118420-49-8; **17r**·2tartrate, 118420-80-7; **17s**, 118420-50-1; **17t**, 118420-51-2; **17u**, 118420-52-3; **17u**·2tartrate, 118420-81-8; **17v**, 118420-53-4; **17v**·2tartrate, 118420-82-9; **17w**, 118420-54-5; **17w**·3oxalate, 118420-83-0; **17x**, 118437-10-8; **18a**, 107755-78-2; **18a**·fumarate, 118420-84-1; **18b**, 107755-79-3; **19a**, 107755-62-4; **19a**·fumarate, 107755-63-5; **19b**, 107755-60-2; **19c**, 118420-55-6; **19c**·1.5fumarate, 118420-71-6; **19d**, 107755-68-0; **19e**, 107755-64-6; **19e**·2oxalate, 107755-65-7; **19f**, 107755-61-3; **20**, 118420-56-7; **20**·3fumarate, 118420-72-7; **21**, 118420-57-8; **22**, 118420-58-9; **23**, 118420-59-0; **24**, 118420-60-3; **25**, 118420-61-4; **26a**, 118420-62-5; **26a**·2oxalate, 118420-73-8; **26b**, 118420-63-6; **26b**·1.5tartrate, 118420-74-9; **26c**, 118420-64-7; **26d**, 117830-04-3; $(EtO)_2P(O)CHMeCO_2Et$, 3699-66-9; $(EtO)_2P(O)CHEtCO_2Et$, 17145-91-4; $(EtO)_2P(O)CH_2CO_2Et$, 867-13-0; $(EtO)_2P(O)CHPrCO_2Et$, 35051-49-1; $(EtO)_2P(O)CHPhCO_2Et$, 31641-78-8; 3-acetylpyridine, 350-03-8; 3-pyridinecarbaldehyde, 500-22-1; 4-bromobutyronitrile, 5332-06-9; 3-cyano-6-propyl-2-pyridone, 24049-25-0; 2-chloro-3-cyano-6-propylpyridine, 118419-88-8; 5-cyano-2-propylpyridine, 118419-89-9; 3-cyano-6-isopropyl-2-pyridone, 5782-69-4; 6-butyl-3-cyano-2-pyridone, 118420-86-3; 3-cyano-5,6-dimethyl-2-pyridone, 72716-80-4; 3-cyano-6-ethyl-2-pyridone, 4241-20-7; 2-chloro-3-cyano-5,6-dimethylpyridine, 65176-93-4; 2-chloro-5-nitropyridine, 4548-45-2; 2-methoxy-5-nitropyridine, 5446-92-4; methyl acrylate, 96-33-3; methyl 2-chloro-3-(6-methoxy-3-pyridyl)propionate, 107756-04-7; triethyl phosphonocrotonate, 10236-14-3; ethyl (*E,E*)-5-(3-pyridyl)-2,4-pentadienoate, 118420-14-7; ethyl 2-methylnicotinate, 1721-26-2; malonic acid, 141-82-2; ethyl 5-chloronicotinate, 20825-98-3; 5-chloro-3-pyridinecarbohydrazonic acid, 117830-18-9; ethyl 6-methylnicotinate, 21684-59-3; phenylacetic acid, 103-82-2; 3-(3-pyridyl)acrylic acid, 1126-74-5; 1-(4-aminobutyl)-4-(diphenylmethyl)piperazine, 101620-10-4; (2-(4-bromobutyl)-1*H*-isoindole-1,3(2*H*)-dione, 5394-18-3; ethyl 5-methoxynicotinate, 20826-01-1; ethyl 6-phenylnicotinate, 57443-68-2; ethyl 2,6-dimethylnicotinate, 1721-13-7; ethyl 5-fluoronicotinate, 22620-29-7; ethyl 5-bromonicotinate, 20986-40-7; 4-(diphenylmethyl)-1-piperazinepropanamine, 50971-75-0.

5-(1-Piperazinyl)-1*H*-1,2,4-triazol-3-amines as Antihypertensive Agents[1]

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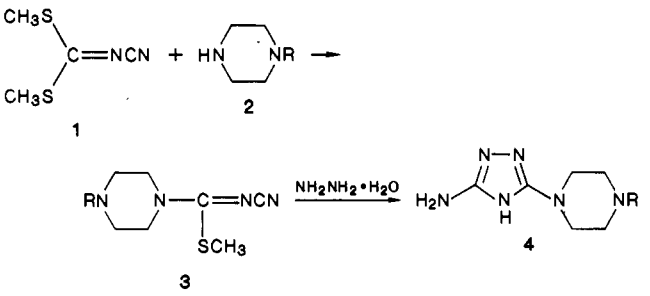
A series of 5-(1-piperazinyl)-1*H*-1,2,4-triazol-3-amines was synthesized and screened for antihypertensive and diuretic activity in spontaneously hypertensive rats (SHR). One compound, 5-[4-[(3-chlorophenyl)methyl]-1-piperazinyl]-1*H*-1,2,4-triazol-3-amine (**8**), was selected to define the mechanism of its antihypertensive activity. Studies in SHR suggest ganglionic blocking activity. Short-lived antihypertensive activity was observed in conscious renal hypertensive dogs.

