Synthesis and Diuretic Activity of Alkyl- and Arylguanidine Analogs of *N,N'*-Dicyclohexyl-4-morpholinecarboxamidine in Rats and Dogs

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Random screening identified *N,N'*-dicyclohexyl-4-morpholinecarboxamidine (U-18177, **1**) as an orally effective nonkaliuretic diuretic in rats. The diuretic profile of **1** and its 1-adamantyl analog (U-37883A, **4**) was confirmed orally in dogs, where they were less potent than standard diuretics but showed furosemide-like natriuresis at ≥100 μmol/kg. However, acute **1** at 61 and 90 μmol/kg iv resulted in lethal cardiac toxicity in dogs. Many analogs of **1** exhibited qualitatively similar diuretic profiles, but none was sufficiently safe to warrant development. Compound **1** also reversed minoxidil's vasodilation in dogs, which led to vascular interaction studies suggesting that analog **4** may block ATP-sensitive K channels. This K channel-blocking mechanism may contribute to the diuretic activity of the series. This is the first report broadly characterizing the diuretic activity of **1** and representative guanidine analogs in rats and dogs and its toxicity and minoxidil-blocking effects in dogs.

Introduction

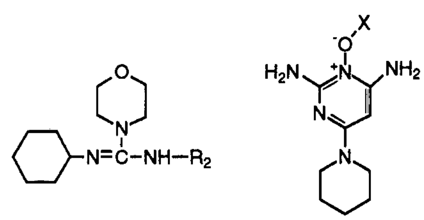
A random screen identified *N,N'*-dicyclohexyl-4-morpholinecarboxamidine (**1**, U-18177; Chart 1) as an orally effective K^+-sparing diuretic in rats. The drug's diuretic profile was confirmed in dogs, but it had a narrow margin between minimum diuretic and lethal doses. An analog program was thus initiated to augment the diuretic potency of the series and eliminate its cardiac toxicity. Toward this goal, over 200 alkyl- and aryl guanidine analogs of **1** were prepared, many of which showed similar or improved diuretic activity in rats. None of these compounds, however, sufficiently improved the margin of safety of **1** to warrant drug development.

During its pharmacologic evaluation, compound **1** was also found to rapidly reverse the vasodilation induced by minoxidil (6-(1-piperidinyl)-2,4–pyrimidinediamine 3-oxide, MNX, **2**) in dogs. When it was later determined that MNX's vasorelaxant metabolite, MNX sulfate (**3**),[1] opens ATP-sensitive K (K_{ATP}) channels,[2] it was reasoned that MNX's reversal by **1** might be due to an interaction at these channels. The 1-adamantyl analog (**4**, U-37883A) was therefore chosen for detailed *in vivo* and *in vitro* vascular interaction studies. Those studies demonstrated that **4** antagonizes the vasodilation induced by chemically dissimilar K channel openers (PCOs),[3] suggesting K_{ATP} channel blockade, an activity which may also be involved in the diuresis of this guanidine series. This paper describes the diuretic activity of **1** and selected guanidine analogs in rats and dogs, the acute toxicity of **1**, and its reversal of MNX's vasodilation in dogs.

Chemistry

The methods used to prepare these guanidine diuretics (Table 1) were based on standard chemical procedures. Thioureas (**5** or **6**) were treated with phosgene to give chloroamidiminium chlorides, which, usually without isolation, were reacted with an amine to give the intended guanidine (Chart 2; procedures A and B). Alternatively, the guanidines were prepared by reacting a carbodiimide (**7**) with an amine. Carbodiimides were obtained commercially (procedure C) or prepared from thioureas (**6**) or ureas (**8**), either by sequential reaction with phosgene and NaOH (procedure D) or by reaction with triphenylphosphine, CCl_4, and triethylamine (procedure E).

Chart 1



1 (R_2 = cyclohexyl) **2** (X = ---)
4 (R_2 = adamantyl) **3** (X = SO_3H)

Pharmacology

Standard Diuretics in Rats. Table 2 summarizes the effects of three standard diuretics orally in rats. Urinary HCO_3^- excretion data has been excluded since it was generally unchanged from control. Hydrochlorothiazide (6-chloro-3,4–dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, HCTZ) was very potent, showing threshold diuresis at 1 μmol/kg. Activity plateaued at 34 μmol/kg with significant kaliuresis. Unlike its extreme potency in dog and man, 30 μmol/kg furosemide (5-(aminosulfonly)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid, FURO) was minimally effective in rats. FURO displayed a very steep dose response, however, yielding 4–5-fold increases in urinary volume (*V*), Na^+, and Cl^- through 302 μmol/kg. Its vigorous diuresis was associated with K^+ loss and an

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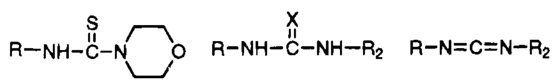
Table 1. Physical and Analytical Data for Alkyl- and Arylguanidines with Diuretic Activity

