2,3-Dihydro-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine]s and 2,3-Dihydro-3-(1-pyrryl)spiro[benzofuran-2,3′-pyrrolidine]s: Novel Antihypertensive Agents

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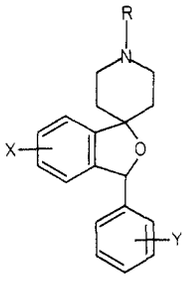
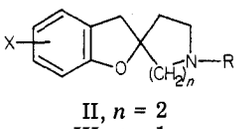
A series of 2,3-dihydro-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine]s (IV) and 2,3-dihydro-3-(1-pyrryl)spiro[benzofuran-2,3′-pyrrolidine]s (V) was synthesized and evaluated for cardiovascular activity. The majority of the compounds displayed good antihypertensive activity in the spontaneous hypertensive rat model at 50 mg/kg po. Compounds **5** (2,3-dihydro-1′-methyl-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine]) and **12a** (2,3-dihydro-1′-ethyl-3-(1-pyrryl)-spiro[benzofuran-2,4′-piperidine]) were selected for a more detailed cardiovascular evaluation in the renal hypertensive rat and for standard cardiovascular challenges in anesthetized dogs and the sinoaortic-deafferented dog.

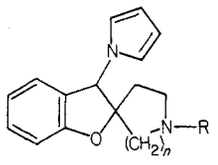
The 3-aryl-1,3-dihydrospiro[isobenzofuran-1(3*H*),4′-piperidine]s (I) have demonstrated marked activity in



I



II, $n = 2$
III, $n = 1$

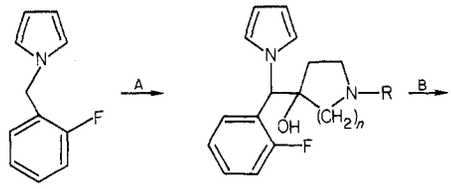


IV, $n = 2$
V, $n = 1$

pharmacological tests, indicating antidepressant,[1] neuroleptic,[2] diuretic,[3] and antihypertensive[4] utility. This, coupled with our successful synthesis of a related series of 2,3-dihydrospiro[benzofuran-2,4′-piperidine]s (II) and 2,3-dihydrospiro[benzofuran-2,3′-pyrrolidine]s (III) via a nucleophilic aromatic fluorine displacement,[5] as well as our interest in pyrrole chemistry, prompted us to explore further this chemistry and prepare a series of 2,3-dihydro-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine]s (IV) and 2,3-dihydro-3-(1-pyrryl)spiro[benzofuran-2,3′-pyrrolidine]s (V) (Table I). As part of our continuing program to find new antihypertensive agents with nonclassical structures that are devoid of the side effects that commonly plague existing blood pressure lowering agents, these compounds were evaluated for antihypertensive activity in the spontaneously hypertensive rat assay, resulting in the selection of **5** and **12a** for further study.

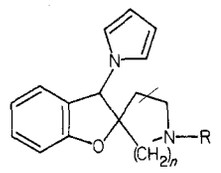
Chemistry. The synthesis of the initial spiro compounds **4** and **5** is shown in Scheme I. 1-(2-Fluorobenzyl)pyrrole (1), prepared by condensation of 2-fluorobenzylamine and 2,5-dimethoxytetrahydrofuran, was metalated with *n*-butyllithium in tetrahydrofuran at −80 °C and then reacted with 1-methyl-4-piperidone or 1-benzyl-3-pyrrolidone (method A) to give the alcohols **2** and **3** in good yield. The alcohols **2** and **3** were cyclized to the spiro compounds **4** and **5** through a base-induced nucleophilic displacement of fluoride with sodium hydride in a

Scheme I



1

2, R = $CH_2C_6H_5$; $n = 1$
3, R = CH_3; $n = 2$



4, R = $CH_2C_6H_5$; $n = 1$
5, R = CH_3; $n = 2$

10% solution of dimethylformamide in benzene at 70 °C (method B).

Aromatic fluoride displacements of this type generally proceed in high yields; the lower yields (30–40%) encountered in this cyclization step are accounted for by a competing reaction in which the alcohol **2** or **3**, in a retro fashion, eliminates the stabilized carbanion of **1** and the corresponding piperidone or pyrrolidone.

A number of attempts were made to minimize this retro elimination and to improve the yield of this cyclization step. Using sodium hydride, but increasing the ratio of dimethylformamide–benzene, or using dimethylformamide alone at various temperatures (30–80 °C), gave lower yields of **4** or **5** with increased retro elimination of **1**. Using tetrahydrofuran or tetrahydrofuran–dimethylformamide mixtures gave similar results. Sodium hydride and benzene alone gave no reaction. Warming the lithium alkoxide of alcohol **3**, generated in the synthesis of **3** (procedure A), at 30–65 °C gave no further reaction. Addition of dimethylformamide to the tetrahydrofuran solution of the lithium alkoxide resulted only in retro elim-

† Chemical Research Department.
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(1) V. J. Bauer, B. J. Duffy, D. Hoffman, S. S. Klioze, R. W. Kosley, Jr., A. R. McFadden, L. L. Martin, H. H. Ong, and H. M. Geyer III, *J. Med. Chem.*, **19**, 1315 (1976).
(2) R. C. Allen, V. J. Bauer, R. W. Kosley, Jr., A. R. McFadden, G. M. Shutske, M. L. Cornfeldt, S. Fielding, H. M. Geyer III, and J. C. Wilker, *J. Med. Chem.*, **21**, 1149 (1978).
(3) S. S. Klioze and W. J. Novick, Jr., *J. Med. Chem.*, **21**, 400 (1978).
(4) S. S. Klioze, R. C. Allen, J. C. Wilker, and D. L. Woodward, *J. Med. Chem.*, **23**, 677 (1980).
(5) R. C. Effland, B. A. Gardner, and J. Strupczewski, *J. Heterocycl. Chem.*, **18**, 811 (1981).

