Novel Linked Antiviral and Antitumor Agents Related to Netropsin and Distamycin: Synthesis and Biological Evaluation

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A group of oligopeptides have been synthesized which are structurally related to the natural antiviral antitumor antibiotics netropsin and distamycin bearing two such moieties linked by polymethylene bridges. Cytostatic activity against both human and murine tumor cell lines and their in vitro activity against a range of viruses are reported. Enhanced antiviral activity was obtained against vaccinia virus. As a result of the introduction of the polymethylene linkers [$(CH_2)_n$, with n = 1, 2, and 6–8], both the antitumor and antivaccinia virus activity was markedly enhanced, relative to that of the parent compounds netropsin and distamycin. The biological activity of these agents is discussed both in terms of their structural differences and in relation to their minor groove binding to duplex DNA.

The family of naturally occurring oligopeptide antibiotics[1] comprises netropsin[2] (1), distamycin[1,3] (2), anthelvencins A and B[4] (3), kikumycins A and B[5] (4), amidinomycin[6] (5), and noformycin[7] (6) (see Figure 1 for structures). Some congeners display antiviral and anticancer properties. The most active of these, netropsin and distamycin, are too toxic for clinical use. Few reports exist of attempted structural modification to increase potency and selectivity of these agents.[8-18] Netropsin and distamycin are among the best characterized agents with respect to their mode of interaction with DNA which is their principal cellular target. These antibiotics bind within the minor groove of B-DNA, where they demand binding sites consisting of four and five AT base pairs, respectively.[19,20] Analysis of the structural and spatial requirements for this specific binding, which is the net result of hydrogen bonding, electrostatic attraction, and van der Waals interactions, led to the concept of lexitropsins, or information-reading ligands.[21-24] Examples of the latter, designed by rational structural modification of netropsin and distamycin, displayed altered sequence recognition.[21-24] A general DNA effector capable of recognizing a unique sequence in the human genome requires a site of 15–16 base pairs.[21] One of the important factors to be considered in this regard is the possible impact of "phasing" or apparent lack of dimensional correspondence between oligonucleotides and their complementary oligopeptides for longer lexitropsins.[23]

We report an initial examination of this problem by synthesizing a series of compounds bearing netropsin moieties joined by a linked which is variable in length. A second consideration, that of promoting cellular uptake,[25] dictated that the linkage be lipophilic, and therefore polymethylene linkers were employed in the prototype structures. We report the synthesis of a group of such novel oligopeptide agents, an examination of their DNA binding properties, and an evaluation of their antiviral and tumor cell cytotoxic properties. A substantial increase in potency, particularly in tumor cell cytotoxicity activity and selectivity toward human tumor cells, was achieved compared with the parent natural products.

Syntheses of Linked Oligopeptide Agents Bearing Mono-, Di-, and Tripyrrole Distamycin-like Moieties. Amino compounds 7–9 (Figure 2) were obtained by catalytic reduction (Pd/C) of the corresponding nitro compounds.[26,27] Several methods were examined to obtain diamide-linked structures of dicarboxylic acids so as to obtain the best yields. Thus the amino compounds were allowed to react with acyl chlorides in the presence of base

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(24) R. E. Dickerson, Molecular Biology Institute, UCLA, has independently suggested the substitution of imidazole for pyrrole in netropsin on the basis of X-ray diffraction analysis of the antibiotic cocrystallized with a dodecamer.[20]
(25) We have obtained independent evidence for the promotion of cellular uptake of oligopeptides by the incorporation of lipophilic moieties in demonstrating rapid uptake of a spin-labeled oligopeptide by living KB cells and concentration in the nucleus (Bailly, C.; Catteau, J.-P.; Henichart, J.-P.; Reszka, K.; Shea, R. G.; Krowicki, K.; Lown, J. W. *Biochem. Pharmacol.* **1989**, *38*, 1625).
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Table I. Analytical and Spectroscopic Data on Linked Oligopeptides and Their Precursors

compd	*n*	method	yield %	mp[a]	formula[b]	anal.
13	0	3	88.6	295–7	$C_{31}H_{34}N_{12}O_5$	C, H, N
13a	0	3	61.6	211–5	$C_{31}H_{41}N_{14}O_5Cl_2$	C, H, N, Cl
14	1	2	40	225–30	$C_{33}H_{36}N_{12}O_6$	C, H, N
14a	1	2	54	281–4	$C_{33}H_{44}N_{14}O_6Cl_2$	C, H, N, Cl
15	2	1	77	29	$C_{34}H_{38}N_{12}O_6$	C, H, N
15a	2	1	34	283–5	$C_{34}H_{46}N_{14}O_6Cl_2$	C, H, N, Cl
16	3	1	51	215–8	$C_{35}H_{40}N_{12}O_6$	C, H, N
16a	3	1	53	204–6	$C_{35}H_{48}N_{14}O_6Cl_2$	C, H, N, Cl
17	4	4	61	244–6	$C_{36}H_{42}N_{12}O_6$	C, H, N
17a	4	4	58.7	195–6	$C_{36}H_{50}N_{14}O_6Cl_2$	C, H, N, Cl
18	5	1	74.5	218–21	$C_{37}H_{44}N_{12}O_6$	C, H, N
18a	5	1	67	195–200	$C_{37}H_{52}N_{14}O_6Cl_2$	C, H, N, Cl
19	6	1	86	228–34	$C_{38}H_{42}N_{12}O_6$	C, H, N
19a	6	1	78	203–5	$C_{38}H_{54}N_{14}O_6Cl_2$	C, H, N, Cl
20	7	1	84	172–5	$C_{39}H_{48}N_{12}O_6$	C, H, N
20a	7	1	34	191–4	$C_{39}H_{56}N_{14}O_6Cl_2$	C, H, N, Cl
21	8	1	82	214–5	$C_{40}H_{50}N_{12}O_6$	C, H, N
21a	8	1	53	190–2	$C_{40}H_{58}N_{14}O_6Cl_2$	C, H, N, Cl
22	9	3	83	205–7	$C_{41}H_{52}N_{12}O_6$	C, H, N
22a	9	3	68	175 (softens)	$C_{41}H_{60}N_{14}O_6Cl_2$	C, H, N, Cl
23	10	3	88.7	204–7	$C_{42}H_{54}N_{12}O_6$	C, H, N
23a	10	3	69	174 (softens)	$C_{42}H_{62}N_{14}O_6Cl_2$	C, H, N, Cl
24	2	1	55	225–31	$C_{22}H_{26}N_8O_4$	C, H, N
24a	2	1	23	207–9	$C_{22}H_{34}N_{10}O_4Cl_2$	C, H, N, Cl
25	2	1	58	286–90 dec	$C_{46}H_{50}N_{16}O_8$	C, H, N
25a	2	1	49	217–20	$C_{46}H_{58}N_{18}O_8Cl_2$	C, H, N, Cl

[a] Uncorrected. [b] All compounds had satisfactory C, H, N (and Cl) elemental analyses within 0.4% of the calculated values and exhibited NMR, IR, and MS data consistent with the structure.

