benzimidazole (**5a**) is given here as a representative example: ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3 H, CH_3), 0.89 (s, 3 H, CH_3), 1.21–3.02 (m, 21 H), 2.59 (s, 1 H, ethynyl-H), 7.31 (t, 1 H, J = 7.68 Hz), 7.46–7.50 (m, 1 H), 7.77 (d, 1 H, J = 8.1 Hz), 7.94 (d, 1 H, J = 8.1 Hz), and 8.39 (s, 1 H).

Receptor Binding Procedures.[12] ^{125}I-Bolton Hunter Substance P used in these experiments was purchased from New England Nuclear. The rat forebrain (whole brain minus cerebellum) of a male Sprague–Dawley rat was homogenized in 20 volumes of ice-cold 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl), pH 7.4, at 4 °C, 5 mM KCl and 120 mM NaCl (wash buffer) with a Tekmar Tissumizer Mark II. The homogenate was centrifuged at 48000g for 10 min, and then the resulting pellet was resuspended in 50 mM Tris·HCl, pH 7.4, at 4 °C, 10 mM EDTA and 30 mM KCl, and incubated for 30 min at 4 °C. The homogenate was then centrifuged as above and washed twice by centrifugation in 50 mM Tris·HCl (pH 7.4, at 25 °C). The final pellet was resuspended in 60 volumes of Tris buffer.

The binding assay mixture (0.25 mL) contained 75–125 μg of membrane protein, 0.1 nM ^{125}I-BHSP, and test compound in 50 mM Tris·HCl, at pH 7.4, at 25 °C, 0.02% bovine serum albumin, 1 μg of chymostatin, 2 μg of leupeptin, 20 μg of bacitracin, 1.2 mM $MnCl_2$. Nonspecific binding was defined with 1 μM substance P. All assays were run in duplicate or triplicate, and the reaction mixtures were incubated for 20 min at 25 °C. The assay mixtures were then diluted with 2 mL wash buffer and filtered through GF/C glass fiber filters presoaked in 0.01% polyethylenimine. The filters were washed seven more times with 2 mL of wash buffer. The radioactivity trapped on these filters was counted in a Packard Cobra Gamma Counter. The competition curve data were analyzed by computer nonlinear least-squares best fit of the data to the Hill equation which determined the IC_{50} values from at least six concentrations of the test compound (1×10^{-10} M to 1×10^{-5} M).

Plasma Extravasation in Rats. Experiments were carried out on male Sprague–Dawley rats (150–175 g). Evans Blue dye (30 mg/kg) was administered iv through the tail vein. Compound **5a** was dissolved in a vehicle of 10% ethanol, 15% propylene glycol, and 75% water containing 0.15% TWEEN 80. The compound was administered (in one-half log unit doses) via the tail vein with the Evans Blue dye. Two minutes later the rats received an intradermal injection of SP (2.5 nM) into the left hind paw. An intradermal injection of saline (50 μL) was administered into the right paw as a vehicle and volume control. The animals were sacrificed 20 min after the intradermal injections and the paws removed at the hair line above the ankle. The paws were minced and placed into 4 mL of 99% formamide and incubated overnight at 60 °C. After incubation the samples were centrifuged at 1800 rpm for 5 min (Sorvall RT6000B). The concentration of extractable Evans blue dye was quantified spectrophotometrically at 620 nm (Thermomax Microplate quantified spectrophotometrically at 620 nm (Thermomax Microplate Reader, Molecular Devices, S/N UVT 05318). Plasma extravasation was assessed in the SP injected paw after subtraction of the Evans Blue value of the saline injected paw. Multiple comparisons were made with a one-way analysis of variance followed by the Dunnett test. Compound **5a** was compared to a control group which received the vehicle and Evans Blue iv.

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Chromophore-Modified Antineoplastic Imidazoacridinones. Synthesis and Activity against Murine Leukemias

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The synthesis of 8-hydroxy and 8-methoxy analogues of some substituted 5-aminoimidazoacridinones (**4**) is described. The synthesis was carried out by a three-step sequence from the corresponding 1-chloro-4-nitro-9(10*H*)-acridinone precursors (**1**). The annulation of the imidazolo ring onto the aminoacridinone chromophore was accomplished by heating the required aminoacridinone (**3**) with formic acid or, in the case of 1-methyl derivatives, with *N*,*N*-dimethylacetamide. Potent cytotoxic activity against L1210 leukemia, as well as antitumor activity against P388 leukemia in mice, was demonstrated for the 8-hydroxy analogues. The corresponding 8-methoxy derivatives were not cytotoxic. However, in some cases, they showed significant in vivo antileukemic activity.