$$\mathrm{R-N=C(R_1)-NH-R_2}$$

no.	R	R_1	R_2	yield %	procedure	m.p. (°C)	recrystn solvt	formula	analyses
1	c-$CH(CH_2)_5$	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_5$	60.5	A	108–111	$MeOH-H_2O$	$C_{17}H_{31}N_3O$	[a]
9	c-$CH(CH_2)_5$	c-$N(CH_2)_5$	c-$CH(CH_2)_5$	51.0	C	158–160	$EtOH-Et_2O$	$C_{18}H_{33}N_{30}{\cdot}HNO_3$	H,N;C[b]
10	c-$CH(CH_2)_5$	c-$N(CH_2CH_2)_2S$	c-$CH(CH_2)_5$	60.7	C[c]	255–256	EtOAc–MeOH	$C_{17}H_{31}N_3S{\cdot}HCl$	C,H,Cl,N,S
11	c-$CH(CH_2)_5$	c-$N(CH_2CH_2)_2NCOOC_2H_5$	c-$CH(CH_2)_5$	52.4	C	239–241	CH_3CN	$C_{29}H_{36}N_4O_2{\cdot}HCl$	H,Cl,N;O[d]
12	c-$CH(CH_2)_5$	c-$N(CH_2CH_2)_2O$	$(CH_2)_2CH(CH_3)_2$	72.0	A[c]	124–126	$MeOH-Et_2O$	$C_{16}H_{31}N_3O{\cdot}C_2H_2O_4$[e]	C,H,N
13	c-$CH(CH_2)_5$	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_4$	66.7	D	168–170	CH_3CN-Et_2O	$C_{16}H_{29}N_3O{\cdot}HCl$	C,H,Cl,N
4	c-$CH(CH_2)_5$	c-$N(CH_2CH_2)_2O$	c-$C_{10}H_{15}$[f]	67.0	B[c]	235–237	CH_3CN-Et_2O	$C_{21}H_{35}N_3O{\cdot}HCl$	C,H,N
14	Ph	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_5$	66.3	B	78–79	hexane	$C_{17}H_{25}N_3O$	C,H,N
15	2,6-diMePh	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_5$	60.0	B	190–191	CH_3CN-Et_2O	$C_{19}H_{29}N_3O{\cdot}HNO_3$	C,H;N[g]
16	3-CF_3Ph	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_5$	70.0	A	150–152	CH_3CN-Et_2O	$C_{18}H_{24}F_3N_3O{\cdot}HNO_3$	C,H;N[h]
17	2-ClPh	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_5$	66.5	A	261–263	$MeOH-Et_2O$	$C_{17}H_{24}ClN_3O{\cdot}HC_1$	C,H,Cl,N
18	3,4-diClPh	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_5$	42.5	B	191–192	$MeOH-Et_2O$	$C_{17}H_{23}Cl_2N_3O{\cdot}HNO_3$	C,H,Cl,N
19	3,4-diClPhCH_2	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_5$	32.5	B	214–215	$MeOH-Et_2O$	$C_{18}H_{25}Cl_2N_3O{\cdot}C_2H_2O_4$[e]	C,H,Cl,N
20	c-$C_{10}H_{15}$[f]	c-$N(CH_2CH_2)_2O$	c-$C_{10}H_{15}$[f]	21	A	154–157	$(CH_3)_2CHOH$	$C_{25}H_{39}N_3O$	C,H,N
21	c-$CH(CH_2)_6$	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_6$	53.7	D[c]	173–174	CH_3CN-Et_2O	$C_{19}H_{35}N_3O{\cdot}HCl$	C,H,Cl,N
22	c-$CH(CH_2)_7$	c-$N(CH_2CH_2)_2O$	c-$CH(CH_2)_7$	33	E[c]	159–161	$(CH_3)_2CO-Et_2O$	$C_{21}H_{39}N_3O{\cdot}HCl$	C,H,Cl,N
23	4-FPh	c-$N(CH_2CH_2)_2O$	4-FPh	27	E	306–307	CH_3CN	$C_{17}H_{17}F_2N_3O{\cdot}HCl$	C,H,Cl,N
24	CH_2Ph	c-$N(CH_2CH_2)_2O$	CH_2Ph	52	E	93–95	hexane	$C_{19}H_{23}N_3O$	C,H,N

[a] Lit.[17] mp 105–105.5, °C. [b] C: calcd, 60.99; found, 60.11. [c] See the Experimental Section. [d] C: calcd, 59.90; found, 59.47. [e] Oxalic acid salt (1:1). [f] 1-Adamantyl. [g] N: calcd, 14.81; found, 15.24. [h] N: calcd, 13.39; found, 13.91.

Chart 2



5 **6**, X = S **8**, X = O **7**

Table 2. Effects of Standard Diuretics in Saline-Loaded Rats[a]

compd[b]	μmol/kg po	V (mL)	Na^+ (mequiv)	K^+ (mequiv)	Cl^- (mequiv)	Na^+/K^+ ratio
HCTZ	0	4.4	0.82	0.27	0.80	3.0
	1.01	6.8*	1.36*	0.32	1.31*	4.3
	3.40	10.0*	1.79*	0.39*	1.91*	4.6
	10.1	10.2*	1.89*	0.37*	1.97*	5.1*
	34.0	11.3*	2.03*	0.40*	2.17*	5.1*
FURO	0	4.4	0.65	0.24	0.74	2.7
	9.06	4.5	0.58	0.23	0.73	2.6
	30.2	6.9*	0.87*	0.30	1.16*	2.9
	90.6	17.3*	2.24*	0.55*	2.82*	4.1
	302.	20.4*	2.52*	0.52*	3.17*	4.8
AMIL	0	4.8	0.73	0.36	0.78	2.0
	1.13	7.2*	1.33*	0.17*	1.14*	7.8*
	3.76	9.1*	1.65*	0.10*	1.29*	16.2*
	11.3	12.6*	2.17*	0.07*	1.73*	30.8*
	37.6	13.9*	2.27*	0.05*	1.86*	45.2*
	113.	9.4*	1.77*	0.05*	1.43*	38.8*

[a] Values are mean 5 h urinary excretion from three cages of paired rats/dose. [b] HCTZ = hydrochlorothiazide; FURO = furosemide; AMIL = amiloride hydrochloride. *$P \leq 0.05$ from parallel control (by ANOVA).

increased Na^+/K^+ ratio. The K^+ retentive diuretic amiloride (3,5-diamino-*N*-(aminoiminomethyl)-6-chloropyrazinecarboxamine, AMIL) was also very potent, giving threshold diuresis, saluresis, and K^+ retention at 1.13 μmol/kg. Maximal 3–fold increases in V and Na^+ were seen at 37.6 μmol/kg, with an 86% reduction in K^+ excretion and increased Na^+/K^+ ratios. Its peak increases in V and Na^+ excretion moderated somewhat at 113 μmol/kg.

Diuretic Effects of 1 and Analogs in Rats. Table 3 summarizes the effects of 1 and 17 of its most active analogs. While broad oral dose responses were examined for each agent (0.3–100 mg/kg), only data for the three highest (active) doses are shown. Compound **1** typified the series in generally requiring 30 μmol/kg to initiate diuresis and being less natriuretic and kaliuretic than HCTZ. The drug maximally doubled Na^+ excretion at 102 μmol/kg with only a 0.05 mequiv K^+ increase. The diuresis and natriuresis seen with **1** were very sensitive to changes in the tertiary amine (R_1; Table 1). Morpholine was the best substituent at this position; however, analogs with 1-piperidinyl (**9**), 4-thiomorpholinyl (**10**), and 4–acetyl-1-piperazinyl (**11**) substituents retained activity. Other modifications such as 1-pyrrolidinyl, 1-hexamethyleneiminyl, diethylamino, 4-methyl-1-piperazinyl, 4–phenyl-1-piperazinyl, and 4-methyl-1-piperidinyl gave analogs with poor diuretic activity (data not shown). Optimal diuresis in this subgroup was achieved with **10**, which gave 2.5-fold increases in V, Na^+, and Cl^- excretion at 87 μmol/kg with only a 0.07 mequiv increase in K^+.

Unilateral cyclohexyl replacement varied peak diuretic efficacy, as shown by analogs **4** and **12–19**. The 3-methylbutyl (**12**) and cyclopentyl (**13**) derivatives were considerably less natriuretic than **1**. The 1-adamantyl analog (**4**) was somewhat less potent but showed good diuretic and saluretic efficacy with no change in K^+. With an unsubstituted phenyl ring, **14** was less effective and more kaliuretic than **1** at higher doses. The 2,6-dimethylphenyl analog (**15**) improved peak diuresis and saluresis, with modest K^+ loss at intermediate doses. The 3-CF_3-phenyl analog (**16**) was also very effective but consistently kaliuretic, while the *o*-chlorophenyl derivative (**17**) proved less potent and effective than **1**. The 3,4-dichlorophenyl analog (**18**) mirrored the high efficacy of **4**, but with modest kaliuresis. Some activity was lost with the related benzyl derivative (**19**), which caused mild K^+ retention at its highest dose. Within this general subgroup, analogs with poor diuretic activity were obtained when cyclohexyl was replaced by methyl, *tert*-butyl, 3,3,5,5-tetramethylcyclohexyl, 4-methoxyphenyl, or 2-pyridinyl (not shown).

