s, 2 H), 6.96–7.49 (m, 3 H), 7.56–7.86 (m, 1 H). Anal. ($C_{15}H_{17}NO_2$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-3-methylpyrrolo-[2,1-*a*]isoquinoline Bis(methylcarbamate) (13a). A solution of **12** (4.76 g, 0.0196 mol) and triethylamine (1.0 mL) was heated (50 °C) and treated portionwise with methyl isocyanate (2.8 g, 0.049 mol) over a period of 4 h. The mixture was stirred 36 h and concentrated in vacuo to give a brown gum, which yielded white crystals overnight from a minimum volume of cold tetrahydrofuran (2.18 g, 31.2%): mp 162–163 °C dec; IR 3400, 3050 (sh), 2990, 1720, 1640, 1530, 1260, 940 (br), 780–680 cm^{-1}; NMR δ 2.26 (s, 3 H), 2.8 (d, J = 6 Hz, 6 H, overlapping t), 2.53–3.2 (t, 2 H), 2.93 (t, J = 6 Hz, 2 H), 4.43 (s, 2 H), 4.56–5.20 (s, 2 H, overlapping s), 5.23 (s, 2 H), 7.00–7.40 (m, 3 H), 7.46–7.73 (m, 1 H). Anal. ($C_{19}H_{23}N_3O_4$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-3-methylpyrrolo-[2,1-*a*]isoquinoline Bis(cyclohexylcarbamate) (13c). A stirred solution of **12** (3.0 g, 0.012 mol) and triethylamine (0.5 mL) in dry tetrahydrofuran (50 mL) heated under reflux was treated with cyclohexyl isocyanate (3.87 g, 0.031 mol) over a period of 45 min. The mixture was stirred 7 h and concentrated in vacuo to give a solid that was recrystallized from tetrahydrofuran–petroleum ether (4:1) to give **13c** as white crystals (2.78 g, 47%): mp 205–208 °C dec; IR 3350, 2950, 2900, 1690, 1530, 1320, 1290, 1250, 1230, 1040 cm^{-1}; NMR δ 0.73–2.20 (m, 20 H), 2.33 (s, 3 H), 3.03 (t, J = 6 Hz, 2 H), 4.60 (s, 1 H, br overlapping s), 4.73 (s, 1 H, br overlapping s), 5.20 (s, 2 H), 5.33 (s, 2 H), 7.09–7.49 (m, 3 H), 7.49–7.75 (m, 1 H). Anal. ($C_{29}H_{39}H_3O_4$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-3-methylpyrrolo-[2,1-*a*]isoquinoline Bis(ethylcarbamate) (13b). A solution of **12** (4.4 g, 0.018 mol) and triethylamine (0.5 mL) in dry tetrahydrofuran (50 mL) was treated portionwise with ethyl isocyanate (3.2 g, 0.045 mol) over a period of 6 h. The mixture was heated under reflux for 26 h and concentrated to give a dark gum. This was crystallized from a minimum volume of ethyl acetate to yield white crystals (2.84 g, 41%): mp 175–175 °C dec; IR 3300, 3060 (w), 3000–2900, 1690, 1540, 1260, 1000 cm^{-1}; NMR δ 1.20 (t, J = 6 Hz, 6 H), 2.29 (s, 3 H), 2.80–3.56 (m, 6 H), 4.93 (t, J = 6 Hz, 2 H), 4.53–5.00 (s, 2 H, br), 5.20 (s, 2 H), 5.33 (s, 2 H), 7.03–7.46 (m, 3 H), 7.49–7.76 (m, 1 H). Anal. ($C_{21}H_{27}N_3O_4$) C, H, N.

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Registry No. **3**, 1700-37-4; **4**, 24550-32-1; **5**, 588-05-6; **6**, 91523-50-1; **7**, 91523-51-2; **8**, 91523-52-3; **9**, 91523-53-4; **10**, 91523-54-5; **11a**, 91523-55-6; **11b**, 91523-56-7; **12**, 91523-57-8; **13a**, 91523-58-9; **13b**, 91523-60-3; **13c**, 91523-59-0; $PhCH_2O$-*m*-C_6H_4-$(CH_2)_2NH_2$, 51061-22-4; $MeOC(O)C{\equiv}CC(O)OMe$, 762-42-5; CH_3NO_2, 75-52-5; *i*-PrNCO, 1795-48-8; MeNCO, 624-83-9; EtNCO, 109-90-0; dimethyl 5,6-dihydro-3-methylpyrrolo[2,1-*a*]isoquinoline-1,2-dicarboxylate, 53927-34-7; glyoxylic acid, 298-12-4; cyclohexyl isocyanate, 109-90-0.

Design and Synthesis of Naltrexone-Derived Affinity Labels with Nonequilibrium Opioid Agonist and Antagonist Activities. Evidence for the Existence of Different μ Receptor Subtypes in Different Tissues

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A series of β-funaltrexamine (**2**, β-FNA) analogues (**3–14**) were synthesized that contain a variety of electrophilic groups attached at the 6β-position of the opiate. The opioid agonist and antagonist activities of these ligands were evaluated in the guinea pig ileum (GPI) and mouse vas deferens (MVD) in vitro assays. Several of the compounds behaved like β-FNA in that they exhibited reversible agonist activity at κ opioid receptors and irreversible antagonist activity at μ opioid receptors. The rank order of irreversible antagonism for a series of related Michael acceptors did not parallel their intrinsic chemical reactivity, confirming that the degree of covalent binding is in part dependent on the spatial disposition of the electrophilic center relative to the receptor nucleophile (secondary recognition). The maleimidoacetamide **8** behaved very differently from β-FNA in that it exhibited considerably greater irreversible μ antagonism in MVD relative to the μ blockage in the GPI. This suggests that different proportions of μ receptor subtypes exist in the two tissues. Several of the agents tested, including some nonreactive control compounds, displayed an unusual type of persistent κ agonist activity in the GPI. This activity, which was reversed by addition of naloxone, reappeared upon washing. Receptor models have been presented to explain this effect. A few of the reactive ligands displayed a true nonreversible κ agonist activity, suggesting a covalent association with the receptor. Of note in this regard was the propiolamide **6**, which appeared to be an irreversible mixed agonist–antagonist at κ and μ receptors.

We have recently reported on the opioid receptor activity of several epimeric pairs of moderately reactive affinity labels derived from naltrexone (**1**) that differed in stereochemistry of attachment of the electrophilic moiety at C-6.[1] Although both α and β epimers were shown to be recognized by different opioid receptor types, covalent bonding capacity resided mainly in the β series and showed a high preference for labeling of the μ receptor system. This behavior was typified by β-funaltrexamine (**2**, β-FNA), which has been shown to be a reversible κ agonist and a specific irreversible μ antagonist in vivo[2] as well as

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