Novel DNA Groove Binding Alkylators: Design, Synthesis, and Biological Evaluation

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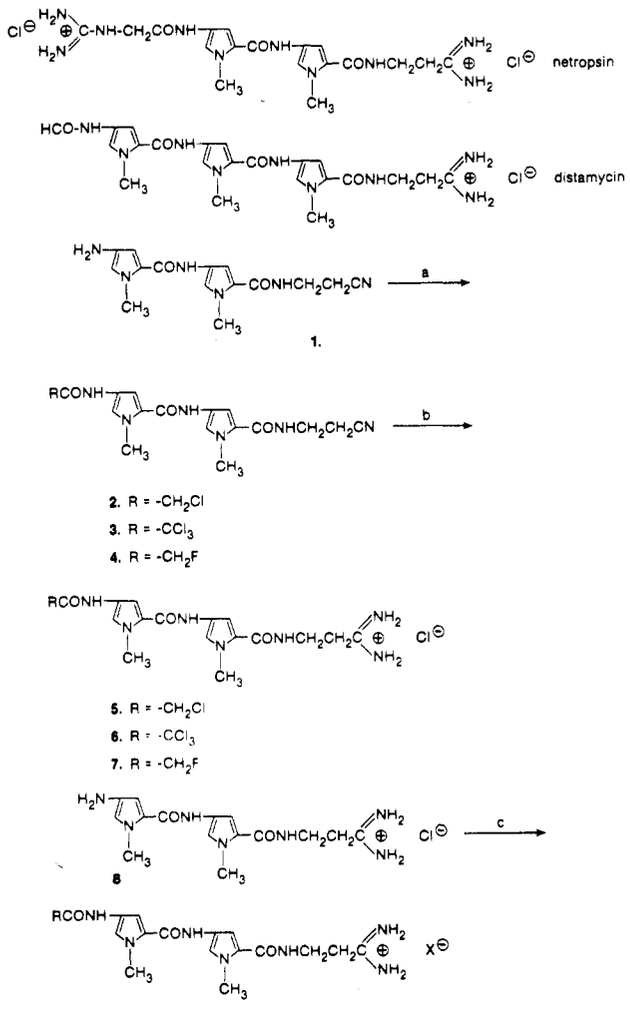
A group of oligopeptides has been synthesized that are structurally related to the natural antiviral antitumor agents netropsin and distamycin but which bear alkylating functions. Cytostatic activity against both human and murine tumor cell lines as well as their in vitro activity against a range of viruses is reported. The biological activity of these agents is discussed both in terms of their alkylating reactivity and of their structural differences. The incorporation of alkylating moieties into these minor groove DNA binders results in up to 45-fold increase in cytostatic activity compared with netropsin and up to 18 times the activity of distamycin.

Alkylating agents as a class have a long history in the treatment of human malignancies.[1] From the early observations of the anticancer activity of nitrogen mustards,[2] systematic development has led to clinically useful agents including cyclophosphamide,[3] (2-haloethyl)nitrosoureas,[4] and certain aryltriazenes.[5] As with any group of xenobiotics, they react chemically at many different sites in the cell.[1] However, compelling biochemical evidence implicates alkylation of nucleic acids as being primarily responsible for the cytotoxicity of alkylating agents.[1,6,7] In view of this, it is logical to try to introduce structural features into alkylating agents that would enhance the selectivity for, and reactivity toward, cellular nucleic acids. There are reports of attempts to incorporate alkylating moieties into the familiar class of DNA intercalating agents as a means of targeting the reactive moieties.[8] However, there exists another important class of DNA effectors, namely, groove-selective binding agents.[9] In general the major groove is utilized by control proteins (repressors and promotors)[10,11] whereas xenobiotics generally show minor-groove selectivity.[9] Among the most widely studied and best defined of the latter class are netropsin[12] and distamycin,[13] members of a modest group of naturally occurring antiviral antitumor antibiotics.[14] Netropsin and distamycin bind within the minor groove where they demand binding sites of $(A{\cdot}T)_4$ and $(A{\cdot}T)_5$, respectively.[9] The latter strict sequence specificity adds another dimension to targeting of reactive groups to predetermined sequences. In this connection, we are developing "lexitropsins" or information-reading oligopeptides which, by rational structural alteration of the lead natural products, are capable of recognizing and binding to quite different sequences from those of netropsin and distamycin.[15,16] These agents have been shown to be minor groove specific.[15] Our long-term goal is to explore systematically the structure–biological activity of sequence-directed DNA effectors,[17] including alkylating agents. Accordingly, in the present work we report the design, synthesis, characterization, and biological evaluation of a group of novel DNA groove binding alkylators. While the primary objective is to develop more effective anticancer agents, comparative antiviral data are included to extend the structure–activity studies.

