

benzimidazole (5a) is given here as a representative example:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.82 (s, 3 H,  $\text{CH}_3$ ), 0.89 (s, 3 H,  $\text{CH}_3$ ), 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.**<sup>12</sup>  $^{125}\text{I}$ -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 Tissuizer 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  $\mu\text{g}$  of membrane protein, 0.1 nM  $^{125}\text{I}$ -BHSP, and test compound in 50 mM Tris-HCl, at pH 7.4, at 25 °C, 0.02% bovine serum albumin, 1  $\mu\text{g}$  of chymostatin, 2  $\mu\text{g}$  of leupeptin, 20  $\mu\text{g}$  of bacitracin, 1.2 mM  $\text{MnCl}_2$ . Nonspecific binding was defined with 1  $\mu\text{M}$  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  $\text{IC}_{50}$  values from at least six concentrations of the test compound ( $1 \times 10^{-10}$  M to  $1 \times 10^{-5}$  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  $\mu\text{L}$ ) 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.

**Acknowledgment.** We would like to acknowledge the assistance of Ms. Barbara Fragale, Ms. Colleen Duggan, and Ms. Cheryl Meravi for the biochemical evaluation and Mr. Larry Wagner for the pharmacological evaluation.

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

Wieslaw M. Cholody,\*<sup>†</sup> Sante Martelli,<sup>‡</sup> and Jerzy Konopa<sup>†</sup>

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, 80952 Gdansk, Poland, and Department of Chemical Sciences, University of Camerino, 62032 Camerino, Italy. Received April 25, 1991

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(10H)-acridinone precursors (1). The annulation of the imidazole 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]-6H-imidazo[4,5,1-de]acridin-6-ones were reported as a novel class of antineoplastic agents.<sup>1</sup> Further research on their structurally close analogues, the 5-[(aminoalkyl)amino]-6H-*v*-triazolo[4,5,1-de]acridin-6-ones, revealed the importance of the OH group at position 8 for their antineoplastic activity.<sup>2</sup> This finding, previously ascertained also for other structural groups of synthetic "DNA complexing agents", for example, the analogues of lucanthone,<sup>3</sup> ellipticine,<sup>4,5</sup> and benzothiopyranindazoles,<sup>6</sup> prompted us to synthesize a number of substituted 5-amino-8-hydroxy-6H-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.<sup>7-10</sup> Additionally, in the case of 5-nitropyrzoloacridines, the methoxy derivatives were found to exhibit

selectivity against solid tumors.<sup>11,12</sup> On the basis of these reports, we synthesized for comparison the corresponding

- (1) Cholody, W. M.; Martelli, S.; Paradziej-Lukowicz, J.; Konopa, J. 5-[(Aminoalkyl)amino]imidazo[4,5,1-de]acridin-6-ones as a Novel Class of Antineoplastic Agents. Synthesis and Biological Activity. *J. Med. Chem.* 1990, 33, 49-52.
- (2) Cholody, W. M.; Martelli, S.; Konopa, J. 8-Substituted 5-[(Aminoalkyl)amino]-6H-*v*-triazolo[4,5,1-de]acridin-6-ones as Potential Antineoplastic Agents. Synthesis and Biological Activity. *J. Med. Chem.* 1990, 33, 2852-2856.
- (3) Archer, S.; Miller, K. J.; Rej, R.; Periana, C.; Fricker, L. Ring-Hydroxylated Analogues of Lucanthone as Antitumor Agents. *J. Med. Chem.* 1982, 25, 220-227.
- (4) Bisagni, E.; Nguyen, C. H.; Pierre, A.; Pepin, O.; de Cointet, P.; Gros, P. 1-Amino-Substituted 4-Methyl-5H-pyrido[4,3-b]indoles (-Carbolines) as Tricyclic Analogues of Ellipticines: A New Class of Antineoplastic Agents. *J. Med. Chem.* 1988, 31, 398-405.
- (5) Honda, T.; Kato, M.; Inoue, M.; Shimamoto, T.; Shima, K.; Nakanishi, T.; Yoshida, T.; Noguchi, T. Synthesis and Antitumor Activity of Quaternary Ellipticine Glycosides, a Series of Novel and Highly Active Antitumor Agents. *J. Med. Chem.* 1988, 31, 1295-1305.