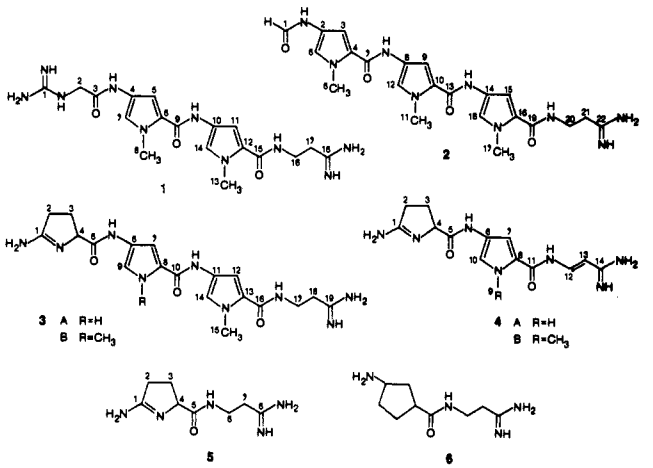

Figure 1. Structures of oligopeptide antibiotics netropsin (**1**); distamycin (**2**); anthelvencins A and B (**3**); kikumycins A and B (**4**); amidinomycin (**5**); and noformycin (**6**).

(method 1); with dicarboxylic acids and dicyclohexylcarbodiimide (DCC) (method 2); with 1,1′-carbonyldiimidazole (method 3); and with dicarboxylic aids via formation of mixed anhydrides with pivalic acid (method 4). The products of these reactions were treated under Pinner reaction conditions[28] to generate amidine hydrochloride terminal groups by treatment with hydrochloric acid in ethanol, followed by ammonia in ethanol, to afford respectively compounds of the type **10–12** (Figure 3).

The final products consisted of oligopeptides bearing aliphatic dicarboxylic acid moieties with $(CH_2)_n$, where n = 0 up to 10. In addition, succinic acid derivatives of the aminomonopyrrole (**11**) and aminotripyrrole (**12**) were obtained for the purpose of comparison. The physical and analytical details of the novel structures are summarized in Table I.

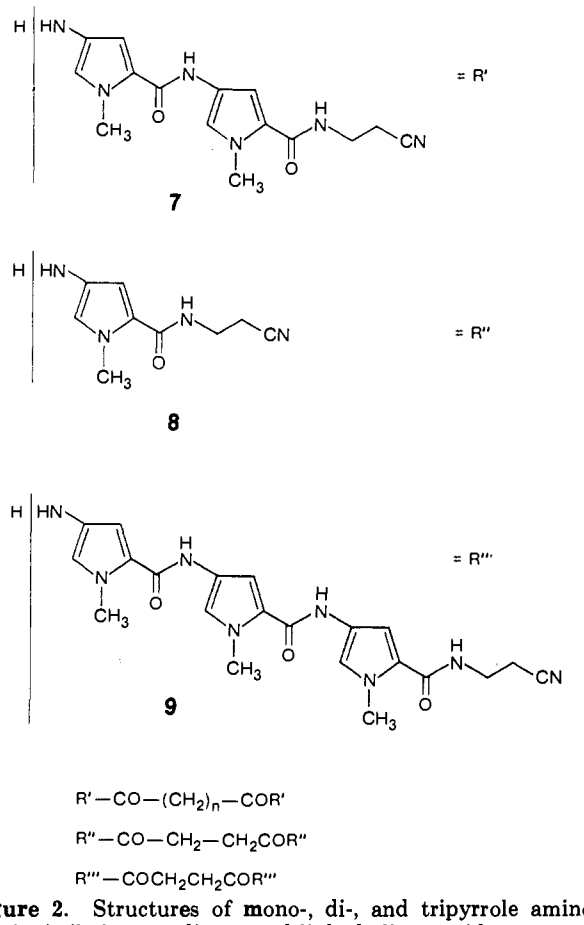

Figure 2. Structures of mono-, di-, and tripyrrole aminopropionitrile intermediates, and linked oligopeptides.

Results

Binding of Linked Oligopeptides to Duplex DNA. The homologous series **13a–23a** bind to duplex DNA but not to single-stranded DNA.[29] Substantial binding is

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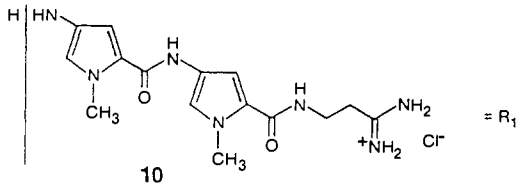

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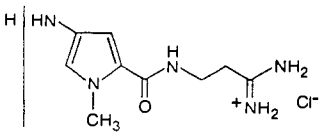

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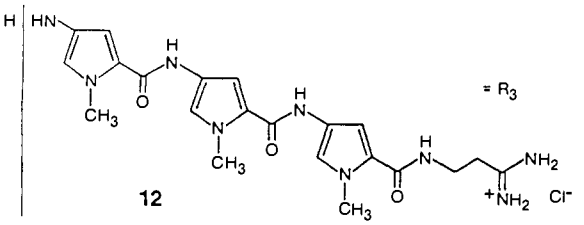

12

R_1—CO—$(CH_2)_n$—COR_1

R_2—CO—CH_2CH_2—CO—R_2

R_3—CO—CH_2CH_2—CO—R_3

13a R_1—CO—R_1
14a R_1—COCH_2CO—R_1
15a R_1—CO$(CH_2)_2$CO—R_1
16a R_1—CO$(CH_2)_3$CO—R_1
17a R_1—CO$(CH_2)_4$CO—R_1
18a R_1—CO$(CH_2)_5$CO—R_1
19a R_1—CO$(CH_2)_6$CO—R_1
20a R_1—CO$(CH_2)_7$CO—R_1
21a R_1—CO$(CH_2)_8$CO—R_1
22a R_1—CO$(CH_2)_9$CO—R_1
23a R_1—CO$(CH_2)_{10}$CO—R_1
24a R_2—CO$(CH_2)_2$CO—R_2
25a R_3—CO$(CH_2)_2$CO—R_3

Figure 3. Structures of general mono-, di-, and tripyrrole linked oligopeptides.

observed with T4 DNA in accord with minor groove specificity of these new agents[30] as expected by analogy with the parent antibiotics netropsin **1**[1,19,20] and distamycin **2**.[1,19] Relative binding constants of **13a–23a** to calf thymus DNA were estimated and compared by measuring the decrease in fluorescence of an ethidium–DNA complex as a result of competitive displacement.[29] The drug concentration that produced 50% inhibition of fluorescence was taken to be inversely proportional to the binding constant. The relative binding constants for this series of linked oligopeptides is shown in Table II. None of the drugs tested interfered with the fluorescence measurements at the levels

(29) The DNA binding constants were determined by the ethidium displacement procedure (Morgan, A. R.; Lee, J. S.; Pulleyblank, D. E.; Murray, N. L.; Evans, D. H. *Nucleic Acids Res.* **1979**, *7*, 547). This procedure gives relative rather than absolute binding constants.