Introduction

In our recent paper, the 5-[(aminoalkyl)amino]-6*H*-imidazo[4,5,1-*de*]acridin-6-ones were reported as a novel class of antineoplastic agents.[1] Further research on their structurally close analogues, the 5-[(aminoalkyl)amino]-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-ones, revealed the importance of the OH group at position 8 for their antineoplastic activity.[2] This finding, previously ascertained also for other structural groups of synthetic "DNA complexing agents", for example, the analogues of lucanthone,[3] ellipticine,[4,5] and benzothiopyranoindazoles,[6] prompted us to synthesize a number of substituted 5-amino-8-hydroxy-6*H*-imidazo[4,5,1-*de*]acridine-6-ones (**4i–p**).

It has been reported in the literature for several different groups of anticancer agents that, beside the hydroxy, the respective methoxy derivatives also show antitumor activity.[7-10] Additionally, in the case of 5-nitropyrazoloacridines, the methoxy derivatives were found to exhibit selectivity against solid tumors.[11,12] On the basis of these reports, we synthesized for comparison the corresponding

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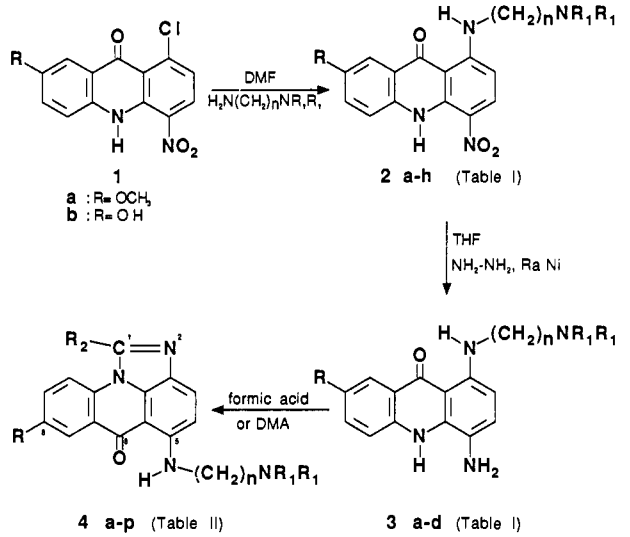
Table I. 1-Substituted 4-Nitro-9(10*H*)-acridinones (2) and 1-Substituted 4-Amino-7-methoxy-9(10*H*)-acridinones (3)

compd	*n*	R	R_1	mp, °C	% yield	molecular formula[a]
2a	2	OCH_3	CH_3	242–243[b]	96	$C_{18}H_{20}N_4O_4$
2b	2	OCH_3	CH_2CH_3	178–179[c]	92	$C_{20}H_{24}N_4O_4$
2c	3	OCH_3	CH_3	165–166	94	$C_{19}H_{22}N_4O_4$
2d	3	OCH_3	CH_2CH_3	153–154[d]	97	$C_{21}H_{26}N_4O_4$
2e	2	OH	CH_3	258–260	90	$C_{17}H_{18}N_4O_4$
2f	2	OH	CH_2CH_3	227–229	94	$C_{19}H_{22}N_4O_4$
2g	3	OH	CH_3	213–214[e]	82	$C_{18}H_{20}N_4O_4$
2h	3	OH	CH_2CH_3	208–210	86	$C_{20}H_{24}N_4O_4$
3a	2	OCH_3	CH_3	240–243 dec	79	$C_{18}H_{22}N_4O_2 \cdot 2HCl$
3b	2	OCH_3	CH_2CH_3	227–231 dec	74	$C_{20}H_{26}N_4O_2 \cdot 2HCl$
3c	3	OCH_3	CH_3	232–235 dec	80	$C_{19}H_{24}N_4O_2 \cdot 2HCl$
3d	3	OCH_3	CH_2CH_3	180–185 dec	84	$C_{21}H_{28}N_4O_2 \cdot 3HCl$

[a] The analyses are within ±0.4% of the theoretical values for C, H and N. [b] Literature[9b] mp 234–237 °C. [c] Literature[9b] mp 179–180 °C. [d] Literature[9b] mp 151–152 °C. [e] Literature[9b] mp 212–213 °C.

Scheme I



methoxy analogues (**4a–h**) although, in the case of triazoloacridinones, the methoxy derivatives were inactive in vivo.[2]

In this paper we report the synthesis and preliminary biological studies for a series of 8-hydroxy and 8-methoxyimidazoacridinones with different (aminoalkyl)amino side chains at the C5 position and a hydrogen or a methyl at C1.