Synthesis. The new oligopeptide agents carry potential alkylating moieties, viz., mono-, di-, and trichloroacetyl, fluoroacetyl, or cyclopropylcarbonyl. All these groups were introduced by allowing the corresponding acyl chlorides to react with 3-[1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido]propionitrile (1) which had been used in our total synthesis of netropsin.[18] The monochloroacetyl derivative **2** obtained by this pro-

Scheme I[a]



[a] Reaction conditions: (a) ROCl and $Et(i\text{-}Pr)_2N$; (b) EtOH and dry HCl and then NH_3; (c) RCOCl and $Et(i\text{-}Pr)_2N$.

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Table I. Inhibitory Effects of Oligopeptide Alkylators on the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A), Human B Lymphoblast (Raji and Namalva), Human T Lymphoblast (Molt/4F), and Human Hepatoma Cells

	ID_{50},[a] μg/mL					
compound	Molt/4F	L1210	FM3A	Raji	Namalva	hepatoma
5, $ClCH_2CO$	28.7 ± 5.3	8.70 ± 5.95	13.3 ± 3.8	3.09 ± 0.13	5.3 ± 2.53	3.63 ± 1.70
9, Cl_2CHO	27.2 ± 2.3	>100	>100	24.1 ± 0.5		
6, Cl_3CCO	84.3 ± 15.0	30.5 ± 8.5	>100	68.7 ± 11.4		
7, FCH_2CO	32.3 ± 2.5	40.3 ± 6.3	≥100	33.1 ± 2.8	32.3 ± 2.5	
10, c-C_3H_7CO	7.8 ± 1.3	>100	>100	26.7 ± 1.5		
11, $Cl(CH_2)_3CO$	>100	>100	74.1 ± 17.8	>100		
distamycin	28.4 ± 8.6	27.1 ± 4.7	30.6 ± 2.4	23.7 ± 3.69	22.5 ± 3.5	54.7 ± 21.8
netropsin	>100	245 ± 92	321 ± 18	139 ± 63	103 ± 46	44.7 ± 12.5

[a] Required to inhibit cell proliferation by 50%.

cedure had been employed in the synthesis of oligopeptides with trimethylammonium and triethylammonium moieties as alternative charged end groups to that of the guanidinylacetyl in the antibiotic netropsin.[17]

After introduction of an acyl group into the oligopeptide, the amidine moiety is normally generated via the Pinner procedure[19] by reacting with ethanol in the presence of hydrogen chloride and subsequent ammonolysis. However, in case of the dichloroacetyl (**9**) and cyclopropylcarbonyl (**10**) derivatives, better results were obtained by direct reaction of the corresponding acyl chlorides with a compound already containing an amidine moiety i.e. 3-[1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)-pyrrole-2-carboxamido]propionamidine hydrochloride (**8**). In condensation of acyl chlorides with amino oligopeptides, Hunig's base (NEt-*i*-Pr_2) or *N*-ethylmorpholine was used as bases. *N*-Ethylmorpholine is especially appropriate in reactions of acyl chlorides with amino oligopeptides containing amidine groups, because the *N*-ethylmorpholine hydrochloride formed is readily soluble in acetonitrile and hydrochloride salts of acylamino oligopeptide amidines formed in the condensation process are insoluble in the same solvent and can be readily isolated in the pure state. The dichloroacetyl derivative was isolated as the tetraethylammonium sulfate. This procedure permitted the elimination of ammonium chloride contaminating the product.