During an ongoing search for effective drugs for the management of hypertension, the piperazinyltriazolamine **5** was synthesized. When administered orally to the conscious, spontaneously hypertensive rat, compound **5** significantly lowered blood pressure without affecting urinary output. The ubiquitous presence of the piperazine nucleus in cardiovascular drugs such as prazosin,[2] lidoflazine,[3] and urapidil[4] encouraged us to undertake, as one aspect of our investigation of this heterocyclic system, the synthesis and biological evaluation of a series of 4-N′-substituted piperazinyltriazolamines related to **5**, which we report in this paper.

Chemistry

The compounds listed in Table I were synthesized by the two-step route outlined in Scheme I. Dimethyl cyanocarboximidodithioate (**1**) reacted smoothly with 1 equiv of **2** in either ethanol or acetonitrile to give thioic acid **3** in high yield. Although **3** could be isolated as a crystalline solid, it was usual to proceed to the final step without isolation of this intermediate. The cessation of methyl mercaptan evolution indicated the completion of step 1. A slight excess of hydrazine hydrate was added and refluxing continued until evolution of the second mole of methyl mercaptan was complete. Ethanol reacted slowly with **1** to give, after reaction with hydrazine hydrate, small quantities of 5-ethoxy-1*H*-1,2,4-triazol-3-amine, which interfered with the purification of the final product; therefore, acetonitrile was the solvent of choice.

Scheme I



Discussion

Blood pressure lowering and diuretic activity was assessed in spontaneously hypertensive rats (SHR). As may be seen from Table I, a number of *N′*-benzyl- and *N′*-alkyl-substituted piperazines show blood pressure lowering properties. Alkyl (**36**, **37**), phenylalkyl (**5**, **6**, **7**, **42**), and phenoxyalkyl (**44**) derivatives, with the exception of those alkyl groups containing nitrogen (**41**, **43**), lower blood pressure as much as 75 mmHg below control levels. Cycloalkyl derivatives **38**, **39**, and **40** show significant but less blood pressure lowering capabilities. Benzylic derivatives indicate varying degrees of potency depending on the substituent and substitution pattern of the phenyl ring.

Thus, while the halogenated benzyl derivatives **8**, **19**, and **20** lower blood pressure markedly, ortho-substituted derivatives **9**, **11**, **14**, **15**, **21**, **29**, **32**, and **47** showed diminished potency. Ring deactivating groups, such as cyano (**24**) and nitro (**33**), suppress activity, whereas the effect of ring activation is less clear. While the *p*-amino (**27**), *p*-dimethylamino (**28**), and 3-bromo-*p*-(dimethylamino)benzyl (**31**) piperazine compounds exhibit blood pressure lowering

(1) Tomcufcik, A. S.; Meyer, W. E.; Dusza, J. P. U.S. Patent 4,421,753, 1983.
(2) Scriabine, A.; Constantine, J. W.; Hess, H.-J.; McShane, N. K. *Experientia* **1968**, *24*, 1150.
(3) Schaper, W. K. A.; Xhonneux, R.; Jageneau, A. H. M.; Janssen, P. A. J. *J. Pharmacol. Exp. Ther.* **1966**, *152*, 265.
(4) Schoetensack, V. W.; Bischler, P.; Dittmann, E. Ch.; Steinijans, V. *Arzneim.-Forsch.* **1977**, *27*(II), 1908.
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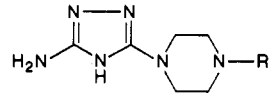
Table I. Antihypertensive Activity of 5-(1-Substituted piperazinyl)-1*H*-1,2,4-triazol-3-amines