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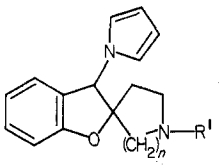
Table I. 2,3-Dihydro-3-(1-pyrryl)spiro[benzofuran-2,4'-piperidine]s and -spiro[benzofuran-2,3'-pyrrolidine]s[a]

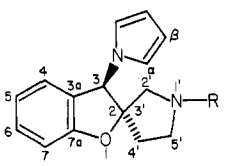


no.	R^1	n	method[b]	mp, °C	yield,[c] %	recrystn solvent[d]	formula	anal.
4	$CH_2C_6H_5$[e]	1	B	136-138	35	A-B	$C_{22}H_{22}N_2O \cdot C_2H_2O_4$[f]	C, H, N
5	CH_3	2	B	217-219	20	A	$C_{17}H_{20}N_2O \cdot HCl$	C, N, H[g]
8	H[e]	1	D	161-163	30	A-B	$C_{15}H_{16}N_2O \cdot C_4H_4O_4$[h]	C, H, N
9	H	2	D	125-126	75	A-B	$C_{16}H_{18}N_2O \cdot C_2H_2O_4$[f]	C, H, N
10	CH_3 (cis)	1	F	82-84	80	C	$C_{16}H_{18}N_2O$	C, H
11	CH_3 (trans)	1	F	149-150	55	A-B	$C_{16}H_{18}N_2O \cdot C_2H_2O_4$[f]	C, H
12a	CH_2CH_3	2	F	115-118	50	A-B	$C_{18}H_{22}N_2O \cdot C_2H_2O_4$[f]	C, H, N
12b	CH_2CH_2OH	2	F	*i*	50	*j*	$C_{18}H_{22}N_2O_2$	C, H
12c	$CH_2CH_2CH_3$	2	F	130-132	51	A	$C_{19}H_{24}N_2O \cdot C_2H_2O_4$[f]	C, H, N
12d	$CH_2CH_2N(CH_2CH_3)_2$	2	E	115-117	83	A	$C_{22}H_{31}N_3O \cdot 2C_2H_2O_4$[f]	C, H, N
12e	$CH_2CH_2C_6H_5$	2	F	112-115	56	A	$C_{24}H_{26}N_2O \cdot C_2H_2O_4 \cdot 0.5H_2O$[f]	C, H, N
12f	$CH_2CH_2CH_2CO(4\text{-}FC_6H_4)$	2	E	120-123	78	A	$C_{26}H_{27}FN_2O_2 \cdot C_2H_2O_4$	C, H, N
13	$C(=NOH)NH_2$	2		160-162	71	C-D	$C_{17}H_{20}N_4O_2$	C, H, N

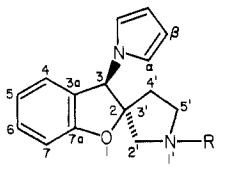
[a] All compounds exhibited IR and ^1H NMR spectra consistent with the structure. [b] See Experimental Section. [c] Isolated yield; no efforts were made to optimize these yields. [d] A = ethyl acetate; B = methanol; C = hexane; D = ethyl ether. [e] Mixture of cis and trans isomers. [f] Acid oxalate salt. [g] H: calcd, 6.95; found, 7.42. [h] Acid maleate salt. [i] Isolated as a heavy oil. [j] Purified by Kugelrohr distillation, 200-210 °C (0.4 mmHg).

ination and the isolation of **1**.

Further transformations of the spiro compounds **4** and



4a, R = $CH_2C_6H_5$, cis
10, R = CH_3, cis



4b, R = $CH_2C_6H_5$, trans
11, R = CH_3, trans

5 are shown in Scheme II. Dealkylation of the tertiary amines to the secondary amines was effected by the reaction of the *N*-benzyl or *N*-methyl tertiary amines **4** or **5** with ethyl chloroformate and potassium carbonate in refluxing benzene to give the corresponding carbamates **6** and **7** (method C). The carbamates **6** and **7** were hydrolyzed by refluxing with 10% aqueous potassium hydroxide solution in 1-propanol to afford the secondary amines **8** and **9** (method D). Reduction of the *cis*- and *trans*-carbamates **6** with lithium aluminum hydride in tetrahydrofuran gave the *cis*- and *trans*-*N*-methylpyrrolidines **10** and **11**.