Cytostatic Activity. All of the compounds tested, **5–7**, **9**, and **10** showed inhibitory effects on the proliferation of at least one of the following tumor cells in culture: murine leukemia (L1210), murine mammary carcinoma (FM3A), human lymphoblast (Raji and Namalva), or hepatoma. The broadest spectrum of activity and greatest potency are shown by the chloroacetyl derivative **5** with ID_{50} values for the different tumor cell lines ranging between 3 and 29 μg/mL (Table I). For the human tumor cell lines Raji, Namalva, and hepatoma, the ID_{50} values of **5** ranged between 3 and 5 μg/mL. The compound was somewhat less active against human T lymphoblast Molt/4F (ID_{50} = 28.7 μg/mL). The dichloroacetyl derivative **9** was active only against Molt/4F and Raji cell lines (ID_{50} = 27 and 29 μg/mL, respectively). The trichloroacetyl **6** and fluoroacetyl **7** compounds showed a similar spectrum of activity inhibiting the growth of all cultured cells with the exception of FM3A. The 3-chlorobutyryl derivative, as expected, was inactive as an anticancer agent because of the insulating intervening methylene group between the halogen and the acyl moiety. The cyclopropyl derivative **10** inhibited only Molt/4F and Raji (ID_{50} = 7.8 and 26.7 μg/mL, respectively). Complementary screens for inhibitory effects in the proliferation of murine leukemias L1210 and P388 at constant drug concentrations of 20 μg/mL (summarized in Table II) are in general accord with the above data. However, in P388 screen, the most active agent was the trichloroacetyl compound **6**, yielding 55% inhibition of cell growth at 20 μg/mL. It may be noted that in general, and especially for **5**, **9**, and **10** toward particular cell lines, the cytotoxicity of the alkylating oligopeptides was much greater than that of the parent antibiotics distamycin and netropsin. The fact that the LD_{50} of chloroacetamide injected iv in mice is 180 mg/kg[20] suggests that the observed high cytostatic activities of chloroacetyl derivatives **5**, **6**, and **9** are not due to nonspecific alkylation by the α-chloroacetamide.

Table II. Inhibitory Effects of Oligopeptide Alkylators on the Proliferation of Murine Leukemias (L1210 and P388)

	% inhibition at 20 μg/mL	
compound	L1210	P388
5, $ClCH_2CO$	29	38
9, Cl_2CHCO	13	28
6, Cl_3CCO	28	55
7, FCH_2CO	0	0
10, c-C_3H_7CO	3	8
11, $Cl(CH_2)_3CO$	0	0

Antiviral Activity. The chloroacetyl and dichloroacetyl derivatives (**5** and **9**, respectively) exhibit antiviral activity against herpes simplex-1 and herpes simplex-2 and vaccinia virus in primary rabbit kidney cell cultures. In addition, the cyclopropyl compound **10** was active against

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Table III. Antiviral Activity of Oligopeptide Agents in Primary Rabbit Kidney Cells, HeLa, WI-38, and Vero Cell Cultures

		min inhibitory concn,[b] μg/mL						
		primary kidney cell cultures			HeLa			
compound	min cytotoxic concn,[a] μg/mL	herpes simplex-1	herpes simplex-2	vaccinia	vesicular stomatitis	vesicular stomatitis	Coxsackie B4	polio-1
5, $ClCH_2CO$	≥100	70	70	10	>200	>100	>100	>100
9, Cl_2CHCO	200	70	70	20–70	>200	>200	>100	>100
6, Cl_3CCO	≥100	>100	>100	>100	>100	>40	>40	>40
10, c-C_3H_7CO	100	20–70	40–70	>100	>100			
7, FCH_2CO	≥200	150	100	7	>100			
11, $Cl(CH_2)_3CO$	≥200	300	150	20	>400			
netropsin	≥400	>10	>10	2	>10			
distamycin	≥200	70	70	0.2	>100			

		min inhibitory concn,[b] μg/mL						
		WI-38				Vero B		
compound	min cytotoxic concn, μg/mL	rhino-1A	rhino-9	para-influenza	Reo-1	Sindbis	Coxsackie B4	Semliki forest
5	≥100			>100	>100	>100	>100	>100
9	≥200	40	>40	>100	>200	>100	>40	>100
6	≥200	>40	>40	>100	>40	>100	>100	>100
10	≥200	>40	20	>100	>100	>100	>100	>100
7	≥100	>200	>200	>40	>100	>200	>200	>200
11	≥200							
netropsin	≥400			>100	>200	>200	>200	>200
distamycin	≥200			>200	>200	>200	>200	>200

[a] Required to cause a microscopically detectable alteration of normal cell morphology. [b] Required to reduce virus-induced cytopathogenicity by 50%.

both herpes simplex virus types, and the fluoroacetyl agent **7** proved specifically active against vaccinia virus (Table III). Similarly, the 3-chlorobutyryl derivative showed its highest activity against vaccinia virus in common with the parent compounds netropsin and distamycin. In general, this group of agents was devoid of activity at subtoxic concentrations against vesicular stomatitis virus and other RNA viruses including polio type 1, Coxsackie type B4, rhino types 1A and 9, reotype 1, parainfluenza type 3, Sindbis, and Semliki forest virus (in either HeLa, WI-38, or Vero cell cultures (Table III)).