compd[a]	R	n[c]	MABP,[d] mmHg	HR,[e] bpm
control[b]		145	153 ± 1	438 ± 3
clonidine		5	102 ± 5	180 ± 15
4	H·2HBr	2	92	410
5	$CH_2C_6H_5$	2	102	360
6	$CH_2CH_2C_6H_5$	2	77	330
7	$CH_2CH_2CH_2C_6H_5 \cdot H_2O$	2	93	380
8	CH_2-3-ClC_6H_4	2	89	300
9	CH_2-2,6-$Cl_2C_6H_3$	2	140	400
10	CH_2-3,4-$Cl_2C_6H_3 \cdot {}^1/_2C_2H_5OH$	2	95	330
11	CH_2-2-$CH_3C_6H_4$	2	131	410
12	CH_2-3-$CH_3C_6H_4 \cdot {}^1/_4H_2O$	2	132	440
13	CH_2-4-$CH_3C_6H_4$	2	112	340
14	CH_2-2,5-$(CH_3)_2C_6H_3$	2	139	400
15	CH_2-2,4,6-$(CH_3)_3C_6H_2 \cdot {}^1/_2H_2O$	2	137	430
16	CH_2-4-$C(CH_3)_3C_6H_4 \cdot {}^1/_4H_2O$	2	130	350
17	CH_2-4-*n*-BuC_6H_4	2	136	360
18	CH_2-3-$CF_3C_6H_4$	2	129	390
19	CH_2-2-$FC_6H_4 \cdot {}^3/_4H_2O$	2	97	350
20	CH_2-4-FC_6H_4	2	97	360
21	CH_2-2-Cl-4-$FC_6H_3 \cdot {}^1/_4H_2O$	2	145	410
22	CH_2-1-naphthyl·2HCl·$1{}^3/_4H_2O$	2	133	350
23	CH_2-4-$C_5H_4N \cdot H_2O$	2	142	380
24	CH_2-4-CNC_6H_4	2	127	420
25	CH_2-2-quinolinyl	2	121	440
26	CH_2-2-furyl	2	114	410
27	CH_2-4-$NH_2C_6H_4$	2	109	380
28	CH_2-4-$N(CH_3)_2C_6H_4$	2	111	380
29	CH_2-2,3,4-$(CH_3O)_3C_6H_2$	2	147	420
30	CH_2-3,4,5-$(CH_3O)_3C_6H_2$	2	132	440
31	CH_2-3-Br-4-$N(CH_3)_2C_6H_3$	2	116	320
32	CH_2-2-Cl-4-$N(CH_3)_2C_6H_3$	2	120	400
33	CH_2-2-$NO_2C_6H_4$	2	135	430
34	CH-(4-ClC_6H_4)(C_6H_5)$\cdot {}^1/_2H_2O$	2	115	350
35	$CH(CH_3)(C_6H_5)$	2	136	370
36	CH_3	2	116	400
37	$CH_2CH{=}CH_2 \cdot H_2O$	2	96	360
38	CH_2-c-C_6H_{11}	2	123	420
39	CH_2-c-C_5H_9	2	114	
40	CH_2-c-C_3H_5	2	104	390
41	$CH_2CH_2CH_2N(CH_3)_2$	2	140	420
42	$CH_2CH{=}CHC_6H_5$	2	101	430
43	CH_2CH_2NHCH-4-C_5H_4N	2	144	440
44	$CH_2CH_2CH_2O \cdot C_6H_5$	2	89	310
45	2-C_5H_4N	2	133	430
46	C_6H_5	2	128	340
47	2-$CH_3OC_6H_4$	2	157	440
48	4-FC_6H_4	2	151	420
49	2-furoyl	2	142	410
50	$COCH(CH_3)_2$	2	141	420
51	CO-4-$C(CH_3)C_6H_4$	2	141	400
52	CO-4-(C_6H_5)C_6H_4	2	138	400
53	$COCH_3$	2	142	440

[a] Doses at 100 mg/kg orally. [b] 3% starch. [c] n = number of SH rats (standard error of the mean is provided for $N > 2$). [d] MABP = mean arterial blood pressure (standard error provided for $n > 2$). [e] HR = mean heart rate in beats per minute rounded to the nearest 10.

properties, the electron-rich alkoxybenzyl derivative **30** is inactive by our criteria.

Substantial blood pressure lowering properties of the 4-N′-unsubstituted piperazine (**4**) suggests that this material may be an active metabolite, resulting from in vivo N-dealkylation of the piperazine ring. The hypotensive activity of the prodrugs would then depend on the ability and rate of N-dealkylation.

Table II. Antihypertensive Evaluation of 5-(1-Substituted piperazinyl)-1*H*-1,2,4-triazol-3-amines

compd	dose, mg/kg	MABP,[a] mmHg	HR,[b] bpm
5	10	118	400
6	10	142	440
7	50	95	520
8	25	132	440
8	50	113	440
13	50	70	350
19	50	79	330
26	10	153	440
26	30	115	440
26	50	102	370
28	50	129	440
36	50	133	440
39	50	117	440

[a] Mean arterial blood pressure; n = 3. [b] Mean heart rate to the nearest 10 bpm; n = 3.

The deletereous effect on blood pressure lowering properties of *o*-phenyl derivatives **11**, **13**, and **47** and the low activity of the methylated benzylic carbon derivative **35** suggest that steric crowding of the benzylic C-nitrogen reduces metabolic N-dealkylation. Although this steric effect may be responsible for the lower activity of the 2-chloro-4-dimethylamino derivative **32** vs the 4-dimethylamino analogue **28** and the large difference between the 4-fluoro (**20**) and the 2-chloro-4-fluoro derivative (**21**), electronic and other steric factors may be contributing significantly in these and other multisubstituted derivatives (**9**, **14**, **15**, and **29**).

The strong blood pressure lowering properties of the 2-fluorobenzyl derivative **19** would not be unexpected since stereochemical effects do not usually dominate the properties of fluorocarbons.