While fewer compounds having variations of both R and R_2 retained the diuretic profile of **1**, **20–24** were typical of the more active analogs. The di-1–adamantyl compound (**20**) had good potency and efficacy and resulted in no K^+ loss, thereby yielding one of the best

Table 3. Diuretic Effects of Alkyl- and Arylguanidines in Saline-Loaded Rats[a]

compd[b]	μmol/kg po	V (mL)	Na^+ (mequiv)	K^+ (mequiv)	Cl^- (mequiv)	Na^+/K^+ ratio
pooled control[c]	0	4.4 ± 0.1	0.71 ± 0.02	0.25 ± 0.01	0.74 ± 0.02	2.9 ± 0.1
1	34.1	6.6*	1.10*	0.31	1.19*	3.6
	102	10.0*	1.56*	0.30	1.65*	5.1*
	341	10.2*	1.73*	0.34*	1.64*	5.0*
9	28.2	6.5*	1.13*	0.29*	1.18*	4.0*
	84.5	7.4*	1.33*	0.35*	1.46*	3.8*
	282	3.7	0.76	0.21	0.77	3.7*
10	28.9	9.1*	1.32*	0.36*	1.49*	3.7
	86.9	11.3*	1.81*	0.32	1.78*	5.7*
	289	9.2*	1.59*	0.27	1.51*	5.5*
11	24.9	5.3	0.87*	0.29	0.98*	3.0
	74.8	8.3*	1.35*	0.33	1.38*	4.1*
	249	10.8*	1.67*	0.42*	1.70*	4.0*
12	27.0	6.6*	0.85	0.31	0.96	2.7
	80.9	8.4*	1.28*	0.33*	1.31*	3.9
	270	5.6	1.12*	0.21	1.01*	5.2*
13	31.6	3.8	0.62	0.28	0.65	2.2
	94.9	6.3*	0.84	0.26	0.92	3.2
	316	8.9*	1.32*	0.32	1.40*	4.1*
4	28.9	5.5	1.00*	0.24	0.97*	4.2*
	86.7	9.9*	1.58*	0.24	1.54*	6.6*
	289	11.3*	1.86*	0.24	1.82*	7.9*
14	34.8	5.6*	0.93	0.27	1.04*	3.4
	105	7.3*	1.02*	0.37*	1.12*	2.8
	348	9.0*	1.37*	0.40*	1.39*	3.4
15	26.5	9.5*	1.44*	0.33*	1.40*	4.4*
	79.4	12.3*	1.71*	0.35*	1.70*	4.8*
	265	17.4*	2.05*	0.29	1.86*	6.1*
16	23.9	6.6*	1.02*	0.39*	1.17*	2.6
	71.8	9.5*	1.46*	0.32*	1.59*	4.6
	239	14.0*	2.02*	0.45*	2.03*	4.5
17	27.9	6.2*	0.72	0.24	0.84	3.0*
	83.8	7.7*	1.01*	0.29	1.06*	3.5*
	279	11.2*	1.45*	0.31*	1.46*	4.7*
18	23.9	7.4*	1.10*	0.27	1.17*	4.1*
	71.6	10.6*	1.41*	0.32*	1.45*	4.4*
	239	13.2*	1.92*	0.33*	1.69*	5.8*
19	21.7	6.4*	0.96*	0.25	1.05*	3.8
	65.2	9.3*	1.55*	0.26	1.44*	5.9*
	217	8.7*	1.59*	0.15*	1.39*	10.9*
20	19.5	10.1*	1.51*	0.23	1.52*	6.6*
	58.4	12.8*	2.04*	0.27	1.97*	7.5*
	195	10.9*	1.90*	0.25	1.66*	7.6*
21	27.9	8.4*	1.21*	0.32	1.37*	3.8
	83.8	9.7*	1.53*	0.31	1.63*	5.0*
	279	9.1*	1.54*	0.28	1.66*	5.6*
22	25.9	8.7*	1.45*	0.26	1.50*	5.5
	77.7	11.8*	1.89*	0.27	1.99*	7.1*
	259	5.0	1.17	0.16	1.05	7.5*
23	28.2	9.2*	1.29*	0.39*	1.32*	3.3*
	84.7	13.6*	1.90*	0.48*	1.82*	4.0*
	282	16.2*	2.05*	0.24	1.70*	8.5
24	32.4	5.4	0.77	0.33	0.86	2.4
	97.1	7.3*	1.13*	0.31	1.14*	3.6*
	324	13.8*	2.25*	0.42*	1.94*	5.4*

[a] Mean 5 h urinary excretion from three cages of paired rats/dose. [b] Test compounds described in Table 1. [c] Pooled control data (mean ± SE) from 15 tests with 18 test compounds (10 control cages/test). *$P \leq 0.05$ from control (by ANOVA).

Na^+/K^+ ratios. The dicycloheptyl and dicyclooctyl analogs (**21** and **22**) resembled **1** in terms of onset, peak activity, and negligible K^+ loss. Most diphenyl derivatives had inferior profiles; however, not shown tabularly, the bis-4-fluorophenyl analog (**23**) was slightly more potent than **1**, inducing threshold diuresis at 8.47 μmol/kg (7.0 mL of urine, 0.84 mequiv of Na^+, and 0.91 mequiv of Cl^-). It also resulted in good peak diuresis and saluresis at its higher doses, with biphasic effects on K^+ excretion. The dibenzyl analog (**24**) was active but kaliuretic at its highest dose. By comparison, compounds with bis-cyclohexylmethyl and bis-4,4-dimethylcyclohexyl had poor diuretic activity (not shown).

Diuretic Effects of 1 and Analogs in Dogs. Compound **1** was first compared to single oral doses of HCTZ and FURO in conscious non-volume-loaded dogs (Table 4). In pilot tests, **1** was well tolerated orally at 10–102 μmol/kg (data for inactive 10 and 17 μmol/kg doses not shown). Compared to pooled placebo control dogs, the drug resulted in significant nonkaliuretic diuresis and saluresis at 34–102 μmol/kg. Its peak 8-fold increase in Na^+ at 102 μmol/kg exceeded a maximally effective dose of HCTZ (10 μmol/kg) but was inferior to 9.6 μmol/kg FURO. To estimate the relative oral absorption of **1**, a follow-up experiment compared its effects at 34 μmol/kg po and iv. This test demonstrated that **1** was about twice as natriuretic iv as when given orally, and equally K^+ sparing.