Chemistry

The synthetic procedure utilized for the preparation of target compounds **4a–p** is summarized in Scheme I. The starting 1-chloro-7-hydroxy(methoxy)-4-nitro-9(10*H*)-acridinones (**1a,b**) were synthesized by the methods of Capps.[9] Condensation of 1 with suitable amines, carried out in *N,N*-dimethylformamide (DMF), gave 1-aminoacridinones **2a-h** in excellent yield and purity. The reduction of the nitro gorup in compounds **2** was carried out with hydrazine hydrate and Raney Ni in THF. Amino intermediates **3**, especially those containing three methylene groups in the side chains, were extremely unstable, due to their sensitivity to oxidation. The 7-methoxy derivatives **3a–d** were isolated and characterized as hydrochloride salts (Table I). The 7-hydroxy derivatives, even as hydrochloride salts, gave unsatisfactory microanalytical results. Additionally, it was impossible to determine their melting points as they decomposed before melting. Because of this, the 4-amino-7-hydroxy intermediates were not purified or characterized, but were used immediately in the next step as crude hydrochloride salts.

The salts of **3**, when refluxed with 95% formic acid, readily formed the imidazoacridinones unsubstituted at the C1 position. Similar reaction with acetic acid was unsuccessful. For the preparation of 1-methylimidazoacridinones, the salts of **3** were refluxed with dimethylacetamide (DMA) for 8–12 h.

Two general methods were used for isolation of the final products. The methoxy derivatives were extracted with benzene or chloroform after the reaction mixture was made alkaline and then converted into hydrochloride salts. The hydroxy compounds were isolated directly from the reaction mixture as hydrochloride salts after acidification with HCl.

All the target imidazoacridinones were recrystallized from methanol–acetone prior to microanalysis and biological evaluation. Some were transformed into free bases for ^1H NMR spectral evaluation. Regardless of the C8 substituent, the chemical shifts for chromophore and side-chain protons were similar, suggesting similar electronic contributions by OH and OCH_3. The physical properties and yields of target compounds are shown in Table II.

Biological Results and Discussion

All the compounds were tested in vitro against L1210 leukemia, and the results are reported in Table II.

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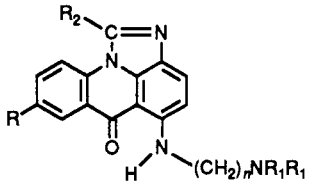
Table II. Activity of Substituted 5-Amino-6*H*-imidazo[4,5,1-*de*]acridin-6-ones against Murine L1210 Leukemia in Vitro and P388 Leukemia in Mice



compd	n	R	R_1	R_2	mp,[a] °C	% yield	formula[b]	L1210 leukemia in vitro: IC_{50} μg/mL[c]	L1210 leukemia in vitro: IC_{50} μM	P388 leukemia in vivo: opt dose, mg/kg per injection	P388 leukemia in vivo: %T/C[d]
ametantrone								1.77(±0.47)	3.65	12.5	300
4a	2	OCH_3	CH_3	H	254–258 dec	90	$C_{19}H_{20}N_4O_2$·1.5HCl·0.75H_2O	0.65(±0.07)	1.65	100	177, 136
4b	2	OCH_3	CH_3	CH_3	255–259 dec	82	$C_{20}H_{22}N_4O_2$·2HCl·H_2O	0.34(±0.09)	0.77	150	127
4c	2	OCH_3	CH_2CH_3	H	250–254 dec[e]	68	$C_{21}H_{24}N_4O_2$·1.75HCl	0.78(±0.02)	1.8	100	183, 209
4d	2	OCH_3	CH_2CH_3	CH_3	238–241 dec[f]	70	$C_{22}H_{26}N_4O_2$·2HCl	0.70(±0.40)	1.55	150	136, 120
4e	3	OCH_3	CH_3	H	237–241 dec	70	$C_{20}H_{22}N_4O_2$·2HCl·0.2H_2O	3.50(±0.75)	8.2	150	164
4f	3	OCH_3	CH_3	CH_3	252–256 dec[g]	66	$C_{21}H_{24}N_4O_2$·1.85HCl	1.40(±0.60)	3.2	150	136
4g	3	OCH_3	CH_2CH_3	H	246–250 dec	72	$C_{22}H_{26}N_4O_2$·1.5HCl	1.10(±0.32)	2.5	150	142
4h	3	OCH_3	CH_2CH_3	CH_3	203–208 dec	64	$C_{23}H_{28}N_4O_2$·2HCl·H_2O	0.70(±0.17)	1.4	150	108
4i	2	OH	CH_3	H	260–264 dec	77	$C_{18}H_{18}N_4O_2$·2HCl·H_2O	0.02(±0.01)	0.048	12.5	210, 210
4j	2	OH	CH_3	CH_3	268–273 dec	68	$C_{19}H_{20}N_4O_2$·2HCl·2H_2O	0.06(±0.03)	0.135	12.5	200, 250
4k	2	OH	CH_2CH_3	H	250–255 dec[h]	72	$C_{20}H_{22}N_4O_2$·2HCl·H_2O	0.013(±0.008)	0.031	5	211, 175
4l	2	OH	CH_2CH_3	CH_3	260–265 dec[i]	76	$C_{21}H_{24}N_4O_2$·1.5HCl·0.5H_2O	0.11(±0.08)	0.25	75	280, 290
4m	3	OH	CH_3	H	247–251 dec	70	$C_{19}H_{20}N_4O_2$·2HCl	0.014(±0.005)	0.034	5	183
4n	3	OH	CH_3	CH_3	268–271 dec	69	$C_{20}H_{22}N_4O_2$·2HCl·0.5H_2O	0.10(±0.07)	0.23	100	255
4o	3	OH	CH_2CH_3	H	269–272 dec[k]	70	$C_{21}H_{24}N_4O_2$·2HCl·H_2O	0.08(±0.05)	0.18	25	309, 230
4p	3	OH	CH_2CH_3	CH_3	238–242 dec[l]	66	$C_{22}H_{26}N_4O_2$·2HCl·H_2O	0.40(±0.21)	0.89	25	150, 155