Significantly, 4-N′-arylated derivatives **45–48** and 4-N′-acylated derivatives **50–53** do not lower blood pressure below control levels.

Compounds selected from Table I for study at lower doses in the SH rat are described in Table II. In addition, derivatives **5**, **7**, **8**, **13**, **20**, **26**, and **36** were tested and found not to produce significant blood pressure lowering in conscious Goldblatt renal hypertensive dogs. Administered at 3 mg/kg or at higher doses orally, intraperitoneally, or intravenously, caused emesis in both normotensive and hypertensive dogs. It was of interest to note, however, that although **5** and **13** caused severe emesis in dogs, 5 mg/kg administered orally to female rhesus monkeys was tolerated, suggesting that emesis might be a species-related phenomenon.

Due to the high antihypertensive efficacy of compound **8** in the SH rat, it was chosen for further biological evaluations. Compound **8** at 25 and 50 mg/kg orally produced maximal mean arterial blood pressure (MABP) lowering of 21 and 40 mmHg, respectively. In conscious Goldblatt renal hypertensive dogs, **8** at 3 mg/kg orally produced about 10–15 mmHg of MABP lowering on day 1 but a second dose of 3 mg/kg given orally 24 h later produced emesis with no significant lowering of blood pressure. At 10 mg/kg orally or intraperitoneally, **8** produced 15–20 mmHg of MABP lowering but caused emesis. Two doses of 5 mg/kg, given intravenously 40 min apart, was not hypotensive in anesthetized normotensive dogs.

In an attempt to define the mechanism of its antihypertensive action, **8** was tested for its effects on the autonomic nervous system and on the vasopressor response of angiotensin II in SHR. It was found that at 50 mg/kg