Discussion

Introduction of an alkylating moiety in place of the charged guanidinium acetyl end group in netropsin proved to be effective in increasing the cytostatic activity against certain human tumor cell lines by a factor of up to 45 compared with the parent natural product netropsin. Especially noteworthy is the chloroacetyl derivative **5**, which was 15–45 times more active than netropsin and 3–18 times more active than distamycin against all murine and human tumor cell lines examined with the exception of Molt/4F, against which it equaled distamycin in activity. It is evident that an active alkylating group is essential for cytostatic activity against both L1210 and P388 leukemias, i.e., it is insufficient solely to bind to DNA as exemplified by control compound **11**, which contains an insulating methylene chain between the halogen and the acyl group. Similarly, the lower alkylating property of the fluoro group is reflected in the relative activities of **5** and **7**. It should be noted, however, that the ability of this class of agents to bind to DNA[15,16] contributes to their cytostatic activity, as attested by the activity of **7** against Molt/4F, Raji, and Namalva, which is comparable with that of distamycin. Similarly the nonalkylating compound **11** shows modest activity against the murine mammary carcinoma FM3A cell line.

Compound **10**, which contains an activated cyclopropane group, was modeled on the potent minor groove DNA binding agent CC-1065. This agent has been shown to bind selectively to $(A{\cdot}T)_4$ sequences, like netropsin does, but to form a covalent bond via the activated cyclopropane moiety.[21] In fact, compound **10** selectively inhibits Molt/4F at a fourfold lower concentration than distamycin.

The other agents tested, **6**, **7**, and **9**, had cytostatic activity comparable with that of distamycin. This indicates the same influence of dichloro, trichloro, or fluoroacetyl groups on the cytostatic activity as the [(formylamino)-pyrrolo]carboxy moiety. It may be noted that one tumor cell line (FM3A) was particularly resistant toward this novel class of agents with the exception of compound **5** (ID_{50} = 13.3 μg/mL).

The question naturally arises of the probable site of attack of these agents in the cell. Several lines of evidence indicate that these oligopeptide agents act at the cell nucleus. We recently synthesized a nitroxide spin-labeled oligopeptide derived from netropsin. EPR studies have shown that this agent enters KB human nasopharangeal tumor cells and concentrates in the nucleus.[21] The closely related (bromoacetyl)distamycin, an anlogous bifunctional groove-binding molecule, has been shown to alkylate DNA sequence specifically in the minor groove.[23] Thus, the structure–activity relations based on the relative alkylating/DNA binding properties may be useful.

Whereas it is not suggested that alkylating agents should be seriously considered as potential antivirals, nevertheless, the comparative antiviral activity of these new agents contribute to an overall description of biological activity for this series. Introduction of an alkylating function does not markedly improve the antiviral activities, which remain at levels comparable with those of distamycin.

Experimental Section

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. The IR spectra were recorded on a Nicolet 7199 F.T. spectrophotometer, and only the principal peaks are reported. The ^1H NMR spectra were recorded on Bruker WH-200 and WH-400 spectrometers. FAB (fast atom bombardment) mass spectra were determined on Associated Electrical Industries (AEI) MS-9 and MS-50 double-focusing

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high-resolution mass spectrometers. Kieselgel 60 (230–400 mesh) from E. Merck was used for flash chromatography, and precoated sheets silica gel 60F-254 from E. Merck were used for TLC. TLC system for (i) covalent peptidic compounds was chloroform–methanol, 9:1, and for (ii) ionic compounds was methanol with some AcOH.