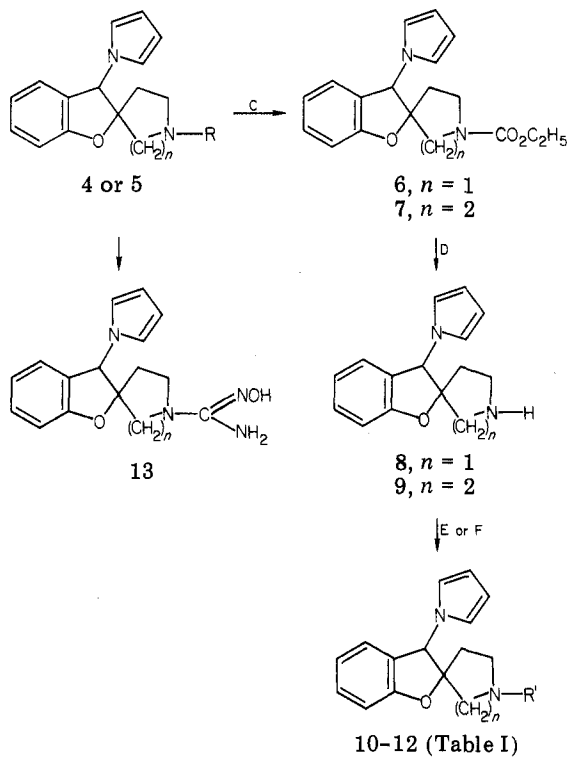
The secondary amines **8** and **9** were either alkylated directly with an alkyl halide in dimethylformamide with potassium carbonate at 70 °C (method E) or acylated with an acid chloride, followed by reduction of the resulting amide with lithium aluminum hydride in tetrahydrofuran (method F), to yield the N-substituted spiro compounds **12a-f**.

Reaction of the *N*-methyl compound **5** with cyanogen bromide and sodium carbonate in refluxing chloroform gave the cyanamide, which was then reacted with hydroxylamine hydrochloride and sodium carbonate in dimethylformamide to give the *N*-amino(hydroxyimino)-methyl **13** in an overall yield of 60%.

The mixture of spiro pyrrolidine diastereoisomers **4** was separated by preparative HPLC. The stereochemical assignments of each diastereoisomer were made based on carbon-13 NMR and proton NMR.

Carbon-13 NMR is a technique that is remarkably sensitive to the orientation of the carbon in a molecule and has been used previously to aid in the assignment of dia-

Scheme II



stereoisomers.[6] When the chemical shifts of the model compounds *N*-methylpyrrolidine,[7] 2,3-dihydro-2,2-dimethylbenzofuran,[8] and benzylamine,[9] in conjunction with the techniques of quaternary enhancement,[10] continuous-wave off-resonance decoupling,[11] and second-order

(6) F. Wehrli and T. Wirthlin, "Interpretation of Carbon-13 Nuclear Magnetic Resonance Spectra", Heyden, London, 1976, p 188.
(7) "Sadtler Standard ^{13}C NMR Spectra", Vol. 46, Sadtler Research Laboratories, Philadelphia, 1980, p. 9049.
(8) Reference 7, Vol. 18, 1978, p 3414.
(9) Reference 7, Vol. 1, 1976, p 140.
(10) I. H. Sadtler, *J. Chem. Soc., Chem. Commun.*, 809 (1973).
(11) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York and London, 1972, p 38.

effects in the latter,[12] were used, all aliphatic and most aromatic carbons were unambiguously assigned (Table II).

The chemical shifts of the ring carbons among isomers **4a**,**b**, **10**, and **11** remain essentially unchanged, with the exception of C-2′ and C-4′, the only carbons influenced significantly by their proximity to the pyrrole ring. This proximity and the resultant steric crowding produce a steric compression shift to higher field of the pyrrolidine ring carbon oriented cis to the 3-pyrryl group.[13] For the *N*-benzyl compounds, the chemical shift of C-2′ in **4a** is 6.63 ppm to higher field than in **4b**, implying that C-2′ is sterically compressed in **4a** and is, therefore, cis to the 3-pyrryl substitutent. The chemical shift of C-4′ in **4b** is 9.51 ppm to higher field than in **4a**, indicating that C-4′ is sterically compressed in **4b**, andd, therefore, C-2′ is trans to the pyrrole ring.

Similar results are obtained for the *N*-methyl compounds, where in **10**, C-2′ is 7.57 ppm to higher field than in **11**, and **11**, C-4′ is 9.39 ppm to higher field than in **10**.

In the 60-MHz proton NMR spectra of isomers **4a** and **10**, the methylene protons at the 2′-position appeared as an AB quartet ($J \approx 11$ Hz, $\Delta\delta \approx 0.55$). This magnetic nonequivalence of the 2′-methylene protons is presumably due to the effect of the anisotropic shielding cone generated by the cis orientation of the pyrrole ring.[14]

In contrast, the 2′-protons of isomer **4b** appear as the predicted singlet at the appropriate chemical shift. However, the 2′-protons in **11** appear not as a singlet but as a multiplet centered at about 2.75 ppm. For both compounds **4b** and **11**, however, the 4′-protons appear as a characteristic pair of shielded multiplets ($J \approx 11$ Hz, $\Delta\delta \approx 0.55$), again due, apparently, to their proximity to the anisotropic field of the pyrrole ring. The 5′-protons in all of the compounds appear to be unaffected and always appear as a multiplet at about 2.7 ppm (Table III).