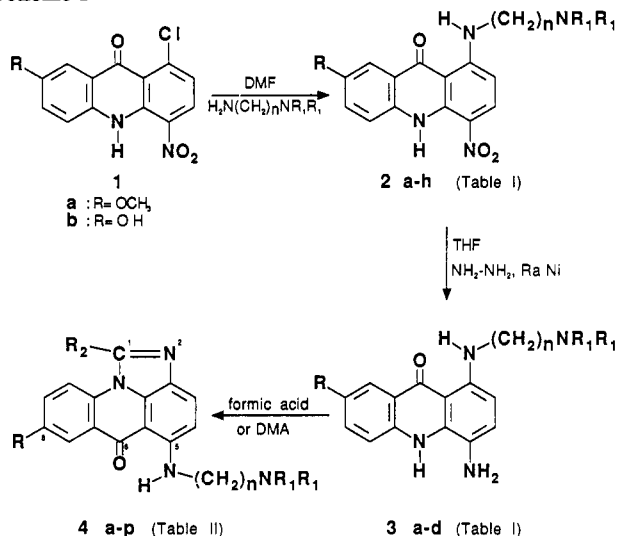
<sup>†</sup> Technical University of Gdansk.

<sup>‡</sup> University of Camerino.

**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 <sub>1</sub>	mp, °C	% yield	molecular formula <sup>a</sup>
2a	2	OCH <sub>3</sub>	CH <sub>3</sub>	242–243 <sup>b</sup>	96	C <sub>18</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub>
2b	2	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	178–179 <sup>c</sup>	92	C <sub>20</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>
2c	3	OCH <sub>3</sub>	CH <sub>3</sub>	165–166	94	C <sub>19</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>
2d	3	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	153–154 <sup>d</sup>	97	C <sub>21</sub> H <sub>26</sub> N <sub>4</sub> O <sub>4</sub>
2e	2	OH	CH <sub>3</sub>	258–260	90	C <sub>17</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub>
2f	2	OH	CH <sub>2</sub> CH <sub>3</sub>	227–229	94	C <sub>19</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>
2g	3	OH	CH <sub>3</sub>	213–214 <sup>e</sup>	82	C <sub>18</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub>
2h	3	OH	CH <sub>2</sub> CH <sub>3</sub>	208–210	86	C <sub>20</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>
3a	2	OCH <sub>3</sub>	CH <sub>3</sub>	240–243 dec	79	C <sub>18</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl
3b	2	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	227–231 dec	74	C <sub>20</sub> H <sub>26</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl
3c	3	OCH <sub>3</sub>	CH <sub>3</sub>	232–235 dec	80	C <sub>19</sub> H <sub>24</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl
3d	3	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	180–185 dec	84	C <sub>21</sub> H <sub>28</sub> N <sub>4</sub> O <sub>2</sub> ·3HCl

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

**Scheme I**

methoxy analogues (4a–h) although, in the case of triazoloacridinones, the methoxy derivatives were inactive *in vivo*.<sup>2</sup>