Table III. Physical Properties of 4–53

compd	R	yield, %	mp, °C	formula	anal.
4	H	64	303–306	$C_6H_{12}N_6 \cdot 2HBr$	C, H, N, Br
5	$CH_2C_6H_5$	89	160–161	$C_{13}H_{18}N_6 \cdot 0.1H_2O$	C, H, N
6	$CH_2CH_2C_6H_5$	30	136–138	$C_{14}H_{20}N_6$	C, H, N
7	$CH_2CH_2CH_2C_6H_5$	27	91–93	$C_{15}N_{22}N_6 \cdot H_2O$	C, H, N
8	CH_2-3-ClC_6H_4	74	118–120	$C_{13}H_{17}N_6Cl$	C, H, N, Cl
9	CH_2-2,6-$Cl_2C_6H_3$	50	171–173	$C_{13}H_{16}N_6Cl_2$	C, H, N, Cl
10	CH_2-3,4-$Cl_2C_6H_3$	77	163–164	$C_{13}H_{16}N_6Cl_2 \cdot 0.5C_2H_5OH$	C, H, N, Cl
11	CH_2-2-$CH_3C_6H_4$	95	169–170	$C_{14}H_{20}N_6$	C, H, N
12	CH_2-3-$CH_3C_6H_4$	80	191–193	$C_{14}H_{20}N_6 \cdot 0.25H_2O$	C, H, N
13	CH_2-4-$CH_3C_6H_4$	53	153–154	$C_{14}H_{20}N_6$	C, H, N
14	CH_2-2,5-$(CH_3)_2C_6H_3$	42	165–167	$C_{15}H_{22}N_6$	C, H, N
15	CH_2-2,4,6-$(CH_3)_3C_6H_2$	72	170–172 dec	$C_{16}H_{24}N_6 \cdot 0.5H_2O$	C, H, N
16	CH_2-4-$C(CH_3)_3C_6H_4$	81	174–175	$C_{17}H_{26}N_6 \cdot 0.25H_2O$	C, H, N
17	CH_2-4-*n*-BuC_6H_5	20	147–149	$C_{17}H_{26}N_6$	C, H, N
18	CH_2-3-$CF_3C_6H_4$	60	180–181	$C_{14}H_{17}N_6F_3$	C, H, N, F
19	CH_2-2-FC_6H_4	87	92–95	$C_{13}H_{17}N_6F \cdot 0.75H_2O$	C, H, N, F[a]
20	CH_2-4-FC_6H_4	33	177–179	$C_{13}H_{17}N_6F$	C, H, N, F
21	CH_2-2-Cl-4-FC_6H_3	74	122–128 dec	$C_{13}H_{16}N_6ClF \cdot 0.25H_2O$	C, H, N, Cl, F
22	CH_2-1-naphthyl	79	142–144 dec	$C_{17}H_{20}N_6 \cdot 2HCl \cdot 1.75H_2O$	C, H, N, Cl[b]
23	CH_2-4-C_5H_4N	93	191–193	$C_{12}H_{17}N_7 \cdot 1.25H_2O$	C, H, N[c]
24	CH_2-4-CNC_6H_4	81	213–215	$C_{14}H_{17}N_7$	C, H, N
25	CH_2-2-quinolinyl	91	149–150	$C_{16}H_{19}N_7$	C, H, N
26	CH_2-2-furyl	61	196–197	$C_{11}H_{16}N_6O$	C, H, N
27	CH_2-4-$NH_2C_6H_4$	49	203–205	$C_{13}H_{19}N_7$	C, H, N[d]
28	CH_2-4-$N(CH_3)_2C_6H_4$	76	161–163	$C_{15}H_{23}N_7$	C, H, N
29	CH_2-2,3,4-$(CH_3O)_3C_6H_2$	76	145–147 dec	$C_{16}H_{24}N_6O_3$	C, H, N
30	CH_2-3,4,5-$(CH_3O)_3C_6H_2$	75	223–225	$C_{16}H_{24}N_6O_3$	C, H, N
31	CH_2-3-Br-4-$N(CH_3)_2C_6H_3$	30	189–190	$C_{15}H_{22}N_7Br$	C, H, N, Br
32	CH_2-2-Cl-4-$N(CH_3)_2C_6H_3$	80	180–181	$C_{15}H_{22}N_7Cl$	C, H, N, Cl
33	CH_2-2-$NO_2C_6H_4$	51	106–110	$C_{13}H_{17}N_7O_2$	C, H, N
34	$CH(4\text{-}ClC_6H_4)(C_6H_5)$	73	138–140 dec	$C_{19}H_{21}N_6Cl \cdot 0.5H_2O$	C, H, N, Cl
35	$CH(CH_3)(C_6H_5)$	69	110–114	$C_{14}H_{20}N_6 \cdot 0.25H_2O$	C, H, N
36	CH_3	69	glass	$C_7H_{14}N_6$	C, H, N
38	CH_2-c-C_6H_{11}	24	175–176	$C_{13}H_{24}N_6$	C, H, N
39	CH_2-c-C_5H_9	65	185–186	$C_{12}H_{22}N_6$	C, H, N
40	CH_2-c-C_3H_5	81	141–142	$C_{10}H_{18}N_6$	C, H, N
41	$CH_2CH_2CH_2N(CH_3)_2$	13	138–141	$C_{11}H_{23}N_7$	C, H, N[e]
42	$CH_2{=}CHC_6H_5$	79	119–121 dec	$C_{15}H_{20}N_6$	C, H, N
43	$CH_2CH_2NHCH\text{-}4\text{-}C_5H_4N$	6	191–193	$C_{14}H_{22}N_8$	C, H, N
44	$CH_2CH_2CH_2OC_6H_5$	77	151–153	$C_{15}H_{22}N_6O$	C, H, N
45	2-C_5H_4N	90	218–220	$C_{11}H_{15}N_7$	C, H, N
46	C_6H_5	72	216–218	$C_{12}H_{16}N_6$	C, H, N
47	2-$CH_3OC_6H_4$	82	190–191	$C_{13}H_{18}N_6O$	C, H, N
48	4-FC_6H_4	33	177–179	$C_{12}H_{15}N_6F$	C, H, N, F
49	2-furoyl	50	167–169	$C_{11}H_{14}N_6O_2$	C, H, N
50	$COCH(CH_3)_2$	36	189–190	$C_{10}H_{18}N_6O$	C, H, N
51	CO-4-$C(CH_3)_3C_6H_4$	78	306–308	$C_{17}H_{24}N_6O$	C, H, N
52	CO-4-$(C_6H_5)C_6H_4$	67	260–262	$C_{19}H_{20}N_6O$	C, H, N
53	$COCH_3$	49	244–246	$C_{18}H_{14}N_6O$	C, H, N

[a] N: calcd, 29.00; found, 29.57. [b] N: calcd, 20.36; found, 19.78. [c] N: calcd, 34.80; found, 35.45. [d] C: calcd, 57.12; found, 56.55. [e] H: calcd, 9.15; found, 8.63.

orally, the major effects of 8 were blocking the vasopressor effects of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), a ganglionic stimulant, and head-up tilting, suggesting that 8 possesses ganglionic blocking activity (Table III). This material did not block the vasopressor response of tyramine, epinephrine, or norepinephrine, suggesting that 8 does not exert neuronal blocking activity or α-adrenoceptor blocking activity. Due to its blocking effects on ganglia and the tilt response, this compound may cause some degree of orthostatic hypotension in a dose-dependent manner.

None of the compounds listed in Table I exhibited diuretic properties.

Experimental Section

Chemistry. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian HA-100 spectrophotometer using tetramethylsilane as an internal standard; typical ^1H NMR shifts are reported. All compounds showed appropriate NMR spectra. Analytical results were within ±0.4% of the theoretical values unless otherwise indicated. Commercially available piperazines were used without further purification.

1-N-Substituted Piperazines. Piperazines not commercially available or previously reported were prepared by reductive alkylations of 1-N-protected piperazines according to the method of Servier[5] followed by deprotection by standard methods. Acylated piperazines were prepared from 1-benzylpiperazines followed by hydrogenolysis of the benzyl protecting group.