3-[1-Methyl-4-[1-methyl-4-(chloroacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (5). A solution of 110 mg (0.28 mmol) of the corresponding nitrile **2**[13] in 5 mL of absolute ethanol was treated with dry HCl gas with cooling, and the mixture was stirred for 1.5 h at room temperature. The solvent was removed, 5 mL of absolute EtOH was added, and then dry NH_3 was condensed into the reaction vessel. After 1 h at room temperature, the solvents were removed, and the residue was extracted with hot 2-propanol. More polar impurities (evidenced by an immobile spot on TLC, silica gel, developed with MeOH and AcOH system) were removed by controlled precipitation with EtOAc. The pure product was obtained by precipitation with a large excess of EtOAc to give 100 mg (80% yield): no definite mp, softens at 165 °C and melts at about 195 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.65 (t, J = 6 Hz, 2 H), 3.50 (q, J = 6 Hz, 2 H), 3.82 and 3.88 (2 s, 6 H), 4.22 (s, 2 H), 6.95 (m, 2 H), 7.21 (s, 2 H), 8.26 (t, J = 6 Hz, 1 H), 8.76 and 9.07 (2 br s, 4 H), 9.97 and 10.49 (2 s, 1 H each); IR (Nujol) ν_{max} 1463, 1530, 1580, 1640, 1690, 3100, 3120, 3270 cm^{-1}; MS-FAB (m/z) 408 $(M - Cl)^+$, 815 $(2\,M - Cl - HCl)^+$. Anal. Calcd for $C_{17}H_{23}Cl_2N_7O_3$: H, 5.2; C, 45.9; Cl, 16.0; N, 22.1. Found: H, 5.4; C, 45.7; Cl, 16.2; N, 22.3.

3-[1-Methyl-4-[1-methyl-4-(dichloroacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Tetraethylammonium Sulfate (9). Compound **8** (227 mg, 0.6 mmol) and Hunig's base (diisopropylethylamine) (214 μL, 1.2 mmol) were dissolved in EtOH (3 mL), and the mixture was cooled to −30 °C. Then, dichloroacetyl chloride (120 μL, 1.2 mmol) in 1 mL of THF was added slowly. The mixture was allowed to attain room temperature, and the solvents were removed in vacuo. Addition of chloroform precipitated the crude product. It was dissolved in MeOH and separated from a more polar impurity by flash chromatography on silica gel with methanol as eluent. The fractions containing the product were collected, and the solvent was removed. The residue was dissolved in absolute EtOH, and some inorganic impurities were removed by filtration. The ethanol was removed in vacuo, and the residual solid was dissolved in 2-propanol and an 2-propanolic solution of bis(tetraethylammonium) sulfate was added to precipitate the pure compound **9**. The latter was collected, washed with 2-propanol and hexane, and dried in vacuo at 80 °C to give 193 mg (48% yield) of **9**: mp 170 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.11 (t, 12 H), 2.66 (br t, 2 H), 3.18 (q, 8 H), 3.50 (br s, 2 H), 3.76 (s, 3 H), 3.82 (s, 3 H), 6.92 (2 d, 2 H), 7.08 (s, 1 H), 7.32 (2 d, 2 H), 8.65 (br s, 1 H), 9.05 (v br s, 2 H), 9.93 (br s, 1 H), 10.38 (v br s, 2 H), 11.7 (br s, 1 H); IR (Nujol) ν_{max} 1376, 1406, 1462, 1540, 1580, 1648, 1693, 3250 cm^{-1}; MS-FAB (m/z) 671 (MH^+ for 2 ^{37}Cl), 669 (MH^+ for 2 ^{35}Cl), 444 $[(M - SO_4NEt_4)^+$ for 2 ^{37}Cl], 442 $[(M - SO_4NEt_4)^+$ for 2 ^{35}Cl]. Anal. Calcd for $C_{25}H_{42}Cl_2N_8O_7S$: C, 44.8; H, 6.3; Cl, 10.6; N, 16.7; S, 4.8. Found: C, 44.4; H, 6.5; Cl, 10.5; N, 16.4; S, 5.0.