- (6) Showalter, H. D. H.; Angelo, M. M.; Berman, E. M.; Kanter, G. D.; Ortwine, D. F.; Ross-Kesten, S. G.; Sercel, A. D.; Turner, W. R.; Werbel, L. M.; Worth, D. F.; Elslager, E. F.; Leopold, W. R.; Shillis, J. L. Benzothioapyranindazoles, a New Class of Chromophore Modified Anthracenedione Anticancer Agents. Synthesis and Activity against Murine Leukemias. *J. Med. Chem.* 1988, 31, 1527–1539.
- (7) Ducrocq, C.; Wendling, F.; Tourbez-Perrin, M.; Rivalle, C.; Tambourin, P.; Pochon, F.; Bisagni, E. Structure-Activity Relationships in a Series of Newly Synthesized 1-Amino-Substituted Ellipticine Derivatives. *J. Med. Chem.* 1980, 23, 1212–1216.
- (8) Larue, L.; Rivalle, C.; Muzard, G.; Paoletti, J. A New Series of Ellipticine Derivatives (1-(Alkylamino)-9-methoxyellipticine). Synthesis, DNA Binding, and Biological Properties. *J. Med. Chem.* 1988, 31, 1951–1956.
- (9) (a) Capps, D. B. Pyrazolo[3,4,5-kl]acridine compounds and pharmaceutical composition comprising them. Eur. Pat. Appl. E P 138,302, 1985; *Chem. Abstr.* 1985, 103, 196074q. (b) Capps, D. B. Substituted 1-amino-4-nitroacridones and pharmaceutical compositions comprising them. Eur. Pat. Appl. E P 145,226, 1985; *Chem. Abstr.* 1985, 103, 215182s.
- (10) Jackson, R. C.; Leopold, W. R.; Sebolt, J. S. Biochemical and Experimental Chemotherapy Studies of the Pyrazoloacridines. *Proc. Am. Assoc. Cancer Res.* 1988, 29, 536–538.
- (11) Sebolt, J. S.; Scavone, S. V.; Pinter, C. D.; Hamelhele, K. L.; Von Hoff, D. D.; Jackson, R. C. Pyrazoloacridines, a New Class of Anticancer Agents with Selectivity against Solid Tumors *in Vitro*. *Cancer Res.* 1987, 47, 4299–4304.
- (12) Sebolt, J.; Havlick, M.; Hamelhele, K.; Nelson, J.; Leopold, W.; Jackson, R. Activity of the pyrazoloacridines against multi-drug-resistant tumor cells. *Cancer Chemother. Pharmacol.* 1989, 24, 219–224.

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.<sup>9</sup> 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 group 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.

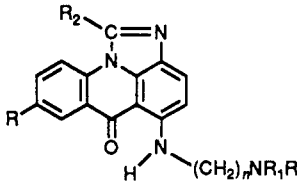
The salts of 3, when refluxed with 95% formic acid, readily formed the imidazoacridinones unsubstituted at the C1 position. Similar reaction with acetic acid was unsuccessful. For the preparation of 1-methylimidazoacridinones, the salts of 3 were refluxed with dimethylacetamide (DMA) for 8–12 h.

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 <sup>1</sup>H 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<sub>3</sub>. 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.

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


compd	n	R	R <sub>1</sub>	R <sub>2</sub>	mp, <sup>a</sup> °C	% yield	formula <sup>b</sup>	L1210 leukemia in vitro: IC <sub>50</sub>		P388 leukemia in vivo	
								μg/mL <sup>c</sup>	μM	opt dose, mg/kg per injection	% T/C <sup>d</sup>
ametantrone								1.77(±0.47)	3.65	12.5	300
4a	2	OCH <sub>3</sub>	CH <sub>3</sub>	H	254–258 dec	90	C <sub>19</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> ·1.5HCl·0.75H <sub>2</sub> O	0.65(±0.07)	1.65	100	177, 136
4b	2	OCH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	255–259 dec	82	C <sub>20</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl·H <sub>2</sub> O	0.34(±0.09)	0.77	150	127
4c	2	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	H	250–254 dec <sup>e</sup>	68	C <sub>21</sub> H <sub>24</sub> N <sub>4</sub> O <sub>2</sub> ·1.75HCl	0.78(±0.02)	1.8	100	183, 209
4d	2	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	238–241 dec <sup>f</sup>	70	C <sub>22</sub> H <sub>26</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl	0.70(±0.40)	1.55	150	136, 120
4e	3	OCH <sub>3</sub>	CH <sub>3</sub>	H	237–241 dec	70	C <sub>20</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl·0.2H <sub>2</sub> O	3.50(±0.75)	8.2	150	164
4f	3	OCH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	252–256 dec <sup>e</sup>	66	C <sub>21</sub> H <sub>24</sub> N <sub>4</sub> O <sub>2</sub> ·1.85HCl	1.40(±0.60)	3.2	150	136
4g	3	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	H	246–250 dec	72	C <sub>22</sub> H <sub>26</sub> N <sub>4</sub> O <sub>2</sub> ·1.5HCl	1.10(±0.32)	2.5	150	142
4h	3	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	203–208 dec	64	C <sub>23</sub> H <sub>28</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl·H <sub>2</sub> O	0.70(±0.17)	1.4	150	108
4i	2	OH	CH <sub>3</sub>	H	260–264 dec	77	C <sub>18</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl·H <sub>2</sub> O	0.02(±0.01)	0.048	12.5	210, 210
4j	2	OH	CH <sub>3</sub>	CH <sub>3</sub>	268–273 dec	68	C <sub>19</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl·2H <sub>2</sub> O	0.06(±0.03)	0.135	12.5	200, 250
4k	2	OH	CH <sub>2</sub> CH <sub>3</sub>	H	250–255 dec <sup>h</sup>	72	C <sub>20</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl·H <sub>2</sub> O	0.013(±0.008)	0.031	5	211, 175
4l	2	OH	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	260–265 dec <sup>i</sup>	76	C <sub>21</sub> H <sub>24</sub> N <sub>4</sub> O <sub>2</sub> ·1.5HCl·0.5H <sub>2</sub> O	0.11(±0.08)	0.25	75	280, 290
4m	3	OH	CH <sub>3</sub>	H	247–251 dec	70	C <sub>19</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl	0.014(±0.005)	0.034	5	183
4n	3	OH	CH <sub>3</sub>	CH <sub>3</sub>	268–271 dec	69	C <sub>20</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl·0.5H <sub>2</sub> O	0.10(±0.07)	0.23	100	255
4o	3	OH	CH <sub>2</sub> CH <sub>3</sub>	H	269–272 dec <sup>k</sup>	70	C <sub>21</sub> H <sub>24</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl·H <sub>2</sub> O	0.08(±0.05)	0.18	25	309, 230
4p	3	OH	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	238–242 dec <sup>l</sup>	66	C <sub>22</sub> H <sub>26</sub> N <sub>4</sub> O <sub>2</sub> ·2HCl·H <sub>2</sub> O	0.40(±0.21)	0.89	25	150, 155