Pharmacology and Discussion of Results

All compounds were evaluated for oral hypotensive activity in spontaneously hypertensive rats (SHR) by using the indirect tail-cuff method (Table IV), and all were significantly active at 50 mg/kg po, except compounds **11**, **12c**, and **13**, which were only marginally active at this dose. In compounds where $n = 2$, the length and size of the *N*-alkyl chain appeared to be important for maintaining hypotensive activity at lower doses. Optimal activity was found in compounds **5** ($R^1 = CH_3$) and **12a** ($R^1 = CH_2CH_3$). Compounds **12b–f**, which possess longer and bulkier side chains, were less active than compounds **5** and **12a**. The pyrrolidine secondary amine **8**, as a mixture of diastereoisomers, and the piperidine **9** were essentially equally potent. Of the two *N*-methylpyrrolidine diastereoisomers, the cis isomer **10** was significantly more active than the trans isomer **11**, and at the screening dose of 50 mg/kg po, it was equal in potency to the *N*-methylpiperidine **5**.

Compounds **5** and **12a** were selected for further study because of their potency, good dose–response, and temporal activity (no decrease in hypotensive response over the duration of treatment) in the SHR. As indicated in Table V, both of these compounds were very active at lowering the blood pressure in renal hypertensive rats [one

Table II. Carbon-13 NMR Chemical Shifts

compd	C-2 (C-3′)	C-3	C-3a	C-4	C-5	C-6	C-7	C-7a	C-2′	C-4′	C-5′	C-α	C-β	R
4a (cis)	97.39	68.17	125.46	130.76	121.37	126.34	110.71	159.04	58.72	39.78	52.70	120.04	108.66	$CH_2C_6H_5$: CH_2 (60.05); C-1″ (138.44); C-2″ (128.17)[a]; C-3″ (128.72)[a]; C-4″ (126.95)
4b (trans)	97.01	67.67	125.29	130.54	121.21	126.34	110.38	159.38	65.35	30.27	52.87	120.10	108.66	$CH_2C_6H_5$: CH_2 (59.94); C-1″ (138.50); C-2″ (128.28)[a]; C-3″ (128.66)[a]; C-4″ (127.06)
10 (cis)	98.11	67.84	125.46	130.76	121.37	126.18	110.76	158.88	60.77	40.44	55.91	119.99	108.72	CH_3: CH_3 (42.10)
11 (trans)	97.83	67.01	125.46	130.60	121.21	126.23	110.54	159.26	68.34	31.05	55.13	120.04	108.72	CH_3: CH_3 (42.10)

[a] Assignment of the C-2″ and C-3″ carbons is tentative and only based upon the relative positions of the equivalent carbons in benzylamine.

Table III. Proton NMR Chemical Shifts

compd	R	H-3	H-4, H-5, H-6, H-7	H-2′	H-4′	H-5′	H-α	H-β	CH_3	$CH_2C_6H_5$	$CH_2C_6H_5$
4a (cis)	$CH_2C_6H_5$	5.35 (s)	6.6–7.5 (m)	2.63 (d), 2.10 (d)	2.15 (m)	2.72 (m)	6.45 (t)	6.09 (t)		3.53 (s)	7.25 (s)
4b (trans)	$CH_2C_6H_5$	5.33 (s)	6.6–7.5 (m)	2.76 (s)	1.88 (m), 1.33 (m)	2.68 (m)	6.49 (t)	6.13 (t)		3.64 (s)	7.30 (s)
10 (cis)	CH_3	5.43 (s)	6.8–7.6 (m)	2.61 (d), 2.00 (d)	2.17 (m)	2.75 (m)	6.56 (t)	6.20 (t)	2.31 (s)		
11 (trans)	CH_3	5.43 (s)	6.8–7.6 (m)	~2.75 (m)	1.92 (m), 1.35 (m)	~2.75 (m)	6.57 (t)	6.20 (t)	2.40 (s)		

(12) F. Wehrli and T. Wirthlin, "Interpretation of Carbon-13 Nuclear Magnetic Resonance Spectra", Heyden, London, 1976, p 72.

(13) G. Levy and G. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, 1972, p 24.

(14) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed., Vol. 5, Pergaman Press, Oxford, 1969, p 94.

Table IV. Hypotensive Activity of 2,3-Dihydro-3-(1-pyrryl)spiro[benzofuran-2,4'-piperidine]s and -spiro[benzofuran-2,3'-pyrrolidine]s in Spontaneously Hypertensive Rats (SHR)

compd	no. of rats	dose, mg/kg po	initial BP, mmHg	ΔBP,[a] mmHg
4	4	50	211	42 ± 15.9
	8	25	185	18 ± 5.4
	8	10	171	10 ± 8.1[b,c]
	8	5	198	27 ± 8.9[c]
5	4	50	202	39 ± 6.3
	8	25	196	58 ± 8.4
	8	10	190	50 ± 5.6
	8	5	184	30 ± 8.8
8	2	50	223	58 ± 1.5
	8	25	181	13 ± 7.9[c]
	7	10	179	36 ± 14.2[c]
	8	5	200	17 ± 10.3[c]
9	4	50	189	43 ± 18.6[c]
		25	200	49
		10	198	33
10	4	50	197	38 ± 11.2
11	4	50	180	14 ± 4.4
12a	4	50	187	71 ± 9.1
	7	30	187	71 ± 5.1
	8	10	180	27 ± 9.4
	8	3	214	38 ± 9.2
12b	3	50	170	39 ± 18.8
12c	4	50	177	14 ± 3.5
12d	4	50	185	38 ± 4.1
12e	4	50	203	59 ± 5.8
	8	25	180	36 ± 8.6
	7	10	180	14 ± 12.7
	8	5	176	26 ± 12.9
12f	4	50	199	37 ± 11.2
13	4	50	209	24 ± 15.6
clonidine		0.25		38

[a] Peak decrease in systolic blood pressure on 3rd day, 2 h postadministration of compound, ±SEM. [b] Increase in systolic blood pressure on 3rd day, 2 h postadministration of compound. [c] Fifth day, 2 h postadministration of compound.

kidney, one wrap (Grollman)]. Compound **5** was particularly active on day 3 of the test, with a decrease of 109 mmHg. The reason for the greater hypotensive activity of compound **5** in RHR vs. SHR is not clear at this time.