[a] All the hydrochlorides were recrystallized from MeOH–acetone and the free bases from benzene–hexane. [b] Microanalyses are within ±0.4% of the theoretical values for C, H, and N. [c] Results represent the mean value from three independent experiments. [d] When two values are given, the second one represents the result obtained during repeated independent multidose assay. [e] Free base mp 191–193 °C. [f] Free base mp 156–158 °C. [g] Free base mp 156–158 °C. [h] Free base mp 239–242 °C. [i] Free base mp 255–258 °C. [j] Free base mp 242–245 °C. [k] Free base mp 222–225 °C. [l] Free base mp 240–243 °C.

The in vitro study revealed clear differences in cytotoxicity between the hydroxy and methoxy analogues. All the 8-hydroxy compounds showed very high cytotoxic activity (IC_{50} = 0.031–0.89 μM). On the contrary, all except one of the methoxy derivatives displayed minimal cytotoxicity (IC_{50} < 1 μM). This is in good agreement with the results of cytotoxic activity against L1210 leukemia reported for the benzothiopyranoindazoles.[6] In addition, all of the 1-unsubstituted 8-hydroxyimidazoacridinones showed higher cytotoxicity than their 1-methylated counterparts (e.g. **4k** vs **4l** or **4m** vs **4n**). There was no significant dependence of cytotoxicity on the length of the side chain.

Antineoplastic activity was evaluated in vivo against murine leukemia P388 (ip/ip; days 1–5).[13] Full drug dose–response studies were carried out, with doses ranging from ineffective to clearly toxic levels. The optimal dose was defined as that which produced the highest T/C value. The results obtained at the optimal doses are summarized in Table II. The hydroxy analogues showed both superior potency and efficacy to their methoxy counterparts. Only in one case did a methoxy derivative exhibit a percent T/C comparable to that of its hydroxy analogue, but only at a 20-fold higher dose (**4c** vs **4k**).

In contrast with the 8-unsubstituted analogues reported earlier,[1] where only the derivatives containing an ethylenediamino side chain had good activity, in the case of 8-hydroxylated compounds both derivatives containing an ethylenediamino and a propylenediamino side chain showed high antileukemic activity. Moreover, the hydroxylated derivatives generally exhibited higher efficacy than the corresponding 8-unsubstituted analogues previously described.[1] We found also that there is complete analogy with the triazoloacridinones[2], where high antileukemic activity was exhibited by 8-hydroxylated compounds with both ethylenediamino and propylenediamino side chains.

The two most cytotoxic compounds (**4k** and **4m**) showed also the highest efficacy in vivo. This suggests, in the case of the hydroxy derivatives, a possible relationship between cytotoxicity in vitro and efficacy in vivo.

For compounds **4c** and **4j–l**, the acute toxicity was evaluated in CDF_1 male mice following a single ip administration and the preliminary results are as follows: **4c** LD_{50} = 250–500 mg/kg; **4j** LD_{50} = 125–250 mg/kg; **4k**, LD_{50} > 200 mg/kg; and **4l**, LD_{50} = 250–500 mg/kg. Surprisingly, despite the strong cytotoxicity against the L1210 cell line, the hydroxylated compounds showed rather moderate toxicity on mice.

It is also worth noting that some of the hydroxy analogues exhibited a wider therapeutic index. For example, the full dose–response studies in the case of **4l** gave the following results (dose mg/kg per injection, percent T/C, respectively): 0.78, 130; 1.56, 150; 3.12, 150; 6.25, 200; 12.5, 250 (1/6 cured); 25, 270; 50, 280 (2/6 cured); 75, 290 (2/6 cured); 100, 80.

Conclusions

Our results indicate that hydroxylation of the C8 position of the previously described imidazoacridinones[1] gives compounds with high cytotoxicity against L1210 leukemia and significant antitumor activity against P388 leukemia in vivo. Methoxylation of the same position, generally,

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abolishes cytotoxicity and diminishes in vivo antitumor activity.