<sup>a</sup> All the hydrochlorides were recrystallized from MeOH-acetone and the free bases from benzene-hexane. <sup>b</sup> Microanalyses are within ±0.4% of the theoretical values for C, H, and N. <sup>c</sup> Results represent the mean value from three independent experiments. <sup>d</sup> When two values are given, the second one represents the result obtained during repeated independent multidose assay. <sup>e</sup> Free base mp 191–193 °C. <sup>f</sup> Free base mp 156–158 °C. <sup>g</sup> Free base mp 156–158 °C. <sup>h</sup> Free base mp 239–242 °C. <sup>i</sup> Free base mp 255–258 °C. <sup>j</sup> Free base mp 242–245 °C. <sup>k</sup> Free base mp 222–225 °C. <sup>l</sup> 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<sub>50</sub> = 0.031–0.89 μM). On the contrary, all except one of the methoxy derivatives displayed minimal cytotoxicity (IC<sub>50</sub> < 1 μM). This is in good agreement with the results of cytotoxic activity against L1210 leukemia reported for the benzothioipyranindazoles.<sup>6</sup> 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).<sup>13</sup> 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,<sup>1</sup> 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.<sup>1</sup> We found also that there is complete analogy with the triazoloacridinones<sup>2</sup>, 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<sub>1</sub> male mice following a single ip administration and the preliminary results are as follows: 4c LD<sub>50</sub> = 250–500 mg/kg; 4j LD<sub>50</sub> = 125–250 mg/kg; 4k, LD<sub>50</sub> > 200 mg/kg; and 4l, LD<sub>50</sub> = 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<sup>1</sup> gives compounds with high cytotoxicity against L1210 leukemia and significant antitumor activity against P388 leukemia in vivo. Methoxylation of the same position, generally,

(13) Geran, R. I.; Greenberg, R. H.; MacDonald, M. M.; Schumacher, A. M.; Abbot, B. J. Protocols for screening chemical agents and natural products against animal tumours and other biological systems. *Cancer Chemother. Rep., Part 3* 1972, 3, 1-103.