In anesthetized neurogenic hypertensive dogs (Table VI), both compounds **5** and **12a** exerted marked hypotensive effects within 5 min of dosage. Both caused moderate bradycardia but had little effect on $dP\ dT^{-1}\ P_{max}^{-1}$, a parameter of cardiac contractility. Compound **12a** did, however, lower the cardiac output somewhat, and this may have contributed to its hypotensive activity.

In anesthetized normotensive dogs in standard cardiovascular challenge experiments, neither of these compounds produced any striking effects on responses to standard challenge drugs, suggesting little interaction with autonomic receptors. Compound **12a** did have a slight tendency to potentiate responses to norepinephrine, epinephrine, and tyramine, but these effects were minimal (Table VII). Additional hemodynamic studies performed in anesthetized, ganglion-blocked dogs suggested that the hypotensive effects were not mediated via the central nervous system, since activity was still present under these conditions.

Experimental Section

The structures of all compounds are supported by their IR (Perkin-Elmer 457) and ^1H NMR (JEOL C6OHL) spectra. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Where analyses

Table V. Hypotensive Activity of Compounds 5 and 12a in Renal Hypertensive Rats (RHR)[a]

compd	no. of rats	dose, mg/kg po	initial BP, mmHg	postdrug BP, mmHg, (day/h)	ΔBP, mmHg
5	4	10	206 ± 13	196 ± 8 (1/2)	37
				184 ± 11 (2/0)	22
				170 ± 12[b] (2/2)	36
				183 ± 6[b] (3/0)	23
				97 ± 12[b] (3/2)	109
12a	4	50	162 ± 8	131 ± 10 (1/2)	31
				110 ± 4 (2/0)	52
				113 ± 5[b] (2/2)	49
				116 ± 5[b] (3/0)	46
				129 ± 23[b] (3/2)	33
captopril	4	10	192 ± 15	181 ± 16 (1/2)	11
				185 ± 9 (2/0)	7
				169 ± 4 (2/2)	23
				163 ± 12 (3/0)	29
				152 ± 12 (3/2)	40

[a] Grollman model of hypertension (one kidney, one wrap). [b] Data for three rats.

are indicated only by symbols of the elements, the analytical results obtained for those elements (performed by Micro-Tech Laboratories, Skokie, IL) were within 0.4% of theoretical values.

Preparative HPLC separation of **4a,b** was performed on a Waters Associates Prep 500 system using a Prep Pak silica cartridge. The mobile phase was ether/hexane (35:65), and detection was by refractive index.

Carbon-13 NMR spectra were recorded as deuteriochloroform solutions at 15.03 MHz on a JEOL FX-60 Fourier-transform spectrometer equipped with an internal deuterium lock. Operating parameters were as follows: pulse width, 3 μs; tip angle, 30°; pulse repetition, 1.5 s; spectral width, 3.4 kHz; data size, 8K. All proton and carbon chemical shifts are reported relative to internal tetramethylsilane.

1-(2-Fluorobenzyl)pyrrole (1). To 120 mL of glacial acetic acid was added 2-fluorobenzylamine (30.0 g, 0.24 mol) with cooling, followed by 2,5-dimethoxytetrahydrofuran (32.0 g, 0.24 mol). After stirring at reflux for 2 h, the cooled solution was concentrated in vacuo to a dark oil, which was added to 100 mL of a 10% solution of sodium carbonate and then extracted with ethyl ether (3 × 100 mL). The dried ($MgSO_4$) etheral solution was concentrated in vacuo to a dark oil, which was distilled to give 27 g (64%), bp 73 °C (0.5 mm), of a water-white liquid. Anal. ($C_{11}H_{10}FN$) C, H, N.

4-[(2-Fluorophenyl)(1-pyrryl)methyl]-1-methyl-4-piperidinol (3). Method A. To 35 mL of THF was added **1** (10.0 g, 0.057 mol), and, after this solution was cooled to −80 °C, a solution of *n*-butyllithium (25 mL of a 2.4 M solution, 0.06 mol) was added dropwise with stirring over a period of 10 min. After the thick solution was stirred at −80 °C for 1 h, a solution of 1-methyl-4-piperidone (6.4 g, 0.055 mol) in 15 mL of THF was added in 5 min. After stirring at ambient temperature for 1 h, the mixture was poured into 250 mL of water, stirred for 10 min, and then extracted with ethyl ether (2 × 50 mL). The organic extracts were washed with water (2 times) then dried ($MgSO_4$) and concentrated under vacuum to a yellow oil, which upon trituration with *n*-hexanes gave **3** as a tan solid: yield 10 g (62%); mp 128–130 °C, 135 °C after crystallization from ethyl acetate/methanol (10:1). Anal. ($C_{17}H_{21}FN_2O\cdot HCl$) C, H, N. Compound **2** was prepared in a similar manner in 58% yield, mp 87–89 °C. Anal. ($C_{22}H_{23}FN_2O$) C, H, N.