(30) Since in T4 DNA glycosylation of the 5-(hydroxymethyl)cytidine residues occludes the major groove (Erickson, R. L.; Szybalski, W. *Virology* **1964**, *22*, 111), the observed binding is consistent with binding in the minor groove as has been established for netropsin.[19,20]

Table II. Relative Binding Constants for Linked Oligopeptides to Calf Thymus DNA Determined by the Ethidium Displacement Assay[a]

compd	n[b]	DNA binding constant, M^{-1}	compd	n[b]	DNA binding constant, M^{-1}
1		1.9×10^7	**18a**	5	1.2×10^7
2		0.8×10^7	**19a**	6	2.5×10^7
13a	0	5.6×10^7	**20a**	7	0.9×10^7
14a	1	3.6×10^7	**21a**	8	1.7×10^7
15a	2	7.2×10^7	**22a**	9	1.9×10^7
16a	3	1.8×10^7	**23a**	10	2.2×10^7
17a	4	1.0×10^7			

[a] Based on a binding constant of ethidium of 10^7 M^{-1} under similar conditions. Binding constant values represent the average of repeat measurements. [b] Number of CH_2 units in the linker.

Table III. Inhibitory Effects of Linked Oligopeptides on the Proliferation of Murine Leukemia (P388) Cells

compd	n[b]	ID_{50},[a] μg/mL	compd	n[b]	ID_{50},[a] μg/mL
2		>20	**19a**	6	0.70
13a	0	8.0	**20a**	7	0.75
14a	1	0.91	**21a**	8	0.98
15a	2	1.75	**22a**	9	18.0
16a	3	6.4	**23a**	10	4.8
17a	4	1.05	**24a**	2	>20
18a	5	4.4	**25a**	2	>20

[a] 50% inhibitory dose measured by using 25000 cells/well and a 72-h incubation at 37 °C with doses of 20, 10, 5, 1, and 0.5 μg/mL. [b] Number of CH_2 units in the linker.

used, and all displaced ethidium from the DNA with relative binding constants ranging from $<0.9 \times 10^7$ to 7.2×10^7 M^{-1}. The binding was influenced by the length of the $-[CH_2]_n-$ linker, with the larger maximum at $n = 2$ and lower maxima at $n = 6$ and $n = 10$.

Antileukemic Activity. Among the linked oligopeptides tested for their inhibitory effects on the proliferation of murine P388[18] compounds **14a**, **19a**, **20a**, and **21a** proved to be the most active with ID_{50} values of 0.91, 0.70, 0.75, and 0.98 μg/mL, respectively (Table III). The most active, **19a**, is approximately 30 times more potent than distamycin in this screen. A fairly marked dependence of antileukemic activity on the length of the linker is observed in this series of homologues, with maxima at $n = 1$ (**14a**) and $n = 6$ (**19a**) reminiscent of the maxima noted above in the DNA binding. The fact that the DNA binding constants of the most potent agents are comparable with (or in some cases lower than) that of netropsin suggests that other pharmacological factors, such as increased lipophilicity of the agents as a result of introduction of the polymethylene linkers, may be contributing to their increased potency.

Cytostatic Activity. Among the linked oligopeptides tested for their inhibitory effects on the proliferation of murine L1210 and FM3A cells and human Raji and Namalva cells, **19a** again proved to be the most potent (Table IV). Its ID_{50} value for the four different cell lines ranged between 1.74 and 3.34 μg/mL. Several agents in this series show activity much higher than that of netropsin and higher than or comparable with that of distamycin, including **21a** (ID_{50} 2.97–4.21 μg/mL) and **20a** (ID_{50} 3.30, 4.36, 10.8 μg/mL). Compounds **14a**, **17a**, and **18a** show high activity against the human tumor cell lines but are only moderately active (or inactive) against the murine cell lines. Netropsin **1** and distamycin **2** are themselves somewhat more active against the human cell lines (ID_{50} 139, 24 μg/mL) than against the murine cell lines (ID_{50} 245, 321, 27, 31 μg/mL). These data indicate that whereas the DNA sequence selectively dominated by the oligopeptide moiety is likely to be similar for this series of agents, the introduction of a lipophilic linker enhances the selectivity

Table IV. Inhibitory Effects of the Linked Oligopeptides on the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A), Human B Lymphoblast (Raji), and Human T-Lymphoblast (Molt/4F) Cells

		ID_{50},[a] μg/mL			
compd	n[b]	L1210	FM3A	Raji	Molt/4F
1 (netropsin)		245 ± 92	321 ± 18	139 ± 63	
2 (distamycin)		27 ± 4.7	31 ± 2.4	24 ± 3.7	
13a	0	>100	>100	>100	>100
14a	1	28.5 ± 9.7	5.87 ± 2.23	3.39 ± 0.69	2.85 ± 0.64
15a	2	>100	47.2 ± 27.8	26.4 ± 2.8	33.8 ± 0.9
16a	3	>100	>100	>100	>100
17a	4	24.3 ± 8.9	57.8 ± 32.7	13.3 ± 5.6	5.62 ± 0.60
18a	5	>100	≥100	11.1 ± 3.2	5.74 ± 1.85
19a	6	3.34 ± 0.27	3.15 ± 0.72	2.14 ± 0.80	1.74 ± 0.28
20a	7	10.8 ± 6.6	32.0 ± 9.7	4.36 ± 0.89	3.30 ± 0.99
21a	8	4.21 ± 1.52	22.1 ± 11.1	3.24 ± 0.44	2.97 ± 0.49
24a	2	>100	>100	>100	>100
25a	2	>100	4.29 ± 1.12	41.4 ± 8.4	2.85 ± 0.64

[a] 50% inhibitory dose. [b] Number of CH_2 units in linker.

Table V. Cytotoxicity and Antiviral Activity of the Linked Oligopeptides in Primary Rabbit Kidney (PRK) Cell Cultures

			min inhibitory concn,[c] μg/mL			
compd	n[a]	min cytotoxic concn,[b] μg/mL	herpes simplex virus 1 (KOS)	herpes simplex virus 2 (G)	vaccinia virus	vesicular stomatitis virus
13a	0	≥40	>10	20	7	>40
14a	1	≥10	>4	>4	0.2	>10
15a	2	≥40	>4	>4	>4	>4
16a	3	≥200	≥200	200	7	>200
17a	4	≥40	>10	>10	0.7	>10
18a	5	400	150	70	2	>200
19a	6	≥10	>10	>10	0.2	>10
20a	7	≥100	>40	>40	0.2	>100
21a	8	≥40	>10	>10	0.7	>10
22a	9	≥40	>10	20	2	>40
23a	10	≥40	>10	7	2	>10
24a	2	≥100	>100	>40	20	>100
tubercidin		≥1	>0.4	>0.4	>0.1	0.2
(S)-DHPA		>400	>400	>400	>70	>70
ribavirin		>400	>400	300	20	>400
carbocyclic 3-deazaadenosine		>400	>400	400	2	0.7

[a] Number of CH_2 groups in the linker. [b] Required to cause a microscopically detectable alteration of normal cell morphology. [c] Required to reduce virus-induced cytopahtogenicity by 50%.

of the oligopeptides toward human tumor cells.