3-[1-Methyl-4-[1-methyl-4-(trichloroacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile (3). The compound **1** (300 mg, 0.95 mmol) and *N*-ethylmorpholine (134 μL, 1.05 mmol) in dry acetonitrile (5 mL) were cooled to −20 °C. Trichloroacetyl chloride (117.5 μL, 1.05 mmol) in 1 mL of dry THF was added. When the solid dissolved, the mixture was allowed to warm up to room temperature. The solvents were removed, and water was added. The solid was collected and recrystallized from acetonitrile to give 355 mg (82%) of **3**: mp 265 °C if heated fast (slow heating produced carbonization without melting); ^{1}H NMR (Me_2SO-d_6) δ 2.72 (t, 2 H), 3.40 (q, 2 H), 3.80 and 3.85 (2 s, 6 H), 6.91 (d, 1 H), 7.12 (d, 1 H), 7.22 (m, 2 H), 8.35 (t, 1 H, 10.00 (s, 1 H), 10.93 (s, 1 H); IR (Nujol) ν_{max} 1377, 1439, 1469, 1521, 1587, 1637, 1661, 1691, 2243, 3200, 3365, 3385 cm^{-1}; MS, m/z (relative intensity) 460.0436 [(13.1) M^+ for 2 ^{35}Cl + 1 ^{37}Cl calcd 460.0398], 458.0459 [(13.14) M^+ for 3 ^{35}Cl calcd 458.0427].

[1-Methyl-4-[1-methyl-4-(trichloroacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (6). Compound **3** (302 mg, 0.66 mmol) was treated with dry ethanol in the presence of HCl at room temperature for 2 h. The solvent was removed, the residue was dissolved in absolute EtOH, and then dry ammonia was condensed into the reaction vessel. After 1 h, the solvents were evaporated in vacuo. The residual solid was dissolved in methanol (2 mL), and the excess of NH_4Cl was precipitated with $CHCl_3$ and EtOAc. The filtrate was concentrated to a small volume, and a crude product was collected. This was dissolved in MeOH, and the operation was repeated. The solid was extracted with acetone. After evaporation of the solvent, the solid was dissolved in methanol, and the product was precipitated with ethyl acetate to give 150 mg (44.5% yield) of **6**: mp 122–125 °C which still contained about 9 mol % of NH_4Cl (estimated by NMR); ^{1}H NMR (Me_2SO-d_6) δ 2.62 (t, 2 H), 3.49 (q, 2 H), 3.78 (s, 3 H), 3.83 (s, 3 H), 6.92 (d, 1 H), 7.12 (d, 1 H), 7.19 and 7.23 (2 d, 2 H), 8.26 (t, 1 H), 8.64, 8.76, and 9.05 (3 br s, together 8 H), 10.00 (s, 1 H), 10.94 (s, 1 H); IR (Nujol) ν_{max} 1377, 1463, 1530, 1580, 1635, 1689, 3250 cm^{-1}; MS-FAB (m/z) 480 for $(M - Cl)^+$ 2 ^{37}Cl + 1 ^{35}Cl, 478 for $(M - Cl)^+$ 1 ^{37}Cl + 2 ^{35}Cl, 476 for 3 ^{35}Cl. Anal. Calcd for $C_{17}H_{21}Cl_4N_7O_3$: C, 39.8; H, 4.1; Cl, 27.6; N, 19.1. Found: C, 39.6; H, 4.3; Cl, 27.9; N, 19.3.

[1-Methyl-4-[1-methyl-4-(fluoroacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile (4). Compound **1** (365 mg, 1.16 mmol) and Hunig's base (309 μL, 1.74 mmol) were dissolved in 7 mL of dry CH_3CN and cooled to −10 °C. Fluoroacetyl chloride (121 μL, 1.74 mmol) was slowly added. The mixture was allowed to reach room temperature when the solid dissolved, followed by precipitation of the product. The mixture was stirred for half an hour, and the product was collected, washed with CH_3CN, water, *i*-PrOH, and hexane, and dried to give 350 mg (80% yield) of **4**: mp 243–245 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.74 (t, 2 H), 3.40 (q, 2 H), 3.82 and 3.85 (2 s, 6 H), 4.84 and 5.07 (d, 2 H, FCH_2), 6.93 (d, 1 H), 7.01 (d, 1 H), 7.22 (m, 2 H), 8.37 (t, 1 H), 9.98 (s, 1 H), 10.18 (s, 1 H); IR (Nujol) ν_{max} 1220, 1265, 1276, 1366, 1376, 1402, 1436, 1464, 1522, 1565, 1581, 1636, 1654, 2250, 3120, 3257, 3320 cm^{-1}; MS, m/z 374.1498 (25) M^+, calcd 374.1502.