2,3-Dihydro-1′-methyl-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine] Hydrochloride (5). Method B. To a suspension of sodium hydride (0.9 g, 0.036 mol) in 50 mL of benzene was added a solution of **3** (9.0 g, 0.03 mol) in 100 mL of benzene. After the solution was heated to reflux, 40 mL of DMF was added slowly, and reflux was continued for 3 h. After cooling, the mixture was poured into 500 mL of water, stirred for 5 min, and then extracted with ethyl ether (2 × 100 mL). The combined organic layer was washed with water (2 times), dried ($MgSO_4$), and then concentrated in vacuo to a brown oil, which was converted to a

Table VI. Hemodynamic Effects in the Sinoaortic-Deafferented Hypertensive Dog[a]

compd	no. of dogs	dose, mg/kg iv	time, min	mean arterial BP, mmHg	heart rate, beats/min	$dP\ dt^{-1}$ P_{max}^{-1}, s^{-1}	cardiac output, mL/min
5	3	5	0	175 ± 15	194 ± 26	24.2 ± 5.8[b]	
			5	123 ± 24[c]	154 ± 32[c]	26.0 ± 0.7[b]	
			30	129 ± 16[c]	174 ± 16[d]	26.2 ± 2.2[b]	
			60	129 ± 19[c]	160 ± 21[c]	23.4 ± 0.2[b]	
12a	3	10	0	165 ± 8.5	170 ± 10	27.2 ± 5.2	2120 ± 350
			5	121 ± 17[c]	134 ± 7[c]	20.3 ± 4.9[d]	1600 ± 260[d]
			30	98 ± 29[c]	126 ± 16[c]	21.2 ± 8.2[d]	1530 ± 610[d]
			60	103 ± 20[c]	132 ± 16[c]	25.6 ± 5.3[d]	1600 ± 560[d]

[a] Data indicated as mean plus or minus SEM. [b] Data from two dogs, no statistics. [c] $p < 0.05$ vs. time 0, analysis of variance (ANOVA) with repeated measures on time factor, Duncan's test. [d] Data not statistically different from time 0, ANOVA with repeated measures, Duncan's test.

Table VII. Effects on Standard Cardiovascular Challenges in Anesthetized Normotensive Dogs

challenge drug		5[a]	12a[a]
epinephrine	pre	+53.7[b]	+44.8
	post	+67.0	+59.7
	Δ	+13.3	+14.9
norepinephrine	pre	+57	+56.7
	post	+58	+66.0
	Δ	+1	+9.3
isoproterenol	pre	−38	−34.3
	post	−30	−39.3
	Δ	−8	+5.0
carbachol	pre	−49.2	−52
	post	−29.7	−37
	Δ	−19.5	−15
tyramine	pre	+52.5	+64.3
	post	+48.0	+84.3
	Δ	−4.5	+20.0
mean arterial BP[c]	pre	117	132.7
	post	82.3	106.3
	Δ	−34.7	−26.4
mean heart rate[d]	pre	147	167
	post	113	126
	Δ	−34	−41

[a] Administered 10 mg/kg iv. [b] mmHg from base line. [c] Fifteen minutes postdose, mmHg. [d] Beats per minute.

crystalline hydrochloride, **5**, in ether. Properties of **5** and **4**, prepared in a similar manner, are included in Table I.

2,3-Dihydro-1′-(ethoxycarbonyl)-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine] (7). Method C. To 200 mL of benzene were added **5** (15.5 g, 0.058 mol) and potassium carbonate (25 g, 0.18 mol), followed by a solution of ethyl chloroformate (8.1 g, 0.75 mol) in 50 mL of benzene. After stirring at reflux for 20 h, the mixture was cooled and filtered, and the filtrate was washed with water and 3 N hydrochloric acid, dried ($MgSO_4$), and then concentrated in vacuo to a brown oil, which upon trituration with hexanes gave **7**: yield 18.6 g (80%); mp 132–133 °C. Anal. ($C_{19}H_{22}N_2O_3$) C, H, N. Compound **6** (both isomers) was prepared in a similar manner in 70% yield.

2,3-Dihydro-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine] Oxalate (9). Method D. To a solution of potassium hydroxide (20.0 g, 0.36 mol) in 6 mL of water and 100 mL of 1-propanol was added a solution of **7** (13.3 g, 0.41 mol) in 150 mL of 1-propanol. After stirring at reflux for 20 h, the mixture was concentrated in vacuo to a dark oil, which was dissolved in $CHCl_3$, washed with water (2 times), and then dried ($MgSO_4$). After concentrating in vacuo, the resultant brown oil was converted to a crystalline oxalate (**9**) in ether. Properties of **9** and **8**, prepared in a similar manner, are included in Table I.

***cis*-2,3-Dihydro-1′-methyl-3-(1-pyrryl)spiro[benzofuran-2,3′-pyrrolidine] (10).** To a suspension of lithium aluminum hydride (1.1 g, 0.029 mol) in 75 mL of THF was added a solution of **6** (cis isomer; 4.5 g, 0.0138 mol) in 25 mL of THF. After stirring at ambient temperature for 3 h, the mixture was diluted with 100 mL of ethyl ether and then quenched with 5 mL of saturated ammonium chloride solution. After the solution was filtered, the organic layer was washed with water (2 times), dried ($MgSO_4$), and then concentrated in vacuo to give a light yellow oil, which crystallized from hexanes. Properties of **10** and **11** (trans isomer), prepared in a similar manner, are included in Table I.