A notable feature of this homologous series is the manner in which the antitumor activity rises and falls according to the length of the linker. As in the cases of the inhibitory effects against P388 murine leukemia (Table III) the human tumor cell cytoxic potency reaches general maxima around the values $n = 1$ and $n = 6$ (Figure 4A,B and Table IV). A similar correspondence between the length of the linker and biological response is seen in the case of associated enzymatic activity, illustrated in Figure 4C for the inhibition of Moloney murine leukemia associated reverse transcriptase for which data are available from $n = 1$ through 10. In the cases of the tumor cells the first maximum appears at $n = 1$, whereas with the antiviral and enzyme inhibitory responses the first maximum (at $n = 2$) corresponds more directly with the DNA binding data for the homologous series. The significance of the size of the oligopeptide moiety may be seen in the subset **24a**, **15a**, and **25a**, which bear 1, 2, and 3 *N*-methylpyrrole units, respectively. One such unit in each "arm" of the bis-linked oligopeptides is insufficient to confer activity (**24a**, ID_{50} >100 μg/mL); two such units immediately confer activity especially against human tumor cells (**15a**, ID_{50} 26.4, 33.8 μg/mL), and three such units maintain and even enhance this activity (**25a**, ID_{50} 41.4, 2.85 μg/mL).

Antiviral Activity. Several linked oligopeptides were selectively active against vaccinia virus; i.e., they inhibited the cytopathic effect of vaccinia virus at a concentration that was significantly lower than their minimum cytotoxic concentration for the host cells [primary rabbit kidney cell cultures (PRK)] (Table V). Thus in order of decreasing antivaccinia potency, **14a = 19a = 20a > 17a = 21a > 1,a = 22a = 23a > 15a > 13a = 16a > 24a**. Some of the oligopeptide agents (i.e., **14a**, **15a**, **23a**) also showed activity against herpes simplex virus type 1 or type 2 and vesicular stomatitis virus, but this activity was seen at a concentration that was less than 10-fold lower than the minimal cytotoxic concentration and, hence, could be attributed to cytotoxicity. Such nonselective antiviral activity was also shown by compounds **14a**, **25a**, and **21a** against vesicular stomatitis virus, Coxsackie virus type B4, and polio virus type 1 in HeLa cell cultures as well as parainfluenze virus type 3, Sindbis virus, and Semliki forest virus in Vero cells (data not shown). As in the cases of the tumor cell cytotoxicity discussed above, significant antiviral activity occurred only when the linker $n = 2$ or 6–8. The antivaccinia and cytotoxic activity of these novel agents was examined more extensively in four different cell cultures (PRK, HeLa, Vero, and E_6^{SM}, the latter being embryonic skin–muscle cells, Table VI). Substantial antivaccinia activity well below the minimum cytotoxic dose was observed for all the homologues (except for **24a**) with potency decreasing in the order of **19a > 17a > 18a > 15a > 14a > 21a > 20a > 25a > 13a > 16a**.

The reference compounds included in the antiviral tests were tubercidin, (*S*)-DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine], ribavirin, and C-c^{3}Ado (carbocyclic 3-deazaadenosine).[31,32] The linked oligopeptides differ from

Table VI. Cytotoxicity and Antiviral Activity of the Linked Oligopeptides against Vaccinia Virus in Different Cell Cultures

		min cytotoxic concn,[b] μg/mL				min inhibitory concn,[c] μg/mL, vaccinia virus					
						PRK			Vero		
compd	n[a]	PRK	HeLa	Vero	E_6^{SM}	expt 1	expt 2	HeLa	expt 1	expt 2	E_6^{SM}
13a	0	40	≥40	≥40	100	7	7	20	20	20	2
14a	1	40	≥40	≥40	≥40	2	2	2	2	2	0.2
15a	2	≥100	≥100	≥200	≥200	2	2	2	2	7	0.2
16a	3	≥200	≥400	≥400	400	20	20	40	70	150	7
17a	4	≥40	≥400	100	100	2	0.7	1	2	7	0.2
18a	5	≥200	>200	>200	200	7	7	20	20	20	0.7
19a	6	≥40	≥200	>40	≥200	0.7	2	2	7	7	0.07
20a	7	≥100	≥200	>200	≥100	7	7	20	40	70	0.7
21a	8	≥40	100	≥40	≥100	2	2	20	20	>40	0.2
22a	9	≥40	100	100	≥40	2		20	>40		0.2
23a	10	≥40	≥40	≥100	≥40	2		2	>40		0.2
24a	(2)	≥400	>400	≥400	>400	300	400	>400	>400	>400	70
25a	(2)	≥4	≥20	≥4	>4	>4	>4	>10	>4	>4	>4
tubercidin		≥0.4	≥1	≥0.4	4	>0.1	>0.1	0.2	0.02	0.02	0.07
(S)-DHPA		>400	>400	>200	>400	100	150	70	20	20	100
ribavirin		>400	≥400	>400	>400	20	7	20	7	20	70
carbocyclic 3-deazaadenosine		>400	>400	>200	>400	2	2	0.7	0.2	0.2	2

[a] Number of CH_2 groups in the linker. [b] Required to cause a microscopically detectable alteration of normal cell morphology. [c] Required to reduce virus-induced cytopathogenicity by 50%.

these reference compounds in that they show a more restricted activity, i.e., primarily against vaccinia. In contrast, tubercidin is active against vaccinia (and several other viruses) at a minimal antiviral concentration which is, as a rule, only slightly lower than its cytotoxic concentration. C-c^{3}Ado inhibits vaccinia and vesicular stomatitis virus to the same extent, and (S)-DHPA and ribavirin, as well as C-c^{3}Ado, are inhibitory to a broad spectrum of viruses, including several RNA viruses, i.e., reo, parainfluenza, and measles (data not shown).

Structure–Activity Relationship. In terms of the relationship between structure and anticancer activity it appears that: (i) Oligopeptides linked by $-(CH_2)_n-$ chains exhibit considerably enhanced antileukemic P388 activity compared with distamycin if n = 0–10, with maximal activity shown at n = 1 and n = 6–8. (ii) The activity against murine tumors (L1210, FM3A) and human tumors (Raji, Molt/4F) is enhanced compared with netropsin by introduction of a $-(CH_2)_n-$ linker, provided n = 1, 2, or 4–8, with maximal activity exhibited if n = 6 or 7. (iii) The polymethylene linker enhances preferential activity against human tumor cell lines compared to murine cells. (iv) In the oligopeptide moiety a minimum of two N-methylpyrrole units is required for significant tumor cell cytotoxic activity and, in the examples examined, three N-methylpyrrole units confer the greatest activity.