Based on these data and the subsequently discovered acute toxicity of **1** (as described in a later section),

Table 4. Effects of Standard and Guanidine Diuretics in Conscious Dogs[a]

compd[b]	μmol/kg	n	V (mL)	Na^+ (mequiv)	K^+ (mequiv)	Cl^- (mequiv)	Na^+/K^+ ratio
control[c]	0	36	26 ± 4	2.8 ± 0.7	1.5 ± 0.3	2.3 ± 0.4	2.4 ± 0.7
HCTZ	10.1 po	5	85 ± 7*	13.7 ± 1.5*	3.4 ± 0.6*	15.6 ± 1.1*	4.4 ± 0.7
FURO	9.6 po	5	333 ± 26*	32.7 ± 3.4*	8.9 ± 0.5*	46.5 ± 3.6*	4.4 ± 0.4
1	34.1 po	11	81 ± 12*	10.6 ± 1.8*	1.2 ± 0.2	9.1 ± 1.2*	9.6 ± 1.6*
	50.8 po	3	62 ± 24	11.9 ± 4.7*	1.9 ± 0.4	9.8 ± 4.7	6.1 ± 1.8
	68.1 po	6	91 ± 17*	14.0 ± 2.1*	2.0 ± 0.3	12.3 ± 1.9*	8.6 ± 2.1
	102 po	3	154 ± 47*	22.5 ± 0.7*	2.1 ± 1.0	19.6 ± 2.4*	17.8 ± 7.4
	34.1 po	8	77 ± 11*	9.9 ± 1.9*	1.3 ± 0.2	9.4 ± 1.7*	9.1 ± 2.2*
	34.1 iv	8	132 ± 12*	18.6 ± 1.3*	1.3 ± 0.2	16.4 ± 0.4*	17.4 ± 4.0*
control[c]	0 iv	80	26 ± 4	3.0 ± 0.7	1.3 ± 0.3	1.4 ± 0.4	2.7 ± 0.8
HCTZ	3.40 iv	11	76 ± 7*	16.1 ± 1.6*	3.3 ± 0.5	17.3 ± 1.3*	5.4 ± 0.8
FURO	9.06 iv	5	263 ± 14*	30.8 ± 2.0*	5.4 ± 0.4*	35.2 ± 1.8*	5.8 ± 0.5*
AMIL	3.76 iv	5	59 ± 18*	9.5 ± 2.2*	0.4 ± 0.2*	4.3 ± 1.8*	58.5 ± 22.3
1	3.03 iv	6	64 ± 10*	8.1 ± 1.4	1.2 ± 0.3	5.4 ± 1.4	8.4 ± 1.9
	9.09 iv	6	87 ± 17*	11.5 ± 1.3*	1.7 ± 0.4	9.6 ± 1.8*	8.7 ± 1.9
	30.3 iv	6	114 ± 19*	13.1 ± 2.6*	1.6 ± 0.2	12.5 ± 1.9*	8.6 ± 2.1
4	7.85 iv	6	62 ± 18	8.4 ± 2.7	1.4 ± 0.4	7.0 ± 2.7	8.8 ± 3.6
	26.2 iv	6	197 ± 22*	27.9 ± 2.4*	2.3 ± 0.5	22.4 ± 1.6*	15.3 ± 2.9*
	52.4 iv	4	218 ± 29*	36.0 ± 2.8*	3.3 ± 0.7	28.8 ± 4.2*	12.0 ± 2.3*
20	5.84 iv	5	79 ± 15*	9.2 ± 1.8	1.4 ± 0.4	5.3 ± 1.4*	8.0 ± 1.6
	19.5 iv	5	134 ± 16*	18.2 ± 2.0*	3.0 ± 0.5	13.3 ± 2.3*	6.8 ± 1.5
24	23.5 iv	5	87 ± 7*	8.5 ± 1.5*	0.6 ± 0.2	7.2 ± 1.6*	16.4 ± 5.0*
	70.6 iv	6	121 ± 14*	16.6 ± 2.3*	0.6 ± 0.2	10.9 ± 1.7*	29.5 ± 10.8*
11	2.49 iv	5	51 ± 7*	8.3 ± 1.6*	2.0 ± 0.3	5.4 ± 1.1*	4.8 ± 1.3
15	7.94 iv	5	76 ± 15*	7.8 ± 1.7	1.3 ± 0.3	6.5 ± 1.5*	6.5 ± 1.4*
18	11.9 iv	6	57 ± 22	8.5 ± 3.2	1.1 ± 0.3	5.7 ± 2.6	7.4 ± 1.8
23	8.47 iv	5	58 ± 6*	6.0 ± 1.4	1.1 ± 0.3	5.7 ± 1.0*	5.7 ± 1.0

[a] Mean ± SE 5 h urinary excretion for n dogs/group. [b] HCTZ = hydrochlorothiazide; FURO = furosemide. Test compounds (compd) described in Table 1. [c] Pooled control data. *$P \leq 0.05$ from parallel control dogs (by ANOVA).

further structure–activity investigations with this guanidine series utilized iv administration to dogs. Doses were carefully selected to be safely tolerated (generally ≤50 μmol/kg), as determined in pilot dogs subjected to slowly stepped iv injections. As shown in Table 4, and consistent with their oral profile, a supramaximal dose of HCTZ and an intermediate dose of FURO resulted in significant diuresis and saluresis, with FURO being most kaliuretic. A supramaximal dose of AMIL was less diuretic and natriuretic and significantly antikaliuretic. By comparison, **1** again increased urinary V, Na^+, and Cl^- excretion devoid of excess K^+ loss. The 1-adamantyl derivative (**4**) was more effective than HCTZ, resulting in a FURO-like diuresis and saluresis at its peak 52 μmol/kg dosage. K^+ excretion was higher than that seen with **1** but less than for HCTZ, as reflected in its augmented Na^+/K^+ ratios. The di-1-adamantyl derivative (**20**) was more diuretic, saluretic, and kaliuretic than **1** but less than **4**. The dibenzyl analog (**24**) was safely tolerated up to 71 μmol/kg, a dosage that was also more effective than the maximally tolerated dose of **1**. Compound **24** reduced K^+ excretion slightly and gave the highest Na^+/K^+ ratios of the series. By comparison, compounds **11**, **15**, **18**, and **23** could only be given at maximal doses of 2.5–12 μmol/kg. While relatively poorly tolerated, each modestly increased V, Na^+, and Cl^- excretion independent of K^+.