1′-[2-(Diethylamino)ethyl]-2,3-dihydro-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine] Dioxalate (12d). Method E. To 100 mL of 1-butanol were added **9** (4.5 g, 0.018 mol), 2-(diethylamino)ethyl chloride (2.7 g, 0.02 mol), K_2CO_3 (20 g, 0.145 mol), and KI (0.01 g). After stirring at reflux for 48 h, the mixture was concentrated in vacuo, dissolved in ethyl ether, washed with water, and then dried ($MgSO_4$). After concentration in vacuo, the oil was converted to a crystalline dioxalate, **12d**, in ether. Properties of **12d,f**, prepared in a similar manner, are included in Table I.

2,3-Dihydro-1′-ethyl-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine] Oxalate (12a). Method F. To 100 mL of CH_2Cl_2 were added **9** (5.5 g, 0.022 mol) and triethylamine (3.8 mL, 0.025 mol), followed by the addition of a solution of acetyl chloride (1.8 mL, 0.025 mol) in 30 mL of CH_2Cl_2 with cooling. After stirring at ambient temperature for 20 h, the mixture was concentrated in vacuo, dissolved in ethyl acetate, washed with water and 2 N hydrochloric acid, and then dried ($MgSO_4$). Concentration in vacuo resulted in a brown semisolid: yield 4.8 g (84%); mp 45 °C. No further purification was attempted. The amide was reduced to the amine as in **10**, to yield a brown oil, which was converted to a crystalline oxalate, **12a**, in ether. Properties of **12a,c,e**, prepared in a similar manner, are included in Table I.

2,3-Dihydro-1′-(2-hydroxyethyl)-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine] (12b). To 50 mL of DMF were added **9** (6.0 g, 0.024 mol), ethyl bromoacetate (4.6 g, 0.028 mol), $NaHCO_3$ (20 g, 0.24 mol), and KI (0.01 g). After stirring at 70 °C for 1 h, the mixture was concentrated in vacuo, dissolved in ethyl acetate, washed with water, dried ($MgSO_4$), and then concentrated in vacuo to give 5 g (62%) of a dark oil. No further purification was attempted. This ester was reduced to the alcohol **12b** as in **10**, to yield a yellow oil. Properties of **12b** are included in Table I.

1′-[Amino(hydroxyimino)methyl]-2,3-dihydro-3-(1-pyrryl)spiro[benzofuran-2,4′-piperidine] (13). To a refluxing solution of cyanogen bromide (3.7 g, 0.035 mol) and K_2CO_3 (20.0 g, 0.145 mol) in 100 mL $CHCl_3$ was added a solution of **5** (8.0 g, 0.03 mol) in 75 mL of $CHCl_3$. After refluxing for 5 h, the mixture was filtered, and the filtrate was concentrated in vacuo to a brown oil, which solidified to a tan solid upon trituration with petroleum ether to give the cyanamide: yield 6.9 g (82%); mp 165–168 °C. Anal. ($C_{17}H_{17}N_3O$) C, H, N.

To 20 mL of DMF were added the cyanamide (6.4 g, 0.023 mol), hydroxylamine hydrochloride (2.6 g, 0.04 mol), and Na_2CO_3 (7.7 g, 0.07 mol). After stirring at 105 °C for 1.5 h, the mixture was poured into 500 mL of iced water, and the resultant tan precipitate was collected and dried to yield 5.1 g of **13**. Properties of **13** are included in Table I.

Hypotensive Activity in Spontaneously Hypertensive Rats (SHR) and Renal Hypertensive Rats (RHR). All compounds were screened for hypotensive activity in spontaneously hypertensive rats of the Okamoto-Aoki strain. The screening dose was 50 mg/kg po. Systolic blood pressures were determined at the following times by tail-cuff plethysmography: day 1, predose and 2 h postdose; day 3, predose and 2 h postdose; day 5, predose and 2 and 4 h postdose. Details of the method are described in Buggy et al.[15] The test compounds were suspended in distilled water

(15) J. Buggy, G. D. Fink, A. K. Johnsen, and M. J. Brody, *Circ. Res., Suppl.*, 40(5), 1977, I-110.

with Tween 80 and administered at either 50, 30, 10, or 3 mg/kg po, unless otherwise indicated. Animals were dosed every day. Four animals were used in the preliminary screen, while four to eight animals per dose were used in the dose–response screen.

Renal hypertension was produced by a modification of the method of Grollman.[15,16] Briefly, male Sprague–Dawley rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and the right kidney was removed through a right subcostal incision. The left kidney was exposed through an incision in the left flank. Silk sutures (size 00) were passed around both poles of the kidney and tightened to produce a visible constriction of the renal parenchyma. Animals were returned to their cages and given food and water ad libitum. After 3 weeks of stabilization, systolic blood pressures were determined as above, and rats with blood pressures >150 mmHg were selected for use in evaluating drugs.