In terms of the relationship between structure and antiviral activity, it appears that: (i) A strong preferential activity against vaccinia virus well below the minimum cytotoxic dose is conferred by oligopeptides linked by $-(CH_2)_n-$, especially when n = 0, 1, or 3–10. Maximum activity is shown for n = 1 and n = 6–8. (ii) Again, in the examples studied a minimum of two N-methylpyrrole units is necessary for antiviral activity and three such units increase the activity. (iii) The extent of antivaccinia activity observed depends on the cell culture system used (with the highest activity seen in E_6^{SM} cells), but the relative ranking is unaltered.

Inhibition of Reverse Transcriptase Activity. Finally, the inhibition of Moloney murine leukemia virus (MLV) associated reverse transcriptase (RNA- and DNA-directed DNA polymerase) activity by the linked oligopeptides was examined (Table VII). Inhibition was observed for the homologous series (n = 1–10) in the range of 9.1–72.5 μg/mL). The greatest extent of inhibition was observed in the following cases (order of decreasing activity): **23a > 22a > 21a > 19a > 15a > 20a > 14a > 16a > 17a > 18a**. Again a correspondence is observed between the length of the linker and the inhibitory activity (Figure 4C).

Table VII. Inhibition of Moloney Murine Leukemia (MLV) Associated Reverse Transcriptase (RNA-Directed and DNA-Directed DNA Polymerase) Activity by Linked Oligopeptides

compd	n[a]	ID_{50},[b] μg/mL (average ± SD)
14a	1	39.0 ± 13.9
15a	2	25.2 ± 11.4
16a	3	39.9 ± 13.3
17a	4	57.2 ± 10.4
18a	5	72.5 ± 6.9
19a	6	21.3 ± 6.1
20a	7	34.2 ± 0.9
21a	8	20.9 ± 9.2
22a	9	10.3 ± 7.5
23a	10	9.1 ± 6.7
24a	2	>400
25a	2	>400
aurintricarboxylic acid		1.42 ± 0.26

[a] Number of CH_2 groups in linker. [b] 50% inhibitory dose, measured after 120-min incubation of the reaction mixtures [MLV, lot 804-84-8A; (^{3}H-*methyl*)dTTP at 10 μCi (sp act. 30 Ci/mmol) per 250 μL of reaction mixture].

Discussion

The results indicate that it is possible, by suitable structural modification, to considerably enhance the tumor cell cytotoxic and antiviral activity of oligopeptide agents based on the natural products netropsin and distamycin. The results permit identification of some of the structural parameters that contribute to enhanced activity. First, binding to DNA of members of this homologous series is comparable ($\sim 10^7$ M^{-1}) both within the series and to that of the parents netropsin or distamycin, so this alone cannot explain the up to 30-fold increase in antileukemic potency (**19a** vs **2**). Similarly, differences in sequence selectivity are unlikely to be a dominant cause given the strict AT binding preference of all N-methylpyrrole-based oligopeptides. The strict AT sequence preference was also

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(32) Mossmann, T. *J. Immunol. Methods* **1983**, *65*, 55.

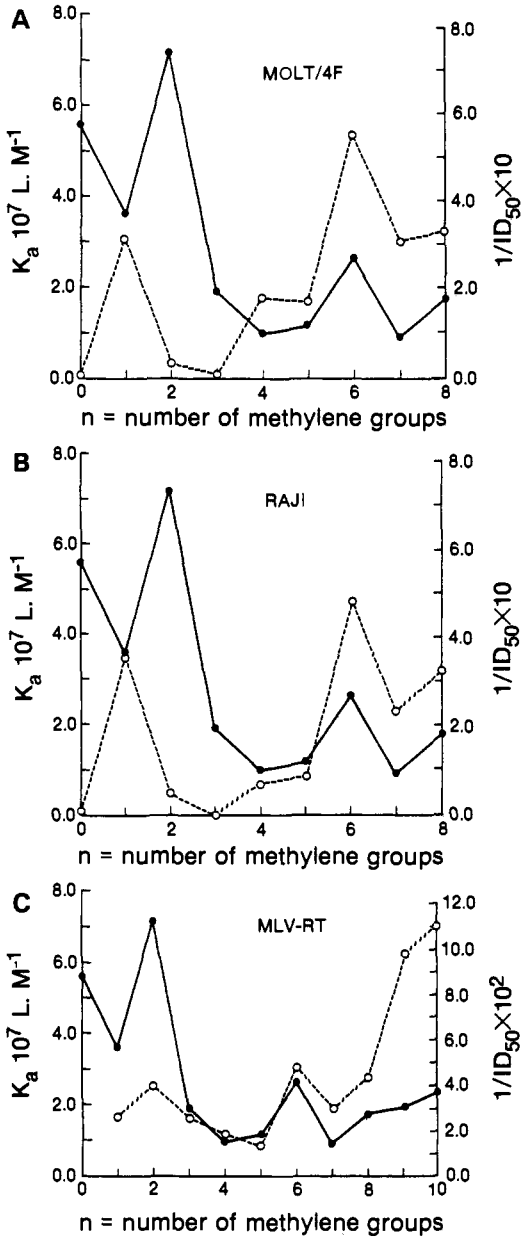

Figure 4. Correlations between DNA binding constants of linked oligopeptides (K_a, solid line) and observed inhibitory properties, expressed as reciprocal of ID_{50} values (dotted lines) for (A) human tumor cell line Molt/4F, (B) human tumor cell line Raji, and (C) inhibition of MLV-associated reverse transcriptase.

found to be an important determinant of biological activity in other novel classes of oligopeptide agents.[17] A more likely factor is the increased lipophilicity promoting cellular uptake as a result of introduction of the polymethylene linkers. Nevertheless, the rise and fall of both tumor cell cytotoxic and antiviral activity with $-(CH_2)_n-$ reflecting maximal activity in the regions n = 1, 2, and 6–8 suggests a subtle interplay of enhanced cellular uptake and phase-dependent binding. The latter aspect, which is beyond the scope of the present study, will require careful quantitative footprinting and thermodynamic evaluation of ligand binding. A serendipitous finding is that introduction of the $-(CH_2)_n$ linker enhances preferential activity against human tumor cells. The introduction of the linker also appears to confer selective antivaccinia activity, but no selectivity toward the other viruses that were examined.

In conclusion, the strict requirements of this class of novel oligopeptides for tight binding to the minor groove of duplex DNA and their predictable AT base preferences may be modulated by the introduction of lipophilic linkers of appropriate length. The marked enhancement in tumor cell cytotoxicity (up to 30-fold) suggests further exploration of compounds of this type to be worthwhile. In particular, the marked and consistent dependence of both anticancer and antiviral activity on the length of the linker warrants more extensive physicochemical examination. Such studies are in progress.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on Nicolet 7199 FT 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 an Associated Electrical Industries (AEI) MS-9 and MS-50 focusing high-resolution mass spectrometers. Kieselgel 60 (230–400 mesh) of E. Merck was used for flash chromatography, and precoated sheets silica gel $60F_{254}$ of E. Merck were used for TLC. TLC system for (i) covalent peptidic compounds was chloroform/methanol, 9:1, that for (ii) ionic compounds with one ionic pair was methanol with some AcOH, and that for (iii) ionic compounds with two ionic pairs was methanol with some formic acid.