Oral Dose Response to 1 and Standard Diuretics in Dogs. On the basis of the oral rat and i.v. dog screening, compounds **1** (10.2–102 μmol/kg) and **4** (39–131 μmol/kg) were selected for oral dose–response comparison to standard diuretics. Figure 1 plots the oral diuretic effects of these cycloalkylguanidines, HCTZ (0.34–3.4 μmol/kg), FURO (0.91–9.1 μmol/kg), and AMIL (1.13–11.3 μmol/kg) in conscious non-volume-expanded beagle dogs. FURO resulted in marked dose-dependent increases in V, Na^+, and K^+ excretion. Not shown, Cl^- excretion closely mirrored the effects on Na^+. HCTZ resulted in its typical plateaued dose response, with peak diuresis and natriuresis seen at 1 μmol/kg. AMIL failed to increase urine V but moderately increased Na^+ excretion with K^+ retention. **1** and **4** were substantially less potent, requiring 60-fold higher oral doses to achieve a HCTZ-like natriuresis. Unlike HCTZ, however, the V and Na^+ dose-response curves for **1** and **4** were much steeper, and their highest doses paralleled FURO's high efficacy. K^+ excretion for **1** was absolutely unchanged from control (dashed line) despite a peak 7-fold increase in V and Na^+ excretion. Compound **4** replicated **1** in terms of V and Na^+ excretion, but its highest doses were kaliuretic.

Compounds **1** and **4** also differed from HCTZ and FURO in terms of their time course of action. In Figure 2A, most of the increased Na^+ and K^+ excreted with 1 μmol/kg HCTZ and 3 μmol/kg FURO occurred within the first 2 h, and both standards were significantly kaliuretic at 2–5 h. This profile contrasts with 102 μmol/kg **1**, which was equally natriuretic and nonkaliuretic during both intervals. Examined at 2 h intervals over 6 h, 98 μmol/kg **4** also had a more prolonged time course, with 54% and 36% of its Na^+ excreted at 2–4 and 4–6 h, respectively, with modest K^+ loss. Thus, both guanidines had a slower onset and were less kaliuretic at later intervals than HCTZ and FURO.

Not shown tabularly, 5 h endogenous creatinine and uric acid clearances (Ccr and Cua) were measured in the dogs treated with **1** and the standard diuretics. Ccr, an index of glomerular filtration rate, ranged from 19 ± 1 mL/min for 9.1 μmol/kg FURO to 31 ± 2 mL/min for 102 μmol/kg **1**. These were the only Ccr values significantly different from control dogs (25 ± 1 mL/min). Paralleling the absolute excretion data, fractional Na^+ clearances (CNa^+/%Ccr) were significantly increased with each drug treatment, and fractional K^+ clearances (CK^+/%Ccr) increased with all doses of FURO (by 2.2–9.2-fold) and 1.0 and 3.4 μmol/kg HCTZ (by 1.7–

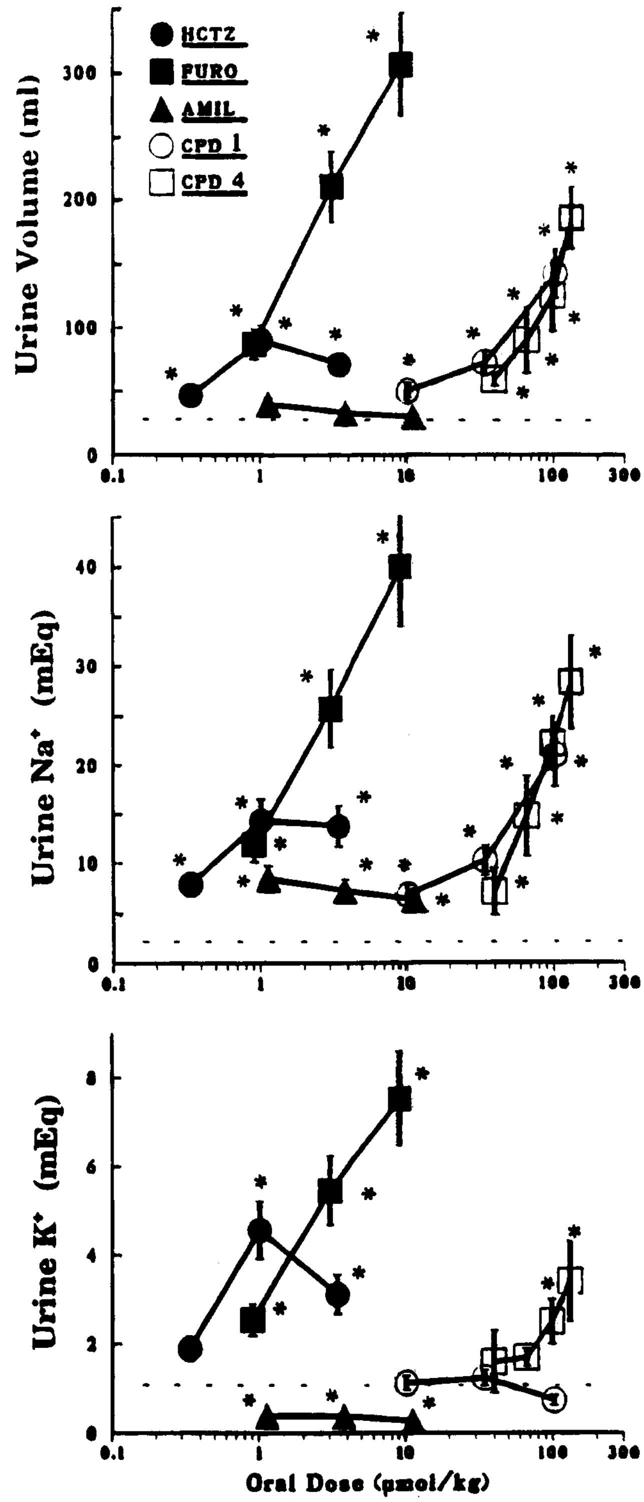


Figure 1. Oral diuretic dose responses to three standard diuretics (HCTZ, closed circles; FURO, closed squares; AMIL, closed triangles) and two guanidine diuretics (**1**, open circles; **4**, open squares) in conscious nonloaded beagle dogs. Values represent mean ± SE 5 h urinary volume (*V*), Na^+, and K^+ excretion for 6 dogs/dose group. Control excretion denoted by dashed line. *$P \le 0.05$ from control.

