1-(2-Aminoethyl)-6-aryl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepines with Diuretic and Natriuretic Activity

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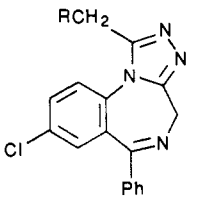
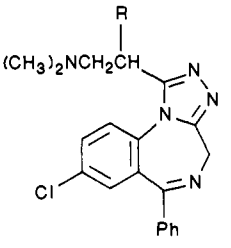
A series of 1-(2-amino-1-phenylethyl)-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepines was prepared and evaluated for diuretic activity. These compounds have diuretic and natriuretic activity but no kaliuretic activity when evaluated by oral administration to the conscious rat. The structure requirements for this activity are discussed. In particular it was found that the 2-aminoethyl side chain at C-1 with hydrogen or methyl substituents on the amino group was required for diuretic activity. A substituent at C-8 was also required; soft substituents such as methylthio and iodo at this position favored activity. Compounds with both phenyl and 2-pyridyl substituents at C-6 were active; substituents on the C-6 phenyl, however, reduced or eliminated the activity. Substituents other than phenyl at the 1-position of the 2-aminoethyl side chain were detrimental to activity; phenyl substitution at this position was required for activity when the substituent at C-8 was chloro but not when it was bromo.

Our investigation of the scope of the acyl-catalyzed Mannich reaction of **1** with dimethylmethyleneammonium chloride to give **2**[1] led to the preparation of several new β-substituted 1-[2-(dimethylamino)ethyl]-8-chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepines which were subjected to random screening. Surprisingly the β-phenyl substituted derivative (**3**) was found to have



1

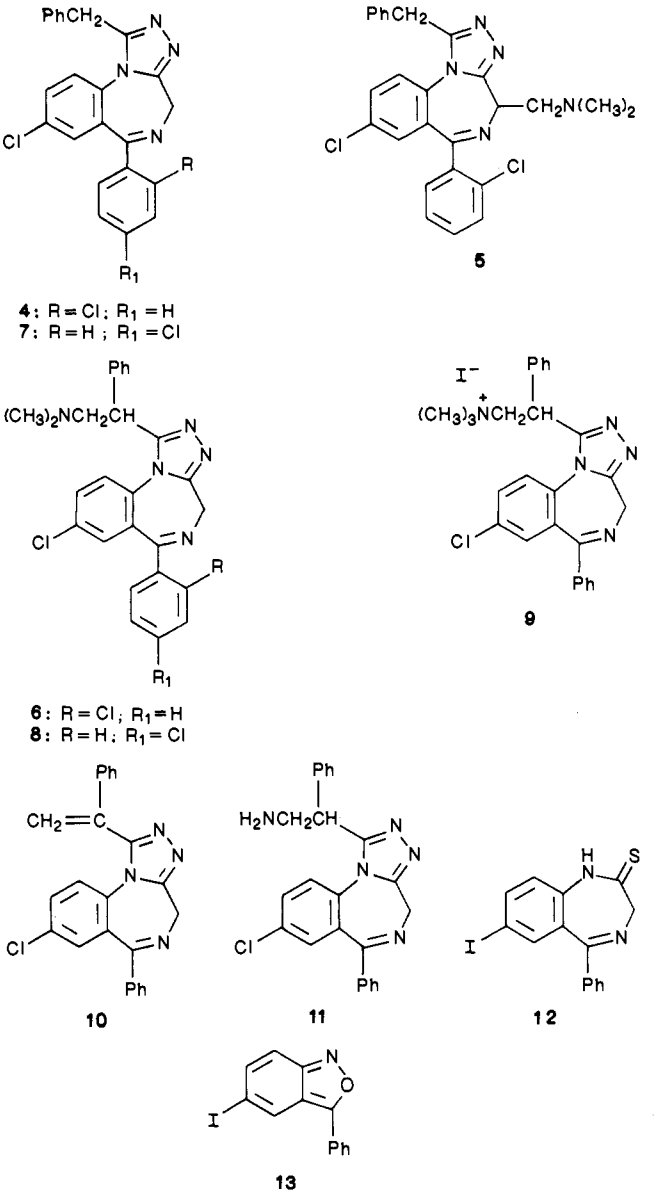


2
3: R = Ph

diuretic and natriuretic activity but no kaliuretic activity after oral administration in animal test systems. This type of activity is of particular interest since among the currently prescribed agents both the loop diuretics and the thiazide-like diuretics cause kaliuresis and are often associated with clinically significant hypokalemia. A compound with useful diuretic and natriuretic activity that did not cause excess potassium excretion would thus be desirable and could have an important place in the treatment of hypertension and congestive heart failure. In addition we were intrigued by the possibility that the activity of this particular lead might be due to an interaction with the high-affinity renal [^{3}H]flunitrazepam binding sites that had recently been investigated.[2] We thus designed a series of 1-(2-amino-1-phenylethyl)-6-aryl-4*H*-[1,2,4]triazolo-[4,3-*a*][1,4]benzodiazepines to study the structural requirements for this type of activity.