3-[1-Methyl-4-[1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile (9). 3-[1-Methyl-4-[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]-pyrrole-2-carboxamido]propionitrile[26,27] (420 mg, 0.9 mmol) was reduced over 5% palladium on charcoal (260 mg) in a mixture of DMF (15 mL) and methanol (5 mL) at 45 °C. After the reduction the solvents were evaporated under reduced pressure. The residue was dissolved in a small amount of acetonitrle (2 mL), and an excess of ethyl acetate (20–30 mL) was added to precipitate some impurities. The filtrate was treated with an excess of hexane to precipitate a white pure product **9** (250 mg, 63.6% yield): mp 155–160 °C; ^1H NMR (DMSO-d_6) δ 2.74 (t, 2 H), 3.42 (q, 2 H), 3.76 (s, 3 H), 3.85 and 3.87 overlapped with a bs (3 s, 8 H), 6.27 (d, 1 H), 6.40 (d, 1 H), 6.95 (d, 1 H), 7.04 (d, 1 H), 7.24 (2 d, 2 H), 8.37 (t, 1 H), 9.66 (s, 1 H), 9.96 (s, 1 H); IR (Nujol) 1260, 1377, 1403, 1464, 1529, 1582, 1646, 2245, 3120, 3310 cm^{-1}; MS (m/z) 436.1981 (calcd 436.1983). Anal. Calcd for $C_{19}H_{26}ClN_9O_3$: C, 52.3; H, 6.0; Cl, 8.1; N, 22.5. Found: C, 52.3; H, 6.0; Cl, 7.9; N, 22.0.

Method 1. *N*,*N*'-Bis[1-methyl-2-[[1-methyl-2-[(2-cyanoethyl)carbamoyl]-4-pyrrolyl]carbamoyl]-4-pyrrolyl]-succinamide (15). The compound **7** (105 mg, 0.33 mmol) and i-Pr_2EtN (65 μL, 0.37 mmol) were dissolved in anhydrous acetonitrile (5 mL) and cooled to −20 °C. Succinyl chloride (18 μL, 0.16 mmol) in anhydrous THF (1 mL) was added. The mixture was allowed to reach ambient temperature. The solvents were evaporated to dryness, water was added, and the resulting solid was collected and washed with hot MeOH. The product dissolved in DMF, and when placed on a TLC plate (SiO_2) with $CHCl_3$ + 15% MeOH system, it gave one spot. For analytical purposes it was purified by dissolution in a small amount of DMF and precipitation with a large amount of EtOH to give 90 mg (77%) of **15**: mp 292 °C; ^1H NMR (DMSO-d_6) δ 2.58 (s, 4 H), 2.74 (t, 4 H), 3.42 (q, 4 H), 3.83 (2 s, 12 H), 6.86, 6.93, 7.17, and 7.22 (4 d, 2 H each), 8.35 (t, 2 H), 9.89 (s, 4 H); IR (Nujol) 1376, 1401, 1447, 1465, 1511, 1535, 1585, 1645, 2245, 3120, 3304 cm^{-1}; MS [m/z (rel intensity)] 396.1543 (9.98) for $C_{19}H_{20}N_6O_4$ which is $(O{=}C{=}CH-M_{1/2})^+$. Anal. Calcd for $C_{34}H_{38}N_{12}O_6$: C, 57.5; H, 5.4; N, 23.6. Found: C, 57.8; H, 5.4; N, 23.3.

***N*,*N*'-Bis[1-methyl-2-[[1-methyl-2-[(2-amidinoethyl)carbamoyl]-4-pyrrolyl]carbamoyl]-4-pyrrolyl]succinamide Dihydrochloride (15a).** A suspension of 130 mg (0.18 mmol) of **15** in anhydrous EtOH was saturated with HCl with cooling. After 1.5 h at room temperature, the solvent was evaporated under reduced pressure. The residue was washed with dry ether, and then ethanol was added followed by some NH_3 condensed into the vessel. After 1 h at room temperature, solvents were removed and the residue was washed with MeOH, EtOH, and hexane to give 116 mg of a solid. The latter was examined by TLC (SiO_2) with MeOH and a drop of formic acid and indicated formation of the product (R_f = 0.3) containing some more polar impurity.

Recrystallization from a small amount of water gave a gel-like precipitate which was washed with EtOH and hexane and dried gave 50 mg (34%) of pure **15a**: mp 283–5° dec; ^{1}H NMR (DMSO-d_6) δ 2.60 (m, 8 H), 3.50 (m, 4 H), 3.83 (s, 12 H), 6.92 (d, 4 H), 7.18 (d, 4 H), 8.25 (t, 2 H, 8.70 (bs, 4 H), 9.02 (bs, 4 H), 9.93 and 9.97 (2 s, 4 H); IR (Nujol) 1352, 1377, 1464, 1521, 1576, 1368, 1700, 3260 cm^{-1}; MS-FAB (m/z) 745 (M – Cl – HCl)$^+$. Anal. Calcd for $C_{34}H_{46}Cl_2N_{14}O_6$: C, 49.94; H, 5.67; N, 23.98; Cl, 8.67. Found: C, 50.3; H, 6.05; N, 22.90; Cl, 8.75.

Method 2. *N*,*N*′-Bis[1-methyl-2-[[1-methyl-2-[(2-cyanoethyl)carbamoyl]-4-pyrrolyl]carbamoyl]-4-pyrrolyl]malonamide (14). The compound **7** (315 mg, 1 mmol) malonic acid (52 mg, 0.5 mmol), and DCC (206 mg, 1 mmol) were stirrred in acetonitrile (6 mL) for 2 h at room temperature, and finally the mixture was heated briefly to boiling to complete the reaction. A solid which contained dicyclohexylurea was collected, and the filtrate was extracted with DMF. The DMF solution was treated with water, and the solid formed was recrystallized from a mixture of acetonitrile (2 mL) and methanol (2 mL) to give pure compound **14**: (140 mg, 40% yield): mp 225–30 °C; ^{1}H NMR (DMSO-d_6) δ 2.73 (t, 2 × 2 H), ~2.40 (q + s overlapped, 2 × 2 H + 2 H), 3.83 and 3.86 (2 s, 2 × 6 H), 6.91 (2 d, 2 × 2 H), 7.18 and 7.22 (2 d, 2 × 2 H), 8.35 (t, 2 × 1 H), 9.91 (s, 2 × 1 H), 10.09 (s, 2 × 1 H); IR (Nujol) 1200, 1264, 1290, 1376, 1401, 1464, 1511, 1532, 1585, 1638, 1662, 2250, 3120, 3305 cm^{-1}; MS-FAB (m/z) 697 (MH$^+$). Anal. Calcd for $C_{33}H_{36}N_{12}O_6$: C, 56.9; H, 5.2; N, 24.1. Found: C, 56.6; H, 5.4; N, 23.9.