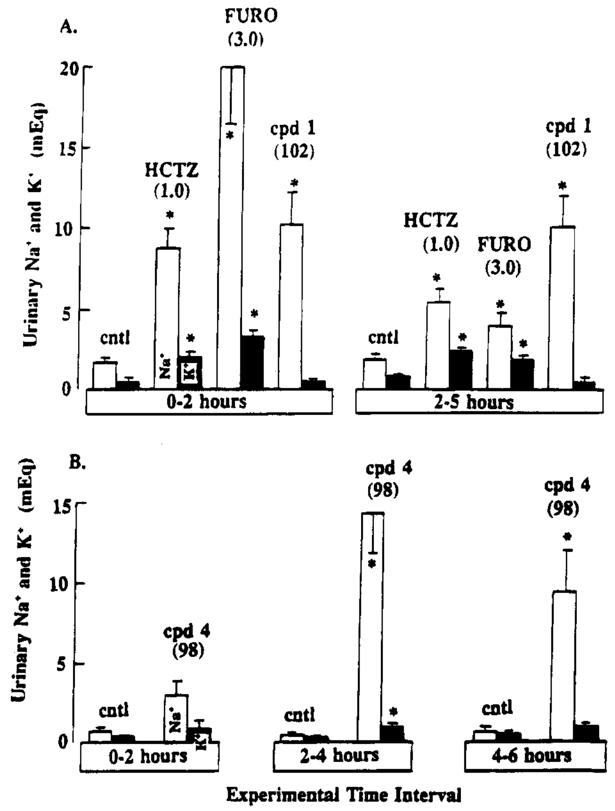


Figure 2. Time course of natriuresis and kaliuresis in conscious nonloaded beagle dogs treated orally with standard diuretics or guanidine diuretics **1** or **4** (6 dogs/dose group). Mean ± SE urinary excretion (mequiv) shown as open bars for Na^+ and closed bars for K^+. Panel A: 0–2 and 2–5 h time course for placebo (cntl), HCTZ (1.0 µmol/kg), FURO (1.0 µmol/kg), or **1** (102 µmol/kg). Panel B: 0–2, 2–4, and 4–6 h time course for placebo (cntl) or **4** (98 µmol/kg). *$P \le 0.05$ from control.

2.9-fold). In contrast, a reduction in CK^+/%Ccr was seen with a high dose of **1** (−47%). While no significant changes were noted in Cua, compound **1** tended to increase and high dose FURO tended to decrease this parameter.

Cardiovascular Effects of 1 in Dogs. During the course of these diuretic studies, pilot tests also evaluated the acute iv effects of **1** on urinary excretion, mean arterial pressure (MAP), heart rate (HR), and the lead II electrocardiogram (ECG) in conscious dogs. Graded 5 min iv infusions of **1** were given at 45 min intervals, thereby resulting in cumulative stepped doses of 3, 9, 21, 30, 61, and 90 µmol/kg over 3.75 h. In the first dog, 3–61 µmol/kg **1** resulted in progressive increases in urine V excretion (from 1 to 6 mL/min) and HR (from 105 to 185 beats/min), with no change in MAP or ECG. After the cumulative 90 µmol/kg dose, however, MAP abruptly fell to <20 mmHg, and despite sustained ECG activity (at 0.5 Hz), the arterial pulse was completely absent. Identical acute diuretic, cardiovascular, and cardiotoxic effects were subsequently seen in a second dog given a cumulative 61 µmol/kg dose of **1**. No gross cardiac pathology was noted in either dog, but significant direct myocardial depression was later confirmed in detailed hemodynamic studies in anesthetized dogs and rats at near diuretic doses of the drug. Thus, although having nonkaliuretic diuretic activity, **1** also proved extremely toxic at acute iv doses of 61 and 90 µmol/kg.

Vascular Interaction between 1 and MNX. Since a primary use of a diuretic developed from this chemical series would have been to prevent the edema incurred with the long-acting vasodilator MNX (**2**),[4] an additional pilot experiment in dogs examined whether 1 could be combined with this potent antihypertensive agent (Table 5). Consistent with earlier reports,[5-7] 4.8 µmol/kg MNX

Table 5. Cardiovascular Interactions between MNX and 1 in Conscious Beagle Dogs

parameter and units[a]	PT[b]	MNX[c]	MNX plus 1[d]
Systemic Cardiovascular Hemodynamics			
mean arterial pressure (MAP; mmHg)	101 ± 5	75 ± 1*	118 ± 5*Δ
heart rate (HR; beats/min)	87 ± 12	138 ± 5*	132 ± 19*
cardiac index (CI; mL/min/kg)	186 ± 23	260 ± 39	138 ± 19Δ
total peripheral resistance (TPR; mmHg/mL/min/kg)	0.56 ± 0.06	0.30 ± 0.05	0.91 ± 0.19Δ
stroke index (SI; mL/beat/kg)	2.2 ± 0.1	1.9 ± 0.2	1.1 ± 0.2*Δ
Tissue Vascular Resistances (unit = mmHg/mL/min/g)			
right atrium	635 ± 95	37 ± 17*	573 ± 87Δ
left ventricle	112 ± 3	10 ± 2*	100 ± 15Δ
large intestine	264 ± 20	116 ± 14*	340 ± 46Δ
pancreas	118 ± 12	43 ± 13*	219 ± 58Δ

[a] Values = mean ± SE of three dogs. [b] PT = pretreatment control. [c] MNX = 3 h after 4.8 μmol/kg MNX po. [d] MNX plus 1 = 45 min after the addition of 34 μmol/kg 1 iv. *$P \leq 0.05$ from PT; Δ = $P \leq 0.05$ from MNX alone (paired *t* test).

reduced MAP and total peripheral resistance (TPR) and increased HR and cardiac index (CI) 3 h after oral dosing. Vascular resistances were significantly reduced in those tissues known to be sensitive to the drug's sustained vasodilation.[6,8]

With MNX's arterial hypotension fully established, the dogs were subsequently given 34 μmol/kg **1** iv, a dose which had been safely tolerated in the diuretic and cardiovascular experiments in non-pretreated dogs. Surprisingly, **1** resulted in the immediate reversal of MNX's hypotension, with MAP actually rising above the pretreatment (PT) level. Despite this abrupt increase in MAP, the MNX-induced tachycardia persisted. At the time of the third hemodynamic determination with radiolabeled microspheres (45 min after compound **1**), MAP was 17 mmHg above PT and left ventricular stroke index (SI) and CI were reduced by 50% and 26%, respectively, from PT. TPR was substantially elevated relative to both PT (+63%) and MNX (+203%) alone. The increase in vascular resistance with **1** was evident in all tissue beds examined. These hemodynamic findings were entirely unexpected since MNX is a very effective, long-acting vasodilator and this type of drug interaction had not been seen with any other diuretics in animals[9,10] or man.[4]

Based on this preliminary test, detailed *in vivo* and *in vitro* vascular interaction studies were subsequently undertaken with **4** in combination with structurally dissimilar vasodilators known to be K_{ATP} channel openers. Those studies demonstrated that **4** is a very potent and specific blocker of PCOs such as MNX, pinacidil, and cromakalim,[3,11] an activity which has also been noted with **1** and many of its guanidine analogs (unpublished observations). Thus, the reversal of MNX by **1** in this pilot test was likely due to the blockade of vascular K_{ATP} channels.

Discussion

We identified **1** as an orally effective nonkaliuretic diuretic in an empiric rat screen. Dog tests confirmed that, while less potent than standard diuretics, **1** and **4** exerted an attractive K^+-sparing diuresis after oral and iv administration. Higher doses of these agents were more effective than HCTZ and resulted in near FURO-like increases in V, Na^+, and Cl^- excretion, which with **1** were independent of changes in Ccr and Cua. Most distinctively, **1** was relatively eukalemic in rats and dogs, proving to be free of the K^+ loss common to HCTZ and FURO and the marked K^+ retention seen with AMIL. Despite its attractive diuretic profile, **1** was also prohibitively toxic in conscious dogs at cumulative iv doses only 6−10 times those producing subthiazide natriuresis. An analog program was thus initiated with the intent of finding a compound which would retain the favored diuretic activity free of cardiac toxicity. Many of the resultant alkyl- and arylguanidines, particularly compounds **4**, **10**, **15**, and **20**, were more effective and equally eukalemic orally in rats. Unfortunately, none of these analogs were sufficiently active in dogs at their maximum safe doses to warrant development.