Additionally it is noteworthy that chromophore hydroxylation results in less critical requirements for side-chain length, both ethylenediamino and propylenediamino derivatives exhibiting high antitumor activity. However, the role played by the hydroxy group in the mechanism of action of these agents remains still unknown.

In vitro, the C1 substituent influences potency but has no significant effect in vivo.

Experimental Section

Melting points were taken on a Büchi 510 capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian VXR-300 spectrometer. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane. NMR abbreviations used are as follows: br (broad), s (singlet), d (doublet), t (triplet), qu (quartet), qt (quintet), m (multiplet), ex (exchangeable with deuterium oxide). Quartets that are transformed into triplets by addition of deuterium oxide are labeled with an asterisk. Single-frequency decoupling was utilized to assign specific protons. Coupling constants are given in hertz. Microanalytical results, indicated by atomic symbols, are within ±0.4% of the theoretical values and were obtained from the Laboratory of Elemental Analyses, Department of Chemical Sciences, University of Camerino.

General Procedure for the Preparation of 7-Substituted 1-[[(Dialkylamino)alkyl]amino]-4-nitro-9(10*H*)-acridinones (2a–h) (Table I). (a) **Example: 1-[[2-(Diethylamino)ethyl]amino]-7-methoxy-4-nitro-9(10*H*)-acridinone (2b).** A mixture of 4.57 g (0.015 mol) of 1-chloro-7-methoxy-4-nitro-9(10*H*)-acridinone,[9] 25 mL of DMF, and 7.00 g (0.06 mol) of 2-(diethylamino)ethylamine was stirred and heated at 60 °C for 30 min. After addition of 100 mL of 40% (v/v) MeOH–water solution, the reaction mixture was heated to a boil and, after cooling, left overnight in a refrigerator. The crystallized product was collected by filtration, washed with water (150 mL) and MeOH (50 mL), and dried to give 5.30 g (92%) of analytically pure **2b** as yellow needles; mp 178–179 °C (lit.[9b] mp 179–180 °C); ^{1}H NMR (Me_2SO-d_6) δ 12.48 (s, 1 H, ex, N10-*H*), 11.90 (t, 1 H, ex, $-NHCH_2-$), 8.34 (d, 1 H, J = 9.8, C3-*H*), 7.93 (d, 1 H, J = 9.0, C5-*H*), 7.56 (d, 1 H, J = 3.0, C8-*H*), 7.40 (dd, 1 H, J = 8.9, J = 3.0, C6-*H*), 6.54 (d, 1 H, J = 9.8, C2-*H*), 3.87 (s, 3 H, OCH_3), 3.49 (qu*, 2 H, $-NHCH_2CH_2-$), 2.73 (t, 2 H, $CH_2CH_2NEt_2$), 2.58 (qu, 4 H, $N(CH_2CH_3)_2$), 1.02 (t, 6 H, $N(CH_2CH_3)_2$).

(b) **Example: 1-[[2-(Diethylamino)ethyl]amino]-7-hydroxy-4-nitro-9(10*H*)-acridinone (2f).** The compound was prepared in the same manner as **2b**: ^{1}H NMR (Me_2SO-d_6) δ 12.40 (s, 1 H, ex, N10-*H*), 11.92 (t, 1 H, ex, $-NHCH_2-$), 9.74 (s, 1 H, ex, O*H*), 8.34 (d, 1 H, J = 9.8, C3-*H*), 7.79 (d, 1 H, J = 8.9, C5-*H*), 7.55 (d, 1 H, J = 2.8, C8-*H*), 7.26 (dd, 1 H, J = 8.9, J = 2.8, C6-*H*), 6.53 (d, 1 H, J = 10.0, C2-*H*), 3.49 (qu*, 2 H, $-NHCH_2CH_2-$), 2.74 (t, 2 H, $CH_2CH_2NEt_2$), 2.58 (qu, 4 H, $N(CH_2CH_3)_2$), 1.02 (t, 6 H, $N(CH_2CH_3)_2$).

General Procedure for the Preparation of 7-Substituted 4-Amino-1-[[(dialkylamino)alkyl]amino]-9(10*H*)-acridinones Hydrochlorides (3). To a mixture of the nitro derivatives **2a–h** (0.01 mol), 200 mL of THF, and about 2.5 g of Raney Ni was added with stirring at room temperature 2 mL of hydrazine monohydrate, and stirring was continued for about 30 min. The catalyst was filtered off and washed with THF (50 mL). The filtrate was quickly treated with 10 mL of concentrated hydrochloric acid and stirred for 10 min. The resultant yellow precipitate was collected and washed with THF. In the case of 7-methoxy derivatives (**3a–d**), the products were recrystallized from a solution of aqueous MeOH (90%)–dioxane made acidic with HCl (pH ~ 2). **3d**: ^{1}H NMR (Me_2SO-d_6 + D_2O) δ 7.80 (d, 1 H, J = 9.0, C5-*H*), 7.58 (d, 1 H, J = 3.0, C8-*H*), 7.55 (d, 1 H, J = 8.8, C3-*H*), 7.43 (dd, 1 H, J = 9.0, J = 3.0, C6-*H*), 6.35 (d, 1 H, J = 8.8, C2-*H*), 3.85 (s, 3 H, OCH_3), 3.35 (t, 2 H, $-NHCH_2CH_2-$), 3.12 (m, 6 H, $CH_2N(CH_2CH_3)_2$), 2.07 (m, 2 H, $-CH_2CH_2CH_2-$), 1.23 (t, 6 H, $N(CH_2CH_3)_2$).