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.  $^1\text{H}$  NMR spectra were recorded on a Varian VXR-300 spectrometer. Chemical shifts are reported as  $\delta$  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  $\pm 0.4\%$  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(10H)-acridinones (2a-h) (Table I).** (a) Example: 1-[[2-(Diethylamino)ethyl]amino]-7-methoxy-4-nitro-9(10H)-acridinone (2b). A mixture of 4.57 g (0.015 mol) of 1-chloro-7-methoxy-4-nitro-9(10H)-acridinone,<sup>9</sup> 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.<sup>9b</sup> mp 179–180 °C);  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  12.48 (s, 1 H, ex, N10-H), 11.90 (t, 1 H, ex,  $-\text{NHCH}_2-$ ), 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,  $\text{OCH}_3$ ), 3.49 (qu\*, 2 H,  $-\text{NHCH}_2\text{CH}_2-$ ), 2.73 (t, 2 H,  $\text{CH}_2\text{CH}_2\text{NET}_2$ ), 2.58 (qu, 4 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 1.02 (t, 6 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ).

(b) Example: 1-[[2-(Diethylamino)ethyl]amino]-7-hydroxy-4-nitro-9(10H)-acridinone (2f). The compound was prepared in the same manner as 2b:  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  12.40 (s, 1 H, ex, N10-H), 11.92 (t, 1 H, ex,  $-\text{NHCH}_2-$ ), 9.74 (s, 1 H, ex, OH), 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,  $-\text{NHCH}_2\text{CH}_2-$ ), 2.74 (t, 2 H,  $\text{CH}_2\text{CH}_2\text{NET}_2$ ), 2.58 (qu, 4 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 1.02 (t, 6 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ).

**General Procedure for the Preparation of 7-Substituted 4-Amino-1-[(dialkylamino)alkyl]amino-9(10H)-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  $\sim$  2). 3d:  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6 + \text{D}_2\text{O}$ )  $\delta$  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,  $\text{OCH}_3$ ), 3.35 (t, 2 H,  $-\text{NHCH}_2\text{CH}_2-$ ), 3.12 (m, 6 H,  $\text{CH}_2\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 2.07 (m, 2 H,  $-\text{CH}_2\text{CH}_2\text{CH}_2-$ ), 1.23 (t, 6 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ).

**General Procedure for the Preparation of 5-[(Dialkylamino)alkyl]amino-8-methoxy-6H-imidazo[4,5,1-de]acridin-6-ones (4a, 4c, 4e, and 4g) (Table II).** Example:

5-[[2-(Diethylamino)ethyl]amino]-8-methoxy-6H-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 ( $2 \times 100$  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:  $^1\text{H}$  NMR (free base) ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  9.13 (s, 1 H, C1-H), 8.98 (t, 1 H, ex,  $-\text{NHCH}_2-$ ), 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,  $\text{OCH}_3$ ), 3.42 (qu\*, 2 H,  $-\text{NHCH}_2\text{CH}_2-$ ), 2.73 (t, 2 H,  $\text{CH}_2\text{CH}_2\text{NET}_2$ ), 2.58 (qu, 4 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 1.02 (t, 6 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ).

**General Procedure for the Preparation of 5-[(Dialkylamino)alkyl]amino-8-methoxy-1-methyl-6H-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-6H-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 ( $2 \times 150$  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:  $^1\text{H}$  NMR (free base) ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  8.98 (t, 1 H, ex,  $-\text{NHCH}_2-$ ), 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,  $\text{OCH}_3$ ), 3.38 (qu\*, 2 H,  $-\text{NHCH}_2\text{CH}_2-$ ), 3.00 (s, 3 H, C1- $\text{CH}_3$ ), 2.72 (t, 2 H,  $\text{CH}_2\text{CH}_2\text{NET}_2$ ), 2.58 (qu, 4 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 1.03 (t, 6 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ).

**General Procedure for the Preparation of 5-[(Dialkylamino)alkyl]amino-8-hydroxy-6H-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:  $^1\text{H}$  NMR (free base) ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  10.00 (s, 1 H, ex, C8-OH), 9.08 (s, 1 H, C1-H), 8.99 (t, 1 H, ex,  $-\text{NHCH}_2-$ ), 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,  $-\text{NHCH}_2\text{CH}_2-$ ), 2.70 (t, 2 H,  $\text{CH}_2\text{CH}_2\text{NET}_2$ ), 2.56 (qu, 4 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 1.01 (t, 6 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ).