[1-Methyl-4-[1-methyl-4-(fluoroacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (7). A suspension of compound **4** (300 mg, 0.8 mmol) in dry EtOH (5 mL) was saturated with dry HCl with cooling, and the mixture was stired at room temperature for 2 h. After evaporation of the solvent, the residue was dissolved in dry EtOH (5 mL), and dry ammonia was condensed into the reaction vessel. After 1 h, the solvent was removed, and the solid was extracted with hot 2-propanol, filtered, and evaporated to a volume of 3 mL. Then, 50 mL of acetonitrile was added to precipitate 250 mg (58.5%) of pure **7**: no distinct mp; ^{1}H NMR (Me_2SO-d_6) δ 2.63 (t, 2 H), 3.51 (q, 2 H), 3.83 and 3.86 (2 s, 6 H), 4.84 and 5.08 (d, 2 H, FCH_2), 6.94 (d, 1 H), 7.02 (d, 1 H), 7.20 (m, 2 H), 8.27 (t, 1 H), 8.73 (s, 2 H), 9.04 (s, 2 H), 9.98 (s, 1 H), 10.24 (s, 1 H); IR (Nujol) ν_{max} 1377, 1443, 1463, 1518, 1543, 1625, 1641, 1692, 3258 cm^{-1}; MS-FAB (m/z) 392 $(M - Cl)^+$. Anal. Calcd for $C_{17}H_{23}ClFN_7O_3$: C, 47.7; H, 5.4; Cl, 8.3; F, 4.4; N, 22.9. Found: C, 47.5; H, 5.7; Cl, 8.4; F, 4.7; N, 23.2.

[1-Methyl-4-(1-methyl-4-cyclopropanecarboxamidopyrrole-2-carboxamido)pyrrole-2-carboxamido]propionamidine Hydrochloride (10). A solution of compound **8** (200 mg, 0.54 mmol) in absolute EtOH (4 mL) was cooled to −30 °C, and *N*-ethylmorpholine (75 μL, 0.6 mmol) in THF was added, followed by cyclopropylcarbonyl chloride (54 μL, 0.6 mmol) in 1 mL of THF. The mixture was allowed to warm up to room temperature. It was cooled again to −30 °C, and the operation was repeated twice with the same amounts of *N*-ethylmorpholine and cyclopropylcarbonyl chloride. The resulting solution was evaporated to dryness, the residue was dissolved in 2-propanol (4 mL), and then acetonitrile (4 mL) was added to precipitate impurities. After filtration, the solvent was removed, and acetonitrile added. The solid was collected to give 117 mg (50% yield) of pure **10**: mp 180 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.72 (m, 4 H), 1.72 (m, 1 H), 2.61 (t, 2 H), 3.48 (q, 2 H), 3.79 (s, 6 H), 6.88 and 6.93 (2 d, 2 H), 7.12 and 7.18 (2 d, 2 H), 8.23 (t, 1 H), 8.70 (s, 2 H), 9.01 (s, 2 H), 9.90 (s, 1 H), 10.14 (s, 1 H); IR (Nujol) ν_{max} 1377, 1463, 1525, 1580, 1641, 1690, 3250 cm^{-1}; MS-FAB (m/z) 400 $(M - Cl)^+$.

3-[1-Methyl-4-[1-methyl-4-[(4-chlorobutyryl)amino]-pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-

amidine Hydrochloride (11). The amino compound 8 (154 mg, 0.5 mmol) was dissolved in absolute ethanol (3 mL), *N*-ethylmorpholine (64 μL, 0.5 mmol) was added, and the mixture was cooled to −10 °C. Then, 4-chlorobutyryl chloride (70.5 mg, 0.5 mmol) was added slowly. After 15 min at room temperature, the solvent was evaporated, and the residue was triturated with acetonitrile to remove the morpholine hydrochloride. The residue was dissolved in absolute ethanol (1 mL) and precipitated with ethyl acetate to give 170 mg of crude product. Flash chromatography on silica gel with methanol afforded 70 mg (30% yield) of a very hygroscopic **11**: mp 62–65 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.02 (m, 2 H), 2.43 (t, 2 H), 2.63 (t, 2 H), 3.52 (q, 2 H), 3.69 (t, 2 H), 3.82 and 3.83 (2 s, 6 H), 6.89 and 6.95 (2 d, 2 H), 7.17 and 7.21 (2 d, 2 H), 8.26 (t, 1 H), 8.71 (s, 2 H), 9.02 (s, 2 H), 9.93 and 9.98 (2 s, 2 H); IR (Nujol) ν_{max} 1269, 1377, 1405, 1445, 1464, 1534, 1582, 1641, 1690, 3100, 3124, 3275 cm^{-1}; MS-FAB, *m/z* (relative intensity) 436 (100) for ^{35}Cl, 438 (37) for ^{37}Cl.