1-[3-Bromo-4-(dimethylamino)benzyl]piperazine. A mixture of 13.0 g (61.3 mmol) of 3-bromo-4-(dimethylamino)benzaldehyde and 10 mL (69.5 mmol) of ethyl 1-piperazinecarboxylate was allowed to react at room temperature for 20 h and then treated with 3.5 g of 99% formic acid at 100 °C such as to maintain a gentle evolution of carbon dioxide. After an additional 2 h of reaction, the reaction mixture was hydrolyzed with 5 N sodium hydroxide for 3 h. The product was extracted into chloroform, which was evaporated to give 10 g of a yellow oil, which was purified by distillation: yield 1.8 g (10%) of a pale yellow syrup, bp 164–167 °C (0.05 mm); ^1H NMR ($CDCl_3$) δ 7.45 (s, br, 1 H, ArH), 7.10 (m, 1 H, ArH), 6.84 (m, 1 H, ArH), 3.31 (s, 2 H, benzylic CH_2), 2.80 (t, 4 H, piperazine CH_2), 2.70 (s, 6 H, $N(CH_3)_2$), 2.31 (t, 4 H, piperazine CH_2).

1-[2-Chloro-4-(dimethylamino)benzyl]piperazine. From 36.8 g (0.20 mol) of 2-chloro-4-(dimethylamino)benzaldehyde, 26.2

Table IV. Effects of 8 on the Cardiovascular Response to Various Challenges in Conscious Spontaneously Hypertensive Rats[a]

drug	n	time	challenges in mean arterial blood pressure: ΔmmHg (mean ± SE)							
			tilt	Tyr	Epi+[b]	Epi++[c]	NE	Iso	Angio	ACH	DMPP
vehicle	6	prevehicle	18.5 ± 3.4	33.0 ± 2.7	26.8 ± 2.0	36.0 ± 2.9	44.8 ± 3.0	−53.8 ± 12.2	42.5 ± 3.0	−60.5 ± 7.9	50.8 ± 13.1
1 mL/100 g po	6	postvehicle[d]	17.3 ± 4.1	34.5 ± 1.7	28.8 ± 4.2	37.3 ± 4.8	45.8 ± 1.1	−38.5 ± 3.1	49.0 ± 2.3	−54.5 ± 5.6	52.5 ± 9.8
8	6	predrug	19.3 ± 2.9	35.3 ± 2.6	27.0 ± 2.1	44.0 ± 3.1	47.8 ± 4.2	−49.8 ± 4.8	55.0 ± 3.0	−68.5 ± 3.9	69.2 ± 10.5
50 mg/kg po	6	postdrug[d]	9.3 ± 2.1[e]	34.8 ± 5.0	29.0 ± 2.6	50.7 ± 5.3	63.0 ± 3.2[e]	−46.7 ± 1.8	70.2 ± 2.3[f]	−47.0 ± 3.9[e]	30.3 ± 6.1[e]

[a] Paired Student's t test was used for statistical analyses. [b] Epi+ = 1 μg/kg iv. [c] Epi++ = 2 μg/kg iv. [d] 1 h postdosing. [e] $p < 0.05$. [f] $p < 0.01$.

g (0.23 mol) of *N*-formylpiperazine, and 12 g of 99% formic acid there was obtained, after hydrolysis, 12.5 g (25%) of colorless crystals, mp 54–56 °C. Anal. ($C_{13}H_{20}ClN_3$) C, H, Cl, N.

1-(2-Chloro-4-fluorobenzyl)piperazine. From 25.8 (0.16 mol) of 2-chloro-4-fluorobenzaldehyde, 20.5 g (0.18 mol) of *N*-formylpiperazine, and 8.3 g of 99% formic acid there was obtained, after hydrolysis, 2.0 g (5%) of a pale yellow syrup: bp 125–130 °C (0.05 mm); ^{1}H NMR ($CDCl_3$) δ 7.11 (m, 2 H, ArH), 6.90 (m, 1 H, ArH), 3.62 (s, 2 H, benzylic CH_2), 2.77 and 2.51 (2 m, 4 H each, piperazine CH_2), 1.69 (s, 1 H, NH).

(4-*tert*-Butylbenzoyl)piperazine. From 31.5 g (0.16 mol) of 4-*tert*-butylbenzoyl chloride and 26.4 g (0.15 mol) of 1-benzylpiperazine in 200 mL of tetrahydrofuran containing 25 g of potassium carbonate was obtained, after hydrogenation in 50% aqueous acetic acid and 5% palladium on charcoal, basification, and extraction into chloroform, 30 g (85%) of a nondistillable light amber syrup.