General Procedure for the Preparation of 5-[[(Dialkylamino)alkyl]amino]-8-methoxy-6*H*-imidazo[4,5,1-*de*]acridin-6-ones (4a, 4c, 4e, and 4g) (Table II). Example: 5-[[2-(Diethylamino)ethyl]amino]-8-methoxy-6*H*-imidazo[4,5,1-*de*]acridin-6-one Dihydrochloride (4c). A mixture of 1.71 g (4 mmol) of **3b** and 20 mL of 95% formic acid was heated at reflux for 6 h. The acid was evaporated and the residue was dissolved in water (100 mL). The solution was made basic (pH 9) by addition of sodium carbonate and the product was extracted with chloroform (2 × 100 mL). The organic extracts were dried and evaporated to give a residue which was dissolved in EtOH. The solution was made acidic with HCl, and the product was crystallized by addition of acetone to give **4c**: ^{1}H NMR (free base) (Me_2SO-d_6) δ 9.13 (s, 1 H, C1-*H*), 8.98 (t, 1 H, ex, $-NHCH_2-$), 8.36 (d, 1 H, J = 9.1, C10-*H*), 7.98 (d, 1 H, J = 8.9, C3-*H*), 7.79 (d, 1 H, J = 3.0, C7-*H*), 7.52 (dd, 1 H, J = 9.1, J = 3.0, C9-*H*), 6.80 (d, 1 H, J = 8.9, C4-*H*), 3.92 (s, 3 H, OCH_3), 3.42 (qu*, 2 H, $-NHCH_2CH_2-$), 2.73 (t, 2 H, $CH_2CH_2NEt_2$), 2.58 (qu, 4 H, $N(CH_2CH_3)_2$), 1.02 (t, 6 H, $N(CH_2CH_3)_2$).

General Procedure for the Preparation of 5-[[(Dialkylamino)alkyl]amino]-8-methoxy-1-methyl-6*H*-imidazo[4,5,1-*de*]acridin-6-ones (4b, 4d, 4f, and 4h) (Table II). Example: 5-[[2-(Diethylamino)ethyl]amino]-8-methoxy-1-methyl-6*H*-imidazo[4,5,1-*de*]acridin-6-one Dihydrochloride (4d). A mixture of 2.14 g (5 mmol) of **3b** and 30 mL of DMA was refluxed for 12 h. Then, 200 mL of water was added and the reaction mixture, made basic with sodium hydroxide, was extracted with benzene (2 × 150 mL). The extracts were evaporated to dryness, and the residue was dissolved in a methanol–dioxane (1:1) mixture. The solution was made acidic with gaseous HCl, and the crystallized product was collected by filtration to give **4d** as yellow crystals: ^{1}H NMR (free base) (Me_2SO-d_6) δ 8.98 (t, 1 H, ex, $-NHCH_2-$), 8.12 (d, 1 H, J = 9.2, C10-*H*), 7.82 (d, 1 H, J = 3.2, C7-*H*), 7.80 (d, 1 H, J = 8.8, C3-*H*), 7.43 (dd, 1 H, J = 9.2, J = 3.2, C9-*H*), 6.70 (d, 1 H, J = 8.8, C4-*H*), 3.91 (s, 3 H, OCH_3), 3.38 (qu*, 2 H, $-NHCH_2CH_2-$), 3.00 (s, 3 H, C1-CH_3), 2.72 (t, 2 H, $CH_2CH_2NEt_2$), 2.58 (qu, 4 H, $N(CH_2CH_3)_2$), 1.03 (t, 6 H, $N(CH_2CH_3)_2$).

General Procedure for the Preparation of 5-[[(Dialkylamino)alkyl]amino]-8-hydroxy-6*H*-imidazo[4,5,1-*de*]acridin-6-ones (4i, 4k, 4m, and 4o) (Table II). A mixture of 5 mmol of the dihydrochloride salt of **3** and 20 mL of 95% formic acid was refluxed for 8 h. Formic acid was evaporated and the residue was dissolved in hot methanol. Then, 3 mL of concentrated hydrochloric acid was added to the hot solution and the product was crystallized by addition of acetone. The product was collected by filtration and recrystallized from a mixture of methanol–acetone.