***N*,*N*′-Bis[1-methyl-2-[[1-methyl-2-[(2-amidinoethyl)carbamoyl]-4-pyrrolyl]carbamoyl]-4-pyrrolyl]malonamide Dihydrochloride (14a).** Compound **14** (160 mg, 0.23 mmol) was suspended in dry ethanol, and the mixture was saturated with dry hydrogen chloride. After 1.5 h at room temperature, the solvent was removed under reduced pressure. The residue was treated with dry ethanol and dry ammonia. After 1 h the solution was decanted from undissolved material and evaporated to dryness. The residue was dissolved in 2 mL of boiling water, and an excess of acetonitrile was added to the hot solution. The precipitate was collected and washed with a small amount of water. The operation was repeated, and pure compound **14a** was collected (100 mg, 59% yield): mp 218–224 °C. The compound, if crystallized from water, precipitates in the form of a jelly. ^{1}H NMR (DMSO-d_6) δ 2.63 (t, 2 × 2 H), ~3.35 (s overlapped with the peak of water), 3.50 (q, 2 × 2 H), 3.80 and 3.83 (2 s, 2 × 6 H), 6.93 (s, 2 × 2 H), 7.20 (s, 2 × 2 H), 8.26 (t, 2 × 1 H), 8.90 (bs, 2 × 4 H), 9.96 (s, 2 × 1 H), 10.28 (s, 2 × 1 H) (D_2O exchange experiments showed the presence of malonyl protons at δ 3.30); IR (Nujol) 1260, 1377, 1405, 1463, 1535, 1580, 1645, 3100, 3270 cm^{-1}; MS-FAB (m/z) 731 (M – Cl – HCl)$^+$. Anal. Calcd for $C_{33}H_{44}N_{14}O_6Cl_2$: C, 49.3; H, 5.5; N, 24.4; Cl, 8.8. Found: C, 49.0; H, 5.7; N, 27.0; Cl, 9.0.

Method 3. *N*,*N*′-Bis[1-methyl-2-[[1-methyl-2-[(2-cyanoethyl)carbamoyl]-4-pyrrolyl]carbamoyl]-4-pyrrolyl]urea (13). Compound **7** (365 mg, 1.16 mmol) and 1,1′-carbonyldiimidazole (94 mg, 0.58 mmol) were allowed to react in boiling acetonitrile (3 mL). A solid which formed was collected, washed with acetonitrile to give 350 mg (88.6% yield) of pure product **13**: mp 295–7 °C; ^{1}H NMR DMSO-d_6) δ 2.74 (t, 4 H), 3.38 (q, 4 H), 3.86 (s, 6 H), 3.88 (s, 6 H), 6.80 (d, 2 H), 6.92 (d, 2 H), 7.02 (d, 2 H), 7.21 (d, 2 H), 8.12 (s, 2 H), 8.25 (t, 2 H), 9.81 (s, 2 H); IR (Nujol) 1199, 1217, 1252, 1378, 1409, 1436, 1465, 1504, 1544, 1589, 1621, 1653, 1672, 2240, 3270, 3424 cm^{-1}; MS-FAB (m/z) 655 (MH$^+$). Anal. Calcd: C, 56.9; H, 5.2; N, 25.7. Found: C, 56.6; H, 5.4; N, 25.5.

***N*,*N*′-Bis[1-methyl-2-[[1-methyl-2-[(2-amidinoethyl)carbamoyl]-4-pyrrolyl]carbamoyl]-4-pyrrolyl]urea Dihydrochloride (13a).** Compound **13** (116 mg, 0.25 mmol) was suspended in dry ethanol and the solution saturated with HCl. After 2 h the solvent was evaporated in vacuo and the residue treated with dry ammonia in ethanol for 1 h. The mixture was decanted from an insoluble residue and the solution evaporated to dryness. The residue was dissolved in 2 mL of methanol, and an excess of acetonitrile was added to precipitate the product. The latter was collected and washed with 1 mL of water when it became jelly-like. It was redissolved in methanol and precipitated with acetonitrile to give the compound **13a** (117 mg, 61.6% yield): mp 211–5 °C; ^{1}H NMR (DMSO-d_6) δ 2.64 (t, 4 H), 3.52 (q, 4 H), 3.84 (2 s, 12 H), 6.82 (d, 2 H), 6.94 (d, 2 H), 7.03 (d, 2 H), 7.20 (d, 2 H), 8.73 (2 s overlapped, 6 H), 9.05 (s, 4 H), 9.88 (s, 2 H); IR (Nujol) 1264, 1377, 1402, 1439, 1489, 1531, 1583, 1640, 1689, 3088, 3279 cm^{-1}; MS-FAB (m/z) 690 (M – Cl – HCl)$^+$. Anal. Calcd for $C_{31}H_{42}Cl_2N_{14}O_5$: C, 48.9; H, 5.6; Cl, 9.3; N, 25.7. Found: C, 48.5; H, 5.7; Cl, 9.7; N, 25.3.

Method 4. *N*,*N*′-Bis[1-methyl-2-[[1-methyl-2-[(2-cyanoethyl)carbamoyl]-4-pyrrolyl]carbamoyl]-4-pyrrolyl]adipamide (17). Adipic acid (29.2 mg, 0.2 mmol) in acetonitrile (0.5 mL) was treated with pivaloyl chloride (50 μL, 0.4 mmol) and Hunig's base (160 μL, 0.9 mmol), and then compound **7** (126 mg, 0.43 mmol) in DMF (0.5 mL) was added. After $^1/_2$ h at room temperature the mixture was evaporated to dryness under reduced pressure. The residue was washed with water and hot acetonitrile. The solid was dissolved in hot DMF and precipitated with an excess of acetonitrile to give **17** (95 mg, 61% yield): mp 244–6 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.60 (s, 4 H), 2.27 (s, 4 H), 2.74 (t, 4 H), 3.40 (q, 4 H), 3.83 (2 s, 12 H), 6.86 (s, 2 H), 6.93 (s, 2 H), 7.17 (s, 2 H), 7.22 (s, 2 H), 8.38 (t, 2 H), 9.82 (s, 2 H), 9.91 (s, 2 H); IR Nujol) 1376, 1400, 1464, 1513, 1533, 1585, 1641, 2258, 3294 cm^{-1}; MS-FAB (m/z) 738 (M$^+$), 739 (MH$^+$). Anal. Calcd: C, 58.5; H, 5.7; N, 22.7. Found: C, 58.9; H, 5.9; N, 22.5.