Methyl *N*-Cyano-4-[(2,6-dichlorophenyl)methyl]-1-piperazinecarboximidothioate. A solution of 18.5 g (76 mmol) of 1-(2,6-dichlorobenzyl)piperazine and 11.1 g (76 mol) of 1 was heated at reflux for 6 h in 100 mL of acetonitrile. The product was collected after evaporation and crystallized from ethanol: yield 12.1 g (46%) of colorless needles, mp 154–156 °C; ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 3 H, ArH), 3.82 (m, 6 H, piperazine and benzylic CH_2), 2.78 (s, 3 H, SCH_3), 2.63 (m, 4 H, piperazine CH_2). Anal. ($C_{14}H_{16}Cl_2N_4S$) CHClNS.

1-(5-Amino-4*H*-1,2,4-triazol-3-yl)-4-(*p*-fluorobenzyl)-piperazine (20). A solution of 19.4 g (0.1 mol) of 2-(fluorobenzyl)piperazine and 14.6 g (0.1 mol) of 1 was refluxed in 150 mL of acetonitrile for 20 h, and then 6 mL (0.12 mol) of hydrazine hydrate was added and refluxing continued for an additional 6 h. The solvent was removed and the residue crystallized by triturating with ether: yield 25.3 g (87%) of granular colorless crystals, mp 92–95 °C; ^{1}H NMR ($CDCl_3$) δ 8.50 (d, 2 H, aromatic), 7.30 (d, 2 H, aromatic), 5.08 (s, br, 2 H, NH_2), 3.55 (s, 2 H, benzylic CH_2), 3.34 (m, 4 H, piperazine CH_2), 2.48 (m, 4 H, piperazine CH_2).

Pharmacology. Antihypertensive Activity. The previously reported procedures of Chan et al.[6] were employed to detect antihypertensive activity and required one to three rats per compound to reach a decision. As the size of the test rats population increased, the stringency for achieved posttreatment blood pressure lessened.

This test used 16-week-old male SH rats (Okamoto strain, Taconic Farms, Germantown, NY) dosed orally by gavage with 100 mg/kg (unless otherwise specified) of test compound dispersed in a starch suspension (3% in normal saline) in a dose volume of 2 mL/kg. Rats were then given an oral load of normal saline (25 mL/kg) and placed in individual metabolism cages, and 0–5-h urine output was collected. Urinary sodium and potassium concentrations were determined by flame photometry. Twenty-four hours after the first dose, rats were redosed, but the 25 mL/kg normal saline load was omitted. Mean arterial blood pressure (MABP) and heart rate (HR) were obtained via direct femoral arterial puncture under local anesthesia 4 h after the second dose. The mean arterial blood pressure of the vehicle-treated SH rats was 153 ± 1.0 mmHg (mean ± SE). Compounds were considered active in the antihypertensive screen when blood pressure in one test SHR had been reduced to 116 mmHg or when the average of two test SHR had been reduced to 122 mmHg. Sodium excretion of ≥1.1 mequiv/5 h was required to be considered active in the diuretic screen.

Additional studies were performed with 8 in a standing colony of chronic phase, two-kidney, one-clip Goldblatt renal hypertensive dogs. Control MABP and HR were obtained by transdermal femoral arterial puncture techniques as reported by Chan et al.,[7] and then the compound was given orally in a gelatin capsule or administered intraperitoneally or intravenously in amounts sufficient to deliver 3 mg/kg based on the daily body weight. Further studies with compound 8 were comprised of a single dose of 10 mg/kg administered orally or intraperitoneally, two doses at 3 mg/kg administered orally at 24-h invervals, and two doses

(6) Chan, P. S.; Poorvin, D. *Clin. Exp. Hypertens.* **1978**, *1*, 817.
(7) Chan, P. S.; Cervoni, P.; Ronsberg, M. A.; Accomando, R. C.; Quirk, G. J.; Scully, P. A.; Lipchuck, L. M. *J. Pharmacol. Exp. Ther.* **1983**, *226*, 726.

of 5 mg/kg given intravenously 40 min apart. Arterial blood pressure was taken at 1-, 3-, and 5-h intervals after dosing to assess drug effects. All animals were habituated to the test procedure through their long history of testing.

To investigate the mechanism of antihypertensive action, male SHR of the Okamoto-Aoki strain (Taconic Farms, Germantown, NY) of approximately 350 g body weight were restrained in a supine position with elastic tape (Elastikon, Johnson & Johnson, New Brunswick, NJ). The area at the base of the tail was locally anesthetized by subcutaneous infiltration with 2% procaine. The ventral caudal artery was isolated and a cannula of PE 10 and PE 20 fused tubing was passed into the lower abdominal aorta. The cannula was secured, heparinized (1000 IU/mL), and sealed and the wound closed. A second cannula was introduced into the lateral caudal vein for the intravenous administration of challenge substances. Control responses to the challenges were recorded prior to drug administration, after which each animal received either vehicle (10 mL/kg) or compound under study orally. One hour later, the challenges were repeated. The animals were tilted 75° for 30 s for effect on BP and the challenges administered intravenously. The challenges consisted of epinephrine (Epi) 1 and 2 μg/kg, norepinephrine (NE) 1 μg/kg, isoproterenol (Iso) 1 μg/kg, acetylcholine (ACh) 2 μg/kg, angiotensin II (Angio) 0.2 μg/kg, tyramine (Tyr) 250 μg/kg, and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) 25 μg/kg. All challenges were calculated as the free base. Each challenge concentration was adjusted such that the volume required was 0.5 mL/kg body weight and followed by 0.1 mL of 0.9% sodium chloride to wash any residual agent from the cannula.