4o:  $^1\text{H}$  NMR (free base) ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  10.02 (br s, 1 H, ex, C8-OH), 9.10 (s, 1 H, C1-H), 8.93 (t, 1 H, ex,  $-\text{NHCH}_2-$ ), 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,  $-\text{NHCH}_2\text{CH}_2-$ ), 2.52 (t, 2 H,  $\text{CH}_2\text{CH}_2\text{NET}_2$ ), 2.48 (qu, 4 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 1.78 (qt, 2 H,  $-\text{CH}_2\text{CH}_2\text{CH}_2-$ ), 0.96 (t, 6 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ).

**General Procedure for the Preparation of 5-[(Dialkylamino)alkyl]amino-8-hydroxy-1-methyl-6H-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:  $^1\text{H}$  NMR (free base) ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  10.00 (s, 1 H, ex, C8-OH), 9.02 (t, 1 H, ex,  $-\text{NHCH}_2-$ ), 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,  $-\text{NHCH}_2\text{CH}_2-$ ), 3.02 (s, 3 H, C1- $\text{CH}_3$ ), 2.72 (t, 2 H,  $\text{CH}_2\text{CH}_2\text{NET}_2$ ), 2.56 (qu, 4 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 1.02 (t, 6 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ).

**4p:**  $^1\text{H}$  NMR (free base) ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  10.0 (br s, 1 H, ex, C8-OH), 8.91 (t, 1 H, ex,  $-\text{NHCH}_2-$ ), 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,  $-\text{NHCH}_2\text{CH}_2-$ ), 3.00 (s, 3 H, C1- $\text{CH}_3$ ), 2.48 (m, 6 H,  $\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 1.76 (qt, 2 H,  $-\text{CH}_2\text{CH}_2\text{CH}_2-$ ).

**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%  $\text{CO}_2$  humidified atmosphere at 37 °C. L1210 mouse leukemia cells were seeded at a density of  $5 \times 10^4$  cells/mL. The test compound, dissolved in 50% ethanol, was added, at four different concentrations, to the cell suspension. The cytotoxic activity ( $\text{IC}_{50}$  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<sub>1</sub> mice were injected ip with  $10^6$  P388 lymphocytic leukemia cells on day 0 and treated ip on days 1-5 in accordance with the protocols described by the National Cancer Institute.<sup>13</sup> 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:

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

**Acknowledgment.** We acknowledge financial support of this work by Polish Project CPBR 11.5 and by Italian C. N. R. Thanks are also due to E. Augustin for the determination of cytotoxic activity, J. Paradziej-Lukowicz for the evaluation of in vivo activity, and F. Lupidi for NMR spectra.

## Synthesis and Some Pharmacological Properties of Potent and Selective Antagonists of the Vasopressor ( $V_1$ -Receptor) Response to Arginine-Vasopressin<sup>†</sup>

Maurice Manning,\* Stoytcho Stoev,<sup>‡</sup> Krzysztof Bankowski,<sup>§</sup> Aleksandra Misicka,<sup>§</sup> and Bernard Lammek<sup>||</sup>

Department of Biochemistry and Molecular Biology, Medical College of Ohio, P.O. Box 10008, Toledo, Ohio 43699-0008

Nga Ching Wo and Wilbur H. Sawyer

Department of Pharmacology, College of Physicians and Surgeons of Columbia University, 630 West 168th Street, New York, New York 10032. Received July 5, 1991