4k: ^{1}H NMR (free base) (Me_2SO-d_6) δ 10.00 (s, 1 H, ex, C8-O*H*), 9.08 (s, 1 H, C1-*H*), 8.99 (t, 1 H, ex, $-NHCH_2-$), 8.26 (d, 1 H, J = 8.9, C10-*H*), 7.95 (d, 1 H, J = 8.8, C3-*H*), 7.72 (d, 1 H, J = 2.8, C7-*H*), 7.33 (dd, 1 H, J = 8.9, J = 2.8, C9-*H*), 6.77 (d, 1 H, J = 8.8, C4-*H*), 3.40 (qu*, 2 H, $-NHCH_2CH_2-$), 2.70 (t, 2 H, $CH_2CH_2NEt_2$), 2.56 (qu, 4 H, $N(CH_2CH_3)_2$), 1.01 (t, 6 H, $N(CH_2CH_3)_2$).

4o: ^{1}H NMR (free base) (Me_2SO-d_6) δ 10.02 (br s, 1 H, ex, C8-O*H*), 9.10 (s, 1 H, C1-*H*), 8.93 (t, 1 H, ex, $-NHCH_2-$), 8.27 (d, 1 H, J = 8.9, C10-*H*), 7.97 (d, 1 H, J = 8.8, C3-*H*), 7.73 (d, 1 H, J = 2.8, C7-*H*), 7.34 (dd, 1 H, J = 8.9, J = 2.8, C9-*H*), 6.81 (d, 1 H, J = 8.8, C4-*H*), 3.42 (qu*, 2 H, $-NHCH_2CH_2-$), 2.52 (t, 2 H, $CH_2CH_2NEt_2$), 2.48 (qu, 4 H, $N(CH_2CH_3)_2$), 1.78 (qt, 2 H, $-CH_2CH_2CH_2-$), 0.96 (t, 6 H, $N(CH_2CH_3)_2$).

General Procedure for the Preparation of 5-[[(Dialkylamino)alkyl]amino]-8-hydroxy-1-methyl-6*H*-imidazo[4,5,1-*de*]acridin-6-ones (4j, 4l, 4n, and 4p) (Table II). A mixture of 5 mmol of the dihydrochloride salt of **3** and 25 mL of DMA was refluxed for 12 h. After evaporation of about 20 mL of solvent, 100 mL of acetone was added to the residue and the resulting solution was acidified with gaseous HCl. The precipitated product was collected by filtration and washed with acetone. The crude product was recrystallized (if necessary twice) from methanol–acetone to give the respective dihydrochloride salt of **4**.

4l: ^{1}H NMR (free base) (Me_2SO-d_6) δ 10.00 (s, 1 H, ex, C8-O*H*), 9.02 (t, 1 H, ex, $-NHCH_2-$), 8.11 (d, 1 H, J = 9.1, C10-*H*), 7.83 (d, 1 H, J = 8.8, C3-*H*), 7.79 (d, 1 H, J = 2.9, C7-*H*), 7.33 (dd, 1 H, J = 9.1, J = 2.9, C9-*H*), 6.72 (d, 1 H, J = 8.8, C4-*H*), 3.38 (qu*, 2 H, $-NHCH_2CH_2-$), 3.02 (s, 3 H, C1-CH_3), 2.72 (t, 2 H, $CH_2CH_2NEt_2$), 2.56 (qu, 4 H, $N(CH_2CH_3)_2$), 1.02 (t, 6 H, $N(CH_2CH_3)_2$).

4p: ^{1}H NMR (free base) (Me_2SO-d_6) δ 10.0 (br s, 1 H, ex, C8-O*H*), 8.91 (t, 1 H, ex, -N*H*CH_2-), 8.08 (d, 1 H, J = 9.1, C10-*H*), 7.80 (d, 1 H, J = 8.8, C3-*H*), 7.77 (d, 1 H, J = 3.0, C7-*H*), 7.32 (dd, 1 H, J = 9.1, J = 3.0, C9-*H*), 6.70 (d, 1 H, J = 8.8, C4-*H*), 3.38 (qu*, 2 H, -NHC$H_2$$CH_2$-), 3.00 (s, 3 H, C1-C$H_3$), 2.48 (m, 6 H, $CH_2CH_2N(CH_2CH_3)_2$), 1.76 (qt, 2 H, -$CH_2CH_2CH_2$-).

Biological Tests. In Vitro Cytotoxicity Evaluation. The mouse L1210 leukemia cells (RPMI) were grown in RPMI 1640 medium supplemented with 5% fetal calf serum and penicillin (10^6 units/L) plus streptomycin (100 mg/L) in controlled air-5% CO_2 humidified atmosphere at 37 °C. L1210 mouse leukemia cells were seeded at a density of 5×10^4 cells/mL. The test compound, dissolved in 50% ethanol, was added, at four different concentrations, to the cell suspension. The cytotoxic activity (IC_{50} value) of the test compound was defined as the concentration causing a 50% growth inhibition after 48 h, measured by cell protein content, and was determined from dose-response curves.