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Synthesis and Hypertensive Activity of Neuropeptide Y Fragments and Analogues with Modified N- or C-Termini or D-Substitutions†

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Porcine neuropeptide Y (NPY), NPY fragments, and analogues with D-Xaa^n, Ala^9, D-Ala^9, and Met^{17} substitutions or modifications to the C- or N-termini were synthesized. The synthesis and purification of these peptides was achieved by using routine laboratory strategies and techniques. The ability of these peptides to alter mean arterial pressure (MAP) and heart rate (HR) in conscious rats was monitored for 15 min following intraarterial administration. Potencies and efficacies of these peptides relative to NPY were determined by comparison of dose–response curves. Administration of 40 μg/kg NPY resulted in a rapid, though short-lived, rise in mean arterial pressure from a basal value of 107.0 ± 2.6 to 157 ± 5.5 mmHg (means ± sem, n = 13). The ED_{50} (±SE) for this response was 3.04 ± 0.88 μg/kg. Peptide YY (PYY) elicited a response that was similar in magnitude but with an ED_{50} (±SE, n = 3) of 0.76 ± 0.24 μg/kg while porcine pancreatic polypeptide (pPP) was inactive when tested at 40 μg/kg (n = 4). Relative potencies for [Ac-Tyr^1]NPY, [Ac-D-Tyr^1]NPY, [des-amino-Tyr^1]NPY, and [Me-Tyr^1]NPY ranged from 1.1 to 2.2. Potencies relative to NPY for D-substitutions at positions 2–6 and 8–13 inclusive ranged from 0.1 to 1.0. Analogues with D-substitutions at positions 1–3 exhibited an extended duration of action. Analogues with D-substitutions at positions 33–35 inclusive were inactive at 40 μg/kg, and [D-Tyr^{36}]NPY was 10-fold less potent than NPY, suggesting that the integrity of the C-terminal region is critical to the overall biological action of NPY. This conclusion is supported by studies with C- and N-terminal deletion peptides. NPY_{2-36} showed full intrinsic activity at 40 μg/kg and retains 40% of the hypertensive potency of NPY. There was a sequential decrease in efficacy upon further N-terminal deletion. In contrast to the finding with NPY_{2-36}, modification of the C-terminus either from the native carboxamide to the free carboxylic acid or by deletion of the C-terminal residue resulted in analogues which were inactive at 40 μg/kg. These data indicate that an essentially full-length, C-terminally amidated NPY structure is required for the hypertensive activity observed in conscious rats upon intraarterial administration of NPY and NPY analogues.

Neuropeptide Y (NPY; structure shown below) is a 36 amino acid, C-terminally amidated peptide that was first isolated from porcine brain by Tatemoto et al.[1,2] in 1982 using a chemical method for the detection of peptide amides.[3] NPY has 69% sequence homology with peptide YY (PYY)[4] and 50% homology with porcine pancreatic polypeptide (pPP).[5]

5 10
Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-
15 20
Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr-
25 30
Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-
35
Ile-Thr-Arg-Gln-Arg-Tyr-NH_2

† Abbreviations: The abbreviations for the amino acids are in accord with the recommendations of the IUPAC–IUB Joint Commission on Biochemical nomenclature (*Eur. J. Biochem.* 1984, *138*, 9–37). The symbols represnt the L-isomer except when indicated otherwise. In addition: NPY, neuropeptide tyrosine; PYY, peptide tyrosine tyrosine; pPP, porcine pancreatic polypeptide; MAP, mean arterial pressure; HR, heart rate; Me-Tyr, *N*-methyltyrosine; Ac-Tyr, *N*-acetyltyrosine; des-amino-Tyr, 4-hydroxyphenylpropanoic acid; BOC, *tert*-butoxycarbonyl; MBHA, 4-methylbenzhydrylamine, CM, chloromethyl; Tos, *p*-toluenesulfonyl; OcHx, cyclohexyl ester; 2ClZ, 2-chlorobenzyloxycarbonyl; Bzl, benzyl ester; 2BrZ, 2-bromobenzyloxycarbonyl; DMF, dimethylformamide; TFA, trifluoroacetic acid; EDT, ethanedithiol; TEA, triethylamine; GRF, growth hormone releasing factor; CRF, corticotropin-releasing factor; NE, norepinephrine; HPLC, high-performance liquid chromatography; C_{18}, octadecyl; TEAP, triethylammonium phosphate; PE, polyethylene.

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