We report the solid-phase synthesis of eight position-9-modified analogues of the potent  $V_1$ -receptor antagonist of arginine-vasopressin, [1-( $\beta$ -mercapto- $\beta$ , $\beta$ -pentamethylenepropionic acid),2- $O$ -methyltyrosine]arginine-vasopressin ( $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ ) (1-8) and five position-9-modified analogues of the closely related  $\beta$ , $\beta$ -dimethyl less potent  $V_1$  antagonist, [1-deaminopenicillamine,2- $O$ -methyltyrosine]arginine-vasopressin ( $\text{dPTyr}(\text{Me})\text{AVP}$ ) (9-13). In  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$  the C-terminal Gly-NH<sub>2</sub> was replaced by (1) ethylenediamine (Eda), (2) methylamine (NHMe), (3) Ala-NH<sub>2</sub>, (4) Val-NH<sub>2</sub>, (5) Arg-NH<sub>2</sub>, (6) Thr-NH<sub>2</sub>, (7) Gly-Eda, (8) Gly- $N$ -butylamide (Gly-NH-Bu); in  $\text{dPTyr}(\text{Me})\text{AVP}$  the C-terminal Gly-NH<sub>2</sub> was replaced by (9) Ala-NH<sub>2</sub>, (10) Val-NH<sub>2</sub>, (11) Thr-NH<sub>2</sub>, (12) Arg-NH<sub>2</sub>, and (13) Tyr-NH<sub>2</sub>. 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- $V_1$   $\text{pA}_2$  values of 8.75, 8.73, 8.86, and 8.78, four of the analogues of  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$  (1-3 and 6) are equipotent with  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$  (anti- $V_1$   $\text{pA}_2 = 8.62$ ) but retain virtually none of the  $V_2$  agonism of  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ . 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  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ . With anti- $V_1$   $\text{pA}_2$  values respectively of 8.16, 8.05, 8.04, 8.52, and 8.25, all five analogues (9-13) of  $\text{dPTyr}(\text{Me})\text{AVP}$  are at least as potent  $V_1$  antagonists as  $\text{dPTyr}(\text{Me})\text{AVP}$  ( $\text{pA}_2 = 7.96$ ) and three of these (9, 12, 13) actually show enhanced  $V_1$  antagonism over that of  $\text{dPTyr}(\text{Me})\text{AVP}$ . In fact, the Arg-NH<sub>2</sub><sup>9</sup> analogue (12) is almost equipotent with  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ . These new  $V_1$  antagonists are potentially useful as pharmacological tools for studies on the cardiovascular roles of AVP. Furthermore the analogues of  $\text{dPTyr}(\text{Me})\text{AVP}$  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-( $\beta$ -mercapto- $\beta$ , $\beta$ -pentamethylenepropionic acid),2- $O$ -methyltyrosine]arginine-vasopressin ( $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ )<sup>3</sup> and [1-deaminopenicillamine,2- $O$ -methyltyrosine]arginine-vasopressin

<sup>†</sup> 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  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ , [1-( $\beta$ -mercapto- $\beta$ , $\beta$ -pentamethylenepropionic acid),2- $O$ -methyltyrosine]arginine-vasopressin;  $\text{dPTyr}(\text{Me})\text{AVP}$ , [1-deaminopenicillamine,2- $O$ -methyltyrosine]arginine-vasopressin; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; BOP, benzotriazol-1-yltris(dimethylamino)phosphonium hexafluorophosphate; Boc, *tert*-butoxycarbonyl; Bzl, benzyl; Tos, tosyl; AcOH, acetic acid; TFA, trifluoroacetic acid; HOBt, *N*-hydroxybenzotriazole; ONp, *p*-nitrophenyl ester; Et<sub>3</sub>N, triethylamine; Eda, ethylenediamine; NHMe, methylamine; NHBu, butylamide; Et<sub>2</sub>O, diethyl ether.

<sup>‡</sup> Visiting Investigator from Institute of Molecular Biology, Bulgarian Academy of Sciences, Bulgaria.

<sup>§</sup> Visiting Investigator from University of Gdansk, Poland.

<sup>||</sup> Visiting Investigator from University of Warsaw, Poland.

- (1) Manning, M.; Sawyer, W. H. Discovery, development and some uses of vasopressin and oxytocin antagonists. *J. Lab. Clin. Med.* 1989, 114, 617-632.
- (2) László, F. A.; László, F., Jr.; De Wied, D. Pharmacology and Clinical Perspectives of Vasopressin Antagonists. *Pharmacol. Revs.* 1991, 43, 73-108.
- (3) Kruszynski, M.; Lammek, B.; Manning, M.; Seto, J.; Haldar, J.; Sawyer, W. H. [1-( $\beta$ -mercapto- $\beta$ , $\beta$ -cyclopentamethylenepropionic acid), 2-( $O$ -methyltyrosine)] arginine-vasopressin and [1-( $\beta$ -mercapto- $\beta$ , $\beta$ -cyclopentamethylenepropionic acid)] arginine-vasopressin, two highly potent antagonists of the vasopressor response to arginine vasopressin. *J. Med. Chem.* 1980, 23, 364-368.