At the time of these investigations, the only clues as to the mode of action of these substituted guanidines were their acute toxicity and the antagonism to MNX by **1** in dogs. Related to this latter observation, a subsequent study by Wendling *et al.* (1979) found that MNX is likewise rapidly reversed by the antidiuretic peptide arginine vasopressin (ADH)[7] but not by other vasoconstrictors such as norepinephrine and angiotensin II. This finding suggested that, like ADH,[12] **1** may increase intracellular Ca^{2+} concentrations in vascular smooth muscle, thereby antagonizing MNX's vasodilation. Similarly, unregulated increases in intracellular Ca^{2+} in the heart likely would impair cardiac function. The key finding by Meisheri *et al.* (1988) that MNX sulfate facilitates ^{86}RB efflux from vascular smooth muscle,[2] indicative of K^+ channel opening, fueled speculation that the *in vivo* reversal of MNX by **1** was due to some type of K^+ channel blockade. Detailed pharmacologic studies subsequently confirmed that **4** potently and specifically antagonizes the vasodilator effects of chemically dissimilar PCOs *in vivo* and *in vitro*,[3,11] an action it shares with many of its structural analogs (unpublished data). Solidifying this conclusion, recent electrophysiological and binding studies by Guillemare *et al.*[13] in follicle-enclosed *Xenopus* oocytes have further shown that, like glyburide, **4** blocks K_{ATP} channels and reduces K_{ATP} channel opening probability without reducing channel conductance, effects which seem to be mediated by a specific receptor for **4**.

These combined data strongly suggest that substituted guanidines such as **1** and **4** specifically block K_{ATP} channels, and it is possible that the eukalemic diuretic activity seen with this series is mediated by K_{ATP} channel blockade in the renal tubule. Relative to this speculation, Giebisch (1992) has theorized that K_{ATP} channel blockade at the apical membrane of the renal tubule should reduce Na^+ reabsorption and restrict K^+ secretion,[14] thereby producing the kind of electrolyte profile seen with these guanidine diuretics. Consistent with this theory, Clark *et al.* (1993)[15] recently reported that high ip (51−202 μmol/kg) and iv (25−51 μmol/kg) doses of the specific K_{ATP} channel blocker glyburide also result in peak 3–4-fold increases in urinary Na^+ excretion in rats, with little K^+ loss. Follow-up renal clearance studies in this species by Ludens *et al.*[16] have further shown that **4** is about 10 times more potent than glyburide in augmenting Na^+ excretion, devoid of hypoglycemia. By comparison, glyburide is about 10 times more potent than **4** in blocking PCOs in vascular smooth muscle[3] and reduces plasma glucose levels at diuretic doses. Thus, while **1** and its analogs have a narrow margin of safety, they nevertheless represent interesting prototypic eukalemic diuretics which may be useful in exploring the role of K_{ATP} channels in regulating Na^+ reabsorption and K^+ secretion in the renal tubule.

Experimental Section

Reagents were purchased from commercial sources. Melting points (mp) were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. NMR spectra were recorded on a Varian HFT-80 instrument and are consistent with the assigned structures.

Procedure A.[17] *N'*-Cyclohexyl-*N*-isopentyl-4-morpholinecarboxamidine Oxalate (1:1) (12). To a stirred ice cold solution of 5.2 g (0.052 mol) of phosgene (Union Carbide) in 100 mL of dry THF was added 10 g (0.044 mol) of *N*-cyclohexyl-4-morpholinethiocarboxamide. The mixture was kept at ambient temperature for 18 h to give a precipitate of *N*-cyclohexyl-4-morpholinecarboximidoyl chloride hydrochloride. This solid was dissolved in 50 mL of $CHCL_3$ and added during 30 min to a stirred ice cold solution of 20 g (0.23 mol) of isoamylamine in 75 mL of dry THF. The reaction mixture was kept at ambient temperature for 2 h, refluxed for 2 h, and concentrated *in vacuo*. The residue was treated with dilute NaOH and extracted with Et_2O; the extract was concentrated *in vacuo*. The oxalic salt was prepared and crystallized from MeOH–Et_2O to give 11.8 g of **12**.

***N*-(1-Adamantyl)-*N'*-cyclohexylthiourea (25).** To a stirred mixture of 19.3 g (0.100 mol) of 1-adamantyl isothiocyanate in 250 mL of Et_2O, under nitrogen, was added 10 g (0.10 mol) of cyclohexylamine. This mixture was kept for 3 h at ambient temperature. The solid was collected by filtration and washed with Et_2O to give 23.5 g (86.9%) of **25**, mp 178–178.5 °C. The analytical sample was crystallized from EtOH and had a mp of 179–180 °C. Anal. ($C_{17}H_{28}N_2S$) C, H, N.

Procedure B.[17] *N*-(1-Adamantyl)-*N'*-cyclohexyl-4-morpholinecarboxamidine Hydrochloride (4). To a stirred ice cold solution of phosgene (21 g, 0.21 mol) in dry THF was added 50 g (0.185 mol) of **25**. The mixture was stirred at ambient temperature for 18 h and concentrated *in vacuo*. The residue was dissolved in 100 mL of $CHCl_3$ and added, under nitrogen, during 45 min, to a stirred ice cold solution of 50 g (0.57 mol) of morpholine in 300 mL of CH_3CN. The mixture was stirred at 0–10 °C for 30 min, at ambient temperature for 3.5 h, and at reflux for 1.5 h. It was kept at ambient temperature for 18 h and concentrated *in vacuo*. The residue was treated with cold dilute NaOH and extracted with Et_2O–CH_2Cl_2. The organic layer was dried (K_2CO_3) and concentrated *in vacuo*. The residue was dissolved in $(CH_3)_2CO$–Et_2O and acidified with a solution of HCl in Et_2O. The resulting solid was crystallized from CH_3CN–Et_2O to give 43.5 g of **4**.

Procedure C.[18] *N,N'*-Dicyclohexyl-4-thiomorpholinecarboxamidine Hydrochloride (10). A mixture of 4.12 g (0.02 mol) of *N,N'*-dicyclohexylcarbodiimide and 2.06 g (0.02 mol) of thiomorpholine in 20 mL of 2–methyl-2-propanol was refluxed for 18 h. The solvent was removed *in vacuo* and the residue was crystallized from MeOH to give 4.3 g of product, mp 84–85 °C. This was dissolved in $CHCl_3$ and acidified with a solution of HCl in Et_2O. The mixture was concentrated *in vacuo* to give a solid that was crystallized from EtOAc–MeOH (9:1) to afford 4.2 g of **10**.