Cytostatic Activity. Mouse leukemic cells (i.e., L1210 and P388D1) were obtained from American Type Tissue Collection (Rockville, MD) and were grown in either McCoy's 5A (L1210) or Fischer's (P388D1) medium supplemented with 10% fetal calf serum (Grand Island Biological Co., Grand Island, NY). Compounds to be tested were dissolved in water; compounds that were poorly water soluble were sonicated and administered as a suspension. Stock solutions were prepared at constant ratios up to 500 times that required in the growth medium so that 10 μL of stock solution could be added to 160 μL of growth medium. Cells were seeded onto 96-well microtiter plates at a concentration of 1×10^5 cells per well and allowed to grow for 72 h in 5% CO_2 at 37 °C in humidified incubator. The cytostatic activity of the drugs was determined by use of a methylenetetrazolium dye (MTT) assay as described by Mossmann.[24] Cell viability was measured as a function of the ability of cells to form a blue formazan product, the optical density of which was determined by a Dynatech Microplate (Model 600) Reader (570 nm; reference set at 630 nm).

The other anticancer assays were performed according to previously established procedures.[25,26]

Antiviral assays were performed as reported previously.[27,28]

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Registry No. **1**, 97950-71-5; **2**, 101772-47-8; **3**, 111770-96-8; **4**, 111770-97-9; **5**, 111770-98-0; **5** (free base), 111771-03-0; **6**, 111770-99-1; **6** (free base), 111771-04-1; **7**, 111771-00-7; **7** (free base), 111771-05-2; **8**, 97950-75-9; **9**, 111793-70-5; **9** (free base), 111771-06-3; **10**, 111771-01-8; **10** (free base), 107580-63-2; **11**, 111771-02-9; **11** (free base), 111771-07-4; $Cl_2CHCOCl$, 79-36-7; Cl_3CCOCl, 76-02-8; FCH_2COCl, 359-06-8; *c*-PrCOCl, 4023-34-1; $Cl(CH_2)_3COCl$, 4635-59-0.

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Design and Synthesis of a Series of Combined Vasodilator/β-Adrenoceptor Antagonists Based on 6-Arylpyridazinones

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A series of new 6-[4-[[(aryloxy)acyl]amino]phenyl]-4,5-dihydropyridazinones have been synthesized and evaluated as combined vasodilator/β-adrenoceptor antagonists and potential antihypertensive agents. Many of the early compounds displayed an unacceptably high level of intrinsic sympathomimetic activity (ISA) and a relatively short duration of action. Disubstitution in the 2,3-positions or in the 4-position of the aryloxy ring gave compounds with low ISA levels and, in some instances, improved duration of action. All of the compounds were vasodilators, but the 5-methylpyridazinone derivatives showed consistently greater antihypertensive activity than their 5-H lower homologues. Further detailed pharmacological investigations led to the selection of 6-[4-[3-[[2-hydroxy-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]propyl]amino]propionamido]phenyl]-5-methyl-4,5-dihydro-3(2*H*)-pyridazinone (**4t**) (SK&F 95018) as a development candidate.

Essential hypertension is characterized by elevated total peripheral resistance which maintains a raised systemic arterial blood pressure while cardiac output and heart rate remain within the normal range.[1] Although the etiology of elevated vascular resistance is unclear, clinical studies have shown that peripheral vasodilators can be used effectively to lower blood pressure. The therapeutic use of vasodilators alone is limited, however, by side effects arising directly from the vasodilator activity of these agents. Thus, reduction in blood pressure following administration of vasodilator agents initiates baroreceptor reflex changes which lead to increased sympathetic drive and activation of β-adrenoceptors in the heart, with a resultant undesirable increase in heart rate and activation of the renin/angiotensin system leading to vasoconstriction and fluid retention.[2] It has been shown that β-adrenoceptor antagonists will inhibit these undesirable effects of vasodilators, with the result that the combined use of vasodilators and β-blockers has been widely adopted for the treatment of hypertension.[3]

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