Standard Cardiovascular Challenges in Anesthetized Dogs. Beagle dogs of either sex were anesthetized with barbital sodium (200 mg/kg iv), thiopental sodium (15 mg/kg iv), and pentobarbital sodium (60 mg iv). The trachea was intubated, and the animals were artificially respired with a Harvard apparatus respirator. A femoral artery and vein were cannulated with PE tubing for measurement of blood pressure and intravenous administration of drugs, respectively. Bilateral vagotomies were performed, and blood pressure and heart rate were continuously displayed on a Beckman R 511 recorder. The effects of various challenge drugs on the blood pressure and heart rate were determined; test drug was administered, and responses to the challenge drugs were again obtained. Each challenge drug was intravenously administered twice predrug to obtain matched responses and once postdrug. Challenge drugs and doses were as follows: epinephrine and norepinephrine, 0.5 to 1.0 μg/kg; isoproterenol and carbachol, 0.25 μg/kg; tyramine, 100 μg/kg. Adjustments were made in test doses to compensate for animal to animal variation. The effect of the test drug on the responses to the challenge drugs was noted, and results were expressed as change from predrug control responses.

Hypotensive Activity in the Sinoaortic-Deafferented Dogs. Adult dogs of either sex were anesthetized with barbital sodium (200 mg/kg iv), thiopental sodium (15 mg/kg iv), and pentobarbital sodium (60 mg iv). A femoral vein and artery were cannulated with PE tubing for intravenous administration of drugs and to record blood pressure and heart rate, respectively. Left ventricular pressure was recorded from a catheter inserted into the left ventricle via the left common carotid artery (postdeafferentation), and the first derivative, dP/dt, was derived from it. Cardiac output was determined by thermodilution technique using ice-cold 5% dextrose in water injected into a Swan–Ganz catheter inserted into the right side of the heart with the tip placed in the pulmonary artery.

Deafferentation was accomplished by clearing both of the carotid arteries up to the internal and external carotid artery bifurcation. The carotid sinus nerves were isolated, ligated, and sectioned, and a bilateral vagotomy was performed to produce neurogenic hypertension (mean arterial pressure >150 mmHg). Dogs were allowed to stabilize for approximately 30 min, and then a bolus intravenous injection of the test compound was administered. Heart rate, arterial pressure, left ventricular pressure, dP/dt, and cardiac output were monitored for 90 min postdose.

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Registry No. 1, 63880-21-7; 2, 79227-66-0; 3·HCl, 79227-46-6; *cis*-4, 82830-95-3; *cis*-4 oxalate, 82830-96-4; *trans*-4, 82831-05-8; *trans*-4 oxalate, 82831-06-9; 5, 79227-49-9; 5·HCl, 79227-48-8; *cis*-6, 82831-04-7; *trans*-6, 82831-03-6; 7, 79227-53-5; *cis*-8, 86632-59-9; *cis*-8 maleate, 86632-60-2; *trans*-8, 86632-61-3; *trans*-8 maleate, 86632-62-4; 9, 79227-51-3; 9 oxalate, 79227-52-4; 10, 82830-99-7; 11, 82830-97-5; 11 oxalate, 82830-98-6; **12a**, 79227-71-7; **12a** oxalate, 79227-72-8; **12a** ($R^1 = COCH_3$), 79227-73-9; **12b**, 79227-75-1; **12b** ($R^1 = CH_2CO_2Et$), 79227-76-2; **12c**, 79227-63-7; **12c** oxalate, 79227-64-8; **12c** ($R^1 = COCH_2CH_3$), 79227-65-9; **12d**, 79227-58-0; **12d** 2-oxalate, 79227-59-1; **12e**, 79227-61-5; **12e** oxalate, 79227-62-6; **12e** ($R^1 = COCH_2C_6H_5$), 79227-60-4; **12f**, 79227-56-8; **12f** oxalate, 79227-57-9; 13, 79227-55-7; 13 (R^1 = CN), 79227-54-6; 2-fluorobenzylamine, 89-99-6; 2,5-dimethoxytetrahydrofuran, 696-59-3; 1-methyl-4-piperidone, 1445-73-4; 1-benzyl-3-pyrrolidinone, 775-16-6; ethyl chloroformate, 541-41-3; 2-(diethylamino)ethyl chloride, 100-35-6; ethyl bromoacetate, 105-36-2; cyanogen bromide, 506-68-3; hydroxylamine, 7803-49-8.

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9-Acridinyl and 2-Methoxy-6-chloro-9-acridinyl Derivatives of Aliphatic Di-, Tri-, and Tetraamines. Chemistry, Cytostatic Activity, and Schistosomicidal Activity

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9-acridinyl derivatives of 1,6-hexanediamine, 1,8-octanediamine, bis(3-aminopropyl)amine, *N,N'*-bis(3-aminopropyl)piperazine, and *N*-ethyl-1,6-hexanediamine in the form of their hydrochlorides were prepared in high yields and converted into potential hetero bis DNA intercalating diacridines. The corresponding potential homo bis DNA intercalating reagents were prepared by heating the above amines with 9-chloroacridines. The chemical stability of the acridines was examined. Their cytostatic activity against Cloudman melanoma cells, in vitro, has been determined. The strongest cytostatic activity was observed for the acridine derivatives of the tri- and tetraamines. The schistosomicidal activity of selected acridine and diacridine derivatives against *Schistosoma mansoni* in mice was found to be insignificant. The *S. mansoni* egg development was apparently suppressed by this treatment.

In the design of biomolecular tools and drugs, it is important to obtain high target specificity. The target could either be an entire organ, a type of tissue, or it could be molecular. Thus, compounds that could be directed not only toward DNA but even toward particular nucleotide sequences in DNA might exhibit high specific biological activity. Such compounds would be potential drugs against tumors,[1] virus,[1,2] bacteria,[3] and parasites.[4]

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