In Vivo Antileukemic Evaluation. BDF_1 mice were injected ip with 10^6 P388 lymphotic leukemia cells on day 0 and treated ip on days 1-5 in accordance with the protocols described by the National Cancer Institute.[13] The mean survival time (MST) for each treatment group (eight mice) was calculated and the percent of T/C was determined by using the following formula:

$$\%\ T/C = [(MST\ treated)/(MST\ control)] \times 100$$

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Synthesis and Some Pharmacological Properties of Potent and Selective Antagonists of the Vasopressor (V_1-Receptor) Response to Arginine-Vasopressin†

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We report the solid-phase synthesis of eight position-9-modified analogues of the potent V_1-receptor antagonist of arginine-vasopressin, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin (d$(CH_2)_5$Tyr(Me)AVP) (**1-8**) and five position-9-modified analogues of the closely related β,β-dimethyl less potent V_1 antagonist, [1-deaminopenicillamine,2-*O*-methyltyrosine]arginine-vasopressin (dPTyr(Me)AVP) (**9-13**). In d$(CH_2)_5$Tyr(Me)AVP the C-terminal Gly-NH_2 was replaced by (**1**) ethylenediamine (Eda), (**2**) methylamine (NHMe), (**3**) Ala-NH_2, (**4**) Val-NH_2, (**5**) Arg-NH_2, (**6**) Thr-NH_2, (**7**) Gly-Eda, (**8**) Gly-*N*-butylamide (Gly-NH-Bu); in dPTyr(Me)AVP the C-terminal Gly-NH_2 was replaced by (**9**) Ala-NH_2, (**10**) Val-NH_2, (**11**) Thr-NH_2, (**12**) Arg-NH_2, and (**13**) Tyr-NH_2. All 13 analogues were tested for agonistic and antagonistic activities in in vivo rat vasopressor (V_1-receptor) and rat antidiuretic (V_2-receptor) assays. They exhibit no evident vasopressor agonism. All modifications in both antagonists were well-tolerated with excellent retention of V_1 antagonism and striking enhancements in anti-V_1/anti-V_2 selectivity. With anti-V_1 pA_2 values of 8.75, 8.73, 8.86, and 8.78, four of the analogues of d-$(CH_2)_5$Tyr(Me)AVP (**1-3** and **6**) are equipotent with d$(CH_2)_5$Tyr(Me)AVP (anti-V_1 pA_2 = 8.62) but retain virtually none of the V_2 agonism of d$(CH_2)_5$Tyr(Me)AVP. They are in fact weak V_2 antagonists and strong V_1 antagonists with greatly enhanced selectivity for V_1 receptors relative to that of d$(CH_2)_5$Tyr(Me)AVP. With anti-V_1 pA_2 values respectively of 8.16, 8.05, 8.04, 8.52, and 8.25, all five analogues (**9-13**) of dPTyr(Me)AVP are at least as potent V_1 antagonists as dPTyr(Me)AVP (pA_2 = 7.96) and three of these (**9**, **12**, **13**) actually show enhanced V_1 antagonism over that of dPTyr(Me)AVP. In fact, the Arg-NH_2[9] analogue (**12**) is almost equipotent with d$(CH_2)_5$Tyr(Me)AVP. These new V_1 antagonists are potentially useful as pharmacological tools for studies on the cardiovascular roles of AVP. Furthermore the analogues of dPTyr(Me)AVP may be useful in studies on the role(s) of AVP in the V_{1b}-receptor-mediated release of ACTH from corticotrophs.

Antagonists of the vasopressor (V_1-receptor) responses to arginine-vasopressin (AVP) originally reported from these laboratories have found widespread use as pharmacological tools in studies on the many putative physiological roles of AVP (for reviews see refs 1 and 2). Among the most widely used are [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin (d$(CH_2)_5$Tyr(Me)AVP)[3] and [1-deaminopenicillamine,2-*O*-methyltyrosine]arginine-vasopressin

† Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* 1984, *138*, 9). All amino acids are in the L configuration unless otherwise noted. Other abbreviations used are d$(CH_2)_5$Tyr(Me)AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin; dPTyr(Me)AVP, [1-deaminopenicillamine,2-*O*-methyltyrosine]arginine-vasopressin; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; BOP, benzotriazol-1-yltris(dimethylamino)phosphonium hexafluorophosphate; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; AcOH, acetic acid; TFA, trifluoroacetic acid; HOBt, *N*-hydroxybenzotriazole; ONp, *p*-nitrophenyl ester; Et_3N, triethylamine; Eda, ethylenediamine; NHMe, methylamine; NHBu, butylamide; Et_2O, diethyl ether.

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