***N*,*N*′-Bis[1-methyl-2-[[1-methyl-2-[(2-amidinoethyl)carbamoyl]-4-pyrrolyl]carbamoyl]-4-pyrrolyl]adipamide Dihydrochloride (17a).** Compound **17** (320 mg, 0.43 mmol) was treated under Pinner reaction conditions as in method 3 above. After evaporation of solvents, water (3.5 mL) was added and a crystalline substance was collected to give **17a** (215 mg, 58.7% yield): mp 195–6 °C; ^{1}H NMR (DMSO-d_6) δ 1.60 (s, 4 H), 2.27 (s, 4 H), 2.62 (t, 4 H), 3.52 (q, 4 H), 3.80 (2 s, 12 H), 6.88 (d, 2 H), 6.95 (d, 2 H), 7.18 and 7.20 (2 d, 4 H), 8.25 (t, 2 H), 8.70 (s, 4 H), 9.00 (s, 4 H), 9.92 (s, 2 H); IR (Nujol) 1208, 1261, 1377, 1404, 1463, 1531, 1579, 1641, 1691, 3256 cm^{-1}; MS-FAB (m/z) 773 (M – HCl – Cl)$^+$. Anal. Calcd: C, 51.1; H, 6.0; N, 23.2; Cl, 8.4. Found: C, 50.9; H, 6.2; N, 23.6; Cl, 8.8.

Estimation of Drug–DNA Binding Constants. Drug–DNA binding constants were estimated as described previously.[29] To 2 mL of Tris–EDTA buffer, pH 8,, containing 1.3 μM ethidium bromide was added calf thymus DNA to give a final concentration of 1.35 μM. The fluorescence was measured after equilibrium for a few minutes, by using a Turner Model 430 spectrofluorometer (Turner Amsco Instruments, Carpinteria, CA) equipped with a 150-W xenon lamp, at an excitation wavelength of 525 nm and an emission wavelength of 600 nm. Aliquots of concentrated drug solutions were added, and the fluorescence was measured. Controls were performed to show that the drugs themselves did not interfere with the fluorescence measurements at the levels employed. From a plot of the decreased fluorescence of the ethidium–DNA complex with increasing dose of drug, the concentration of drug needed to reduce the fluorescence by 50% was determined and used to calculate a relative binding constant for the drug, given the binding constant of ethidium to be 10^7 M^{-1} under similar conditions.[31] This method allows rapid comparison of a series of related compounds, but the individual binding constants may not correspond to values obtained by using other methods.[29]

Cytostatic Activity. Mouse leukemic cells (i.e., L1210 and P388) were obtained from American Type Tissue Collection (Rockville, MD) and were grown in either McCoy's 5A (L1210) or Fischer's (P388) 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 a humidified incubator. The cytostatic activity of the drugs was determined by use of a methylenetetrazolium dye (MTT) assay as described by Mossmann.[32] 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 MR600) reader (570 nm; reference set at 630 nm).

The other tumor cell cytotoxic assays were performed according to previously established procedures.[33,34]

Antiviral Assays. The were performed as reported previously.[35-37]

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Registry No. 7, 97950-71-5; 8, 97950-77-1; 9, 121541-72-8; 9 (4-nitro derivative), 2522-28-3; 13, 121541-73-9; 13a·2HCl, 121541-86-4; 13a (free base), 121541-99-9; 14, 121541-74-0; 14a·2HCl, 121541-87-5; 14a (free base), 121542-00-5; 15, 121571-69-5; 15a·2HCl, 121541-88-6; 15a (free base), 121542-01-6; 16, 121541-75-1; 16a·2HCl, 121541-89-7; 16a (free base), 121542-02-7; 17, 121541-76-2; 17a·2HCl, 121541-90-0; 17a (free base), 121542-03-8; 18, 121541-77-3; 18a·2HCl, 121541-91-1; 18a (free base), 121542-04-9; 19, 121541-78-4; 19a·2HCl, 121541-92-2; 19a (free base), 121542-05-0; 20, 121541-79-5; 20a·2HCl, 121541-93-3; 20a (free base), 121542-06-1; 21, 121541-80-8; 21a·2HCl, 121541-94-4; 21a (free base), 121542-07-2; 22, 121541-81-9; 22a·2HCl, 121541-95-5; 22a (free base), 121571-70-8; 23, 121541-82-0; 23a·2HCl, 121541-96-6; 23a (free base), 121542-08-3; 25, 121541-84-2; 25a·2HCl, 121541-98-8; 25a (free base), 121542-10-7; $ClCOCH_2CH_2COCl$, 543-20-4; $ClCO(CH_2)_3COCl$, 2873-74-7; $ClCO(CH_2)_5COCl$, 142-79-0; $ClCO(CH_2)_6COCl$, 10027-07-3; $ClCO(CH_2)_7COCl$, 123-98-8; $ClCO(CH_2)_8COCl$, 111-19-3; $CH_2(COOH)_2$, 141-82-2; $HOOC(CH_2)_4COOH$, 124-04-9; R″-$COCH_2CH_2CO$-R″, 121541-83-1; R′-(Z)-COCH=CHCO-R′, 121541-85-3; R_2-$COCH_2CH_2CO$-R_2·2HCl, 121541-97-7; R_2-$COCH_2CH_2CO$-R_2 (free base), 121542-09-4; R_1-(Z)-COCH=CHCO-R_1·2HCl, 121542-11-8; maleic anhydride, 108-31-6.

Supplementary Material Available: Table giving analytical and spectroscopic data on linked oligopeptides and their precursors (5 pages). Ordering information is given on any current masthead page.

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Synthesis and Pharmacological Evaluation of CNS Activities of [1,2,3]Triazolo[4,5-*b*][1,5]-, Imidazolo[4,5-*b*][1,5]-, and Pyrido[2,3-*b*][1,5]benzodiazepines. 10-Piperazinyl-4*H*-1,2,3-Triazolo[4,5-*b*][1,5]benzodiazepines with Neuroleptic Activity[1]

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The synthesis of [1,2,3]triazolo[4,5-*b*][1,5]-, imidazolo[4,5-*b*][1,5]-, and pyrido[2,3-*b*][1,5]benzodiazepines is described. The antidopaminergic and anticholinergic activities of the compounds have been examined by the respective in vitro [^{3}H]spiperone and [^{3}H]QNB receptor binding assay. The neuroleptic potential has been further evaluated in terms of their ability to produce hypothermia and catalepsy in mice and a conditioned avoidance response in rats. Only compounds from the triazolobenzodiazepine series show antipsychotic potential. The lack of activity in the imidazolo- and pyridobenzodiazepine series indicates that the basicity of the heteroarene moiety may be determinant for activity.

It is known that a change in the electronic distribution in the two phenyl rings of the dibenzo–epine class of neuroleptics leads to a profound alteration in activity profile.[2-6] For example, clozapine (1) is a clinically effective antipsychotic which differs from typical neuroleptics by producing only minimal extrapyramidal symptoms (EPS), whereas its 2-chloro isomer HF-2046 (2) has a classical profile of activity.[7,8] In a previous publication[5] we reported that a profile of activity, in animal tests, similar to that of clozapine could be obtained if the relatively electron-rich phenyl ring, C, is replaced with an isosteric thiophene ring to give a corresponding thieno-[2,3-*b*][1,5]benzodiazepine. One of these compounds,

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