Procedure D.[17] *N,N'*-Dicycloheptyl-4-morpholinecarboxamidine Hydrochloride (21). To a stirred solution of 5 g (0.05 mol) of phosgene in 50 mL of THF at 10–20 °C was added, during 5 min, 10 g (0.04 mol) of *N,N'*-dicycloheptylurea. The resulting solution was kept at ambient temperature for 3 h and then concentrated *in vacuo*. The residue was dissolved in 25 mL of $CHCl_3$ and added during 20 min to a stirred ice cold solution of 5 g of NaOH in 25 mL of H_2O. After an additional 10 min, the layers were separated and the aqueous layer was extracted with $CHCl_3$; the organic extracts were combined, dried (K_2CO_3), and concentrated *in vacuo*. The residue was distilled to give 5.25 g (56%) of dicycloheptylcarbodiimide, bp 152–157 °C (0.1 kPa). A stirred mixture of 5.2 g (0.022 mol) of dicycloheptylcarbodiimide and 4.0 g (0.046 mol) of morpholine in 10 mL of 2-methyl-2-propanol was refluxed for 4.5 h and concentrated *in vacuo*. The residue was dissolved in Et_2O and acidified with HCl to give the salt, which was recrystallized from CH_3CN–Et_2O to give **21**.

Procedure E.[19] *N,N'*-Dibenzyl-4-morpholinecarboxamidine (24). To a stirred mixture of 25.6 g (0.010 mol) of *N,N'*-dibenzylthiourea and 31.4 g (0.12 mol) of triphenylphosphine in 500 mL of CH_2Cl_2 were added 10.0 g (0.10 mol) of triethylamine and 15.4 g (0.10 mol) of CCl_4. The reaction mixture was warmed on a water bath at 40–45 °C for 3 h, kept at ambient temperature for 90 min, and concentrated *in vacuo*. A stirred solution of the residue in 100 mL of benzene was treated with 75 mL of morpholine (0.86 mol) and refluxed for 18 h. The mixture was concentrated *in vacuo*, and the residue was combined with 300 mL of Et_2O and extracted with 750 mL of 1.5 M HCl. The aqueous extract was made basic with 2 N NaOH. Nitrogen was bubbled through this mixture to give a precipitate which was collected by filtration and crystallized from hexane to give 16.0 g of **24**.

***N'*-Cyclohexyl-*N*-(3,4-dichlorophenyl)thiourea (26).** A mixture of 3,4-dichloroaniline (16.2 g, 0.100 mol) and cyclohexyl isothiocyanate (14.1 g, 0.100 mol) was warmed on a steam bath for 4 h. The resulting solid was recrystallized from EtOH-H_2O to give 18.0 g (59.0%) of **26**, mp 162–165 °C. Anal. ($C_{13}H_{16}Cl_2N_2S$) C, H, N.

***N'*-Cyclohexyl-*N*-[(3,4-dichlorophenyl)methyl]thiourea (27).** A mixture of cyclohexyl isothiocyanate (7.0 g, 0.050 mol) and 3,4-dichlorobenzylamine (9.0 g, 0.051 mol) in Et_2O (75 mL) was allowed to react at ambient temperature. The solvent was evaporated, and the residue was crystallized from Et_2O–hexane to give 12.2 g (77.0%) of **27**, mp 104–106 °C. Anal. ($C_{14}H_{18}O_2N_2S$) C, H, N.

***N*-(1-Adamantyl)-4-morpholinethiocarboxamide (28).** A mixture of 1–adamantyl isothiocyanate (5.0 g, 0.026 mol) and morpholine (2.5 g, 0.029 mol) in Et_2O (250 mL) was kept at ambient temperature for 1 h. The yield of **28** was 6.65 g (91.0%), mp 148–150 °C. Anal. ($C_{15}H_{24}N_2OS$) C, H, N.

Diuretic Activity in Rats.[20] Male Sprague–Dawley rats (160–200 g) were fasted for 16 h and water deprived for 1.5 h prior to administering 25 mL/kg of drug suspension or vehicle (0.5% carboxymethyl cellulose in 0.9% NaCl) by oral gavage. Pairs of identically treated rats were housed in metabolism cages to collect voided urine for 5 h. All test agents were first screened for diuretic activity at 40 mg/kg. Agents increasing the treated/control (T/C) urine V ratio to 1.67, or having a *T/C* 1 x *T/C* 2 value of 3.35 on retest, were declared active and electrolyte profiled at 0.3–100 mg/kg po. For these latter tests, urine samples were retained for Na^+, K^+, Cl^-, and HCO^- analysis to calculate their total electrolyte excretions, *T/C* ratios, and the Na^+/K^+ ratio.

Diuretic Activity in Dogs.[20] Female beagle dogs (9–12 kg) were fasted for 16 h prior to bladder catheterization to eliminate PT urine. Drug was given orally in gelatin capsules or iv in 2.5 mL/kg 0.9% NaCl, and the dogs were housed in metabolism cages to monitor 5 h urinary excretion. The guanidines were solubilized as their acid-derived salts, while HCTZ and FURO were dissolved in 1.5% $NaHCO_3$. At 5 h, the dogs were recatheterized, Vs were recorded, and aliquots were retained for electrolyte analysis. In some tests, urinary and plasma creatinine and uric acid levels were also measured to calculate clearances by the UV/P formula.[21] In those instances, heparinized pre- and posttreatment blood samples were drawn by 21 ga jugular venipuncture.

Hemodynamic Monitoring in Dogs.[6,8] Beagle dogs were anesthetized with 30 mg/kg pentobarbital, and sterile catheters were implanted in the abdominal aorta and a jugular vein to monitor MAP with a Grass polygraph and to inject drug, respectively. Some dogs also were equipped with a left ventricular (LV) cannula to inject 15 μm diameter ^{125}I-, ^{141}Ce-, or ^{85}Sr-radiolabeled tracer microspheres (MS; 3M Co.). The dogs were then allowed 3 days of postsurgical recovery. After an overnight fast, the dogs were placed in slings and equilibrated for 2 h prior to drug administration. For the blood flow tests, three injections of 100 000 MS/kg were given during reference arterial sampling to measure CI and tissue blood flows (ml/min/g) and vascular resistances (MAP/ml/min/g). These dogs were sacrificed after the tests with excess pentobarbital to sample selected tissues for MS radioactivity, as detected with a Packard multichannel γ spectrometer.

Biochemical and Statistical Analyses. Urinary and plasma electrolyte, creatinine, and uric acid concentrations were determined with a Technicon auto analyzer. A computer program calculated mean $\pm$ standard error (SE) excretions and clearances, along with statistical differences from control by analysis of variance (ANOVA). For the MNX interaction test, treatment differences were assessed by paired t test. In all cases, a P value of ≤ 0.05 was deemed statistically significant.

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