Two synthetic methods were used to prepare the members of this series, which are presented in Table I. The *N*,*N*-dimethyl derivatives (Table I, R_1 = $(CH_3)_2N$) were prepared by the reaction of the appropriate 6-phenyl-1-(phenylmethyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (Table II) with *N*,*N*,*N*′,*N*′-tetramethyldiaminomethane and excess acetyl chloride (procedure A).[1] In general the 1-[2-(dimethylamino)ethyl] derivatives listed in Table I were the only products isolated from this reaction. However, when the C-6 phenyl carried an *o*-chloro substituent (see **4**, Chart I), this reaction gave 27% of the 4-substituted product (**5**) in addition to a 41% yield of the

Chart I



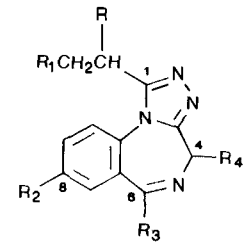
4: R = Cl; R_1 = H
7: R = H; R_1 = Cl
5
6: R = Cl; R_1 = H
8: R = H; R_1 = Cl
9
10
11
12
13

expected product (**6**). This is especially interesting in view of the fact that the corresponding *p*-chloro derivative **7** gave **8**, the only isolated product, in 83% yield. The nature of this effect is not known. Compounds with amino substituents other than *N*,*N*-dimethyl were prepared by the reaction of the quaternary ammonium salt (**9**) with the appropriate amine at elevated temperatures (procedure B).

(1) Hester, J. B., Jr. *J. Org. Chem.* **1979**, *44*, 4165.
(2) Regan, J. W.; Yamamura, H. I.; Yamada, S.; Roeske, W. R. *Life Sci.* **1981**, *28*, 991.

0022-2623/89/1832-1157$01.50/0

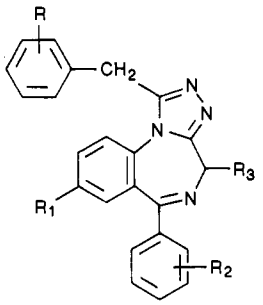
Table I. Physical and Analytical Data for the 1-(2-Aminoethyl)-6-aryl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepines and Diuretic Screening Data



no.	R	R_1	R_2	R_3	R_4	yield, %	procedure	mp, °C	recrystn solvent	formula	analyses	dose[r]	T/C 1[i]	T/C 1.2[i]
3	Ph	$(CH_3)_2N$	Cl	Ph	H	*a*						90.5	2.10	5.27
6	Ph	$(CH_3)_2N$	Cl	2-ClPh	H	40.9[f]	A	122–128[b]	Et_2O–pentane	$C_{26}H_{23}Cl_2N_5$	C,H,Cl,N	84.0	1.87	2.66
8	Ph	$(CH_3)_2N$	Cl	4-ClPh	H	83.1	A	118.5[b]	CH_2Cl_2-Et_2O	$C_{26}H_{23}Cl_2N_5$	C,H,Cl,N	84.0	1.49	
10												100.8	0.76	
11	Ph	H_2N	Cl	Ph	H	34.4	C[f]	270–273	CH_3CN	$C_{24}H_{20}ClN_5$·HI	C,H,Cl,N	73.8	2.53	5.92
14												103.9	0.96	
15	Ph	c-$O(CH_2CH_2)_2N$	Cl	Ph	H	51.4	B[f]	120–121	MeOH–H_2O	$C_{28}H_{26}ClN_5O$·CH_3OH	C,H,Cl,N[p]	77.5	1.46	
16	Ph	c-C_4H_8N	Cl	Ph	H	54.5	B[j]	127–130[b]	MeOH	$C_{28}H_{26}ClN_5$	C,H,Cl,N[k]	80.0	1.33	
17	Ph	Ph-c-$N(CH_2CH_2)_2N$	Cl	Ph	H	44.9	B[l]	213–214[b]	CH_3CN	$C_{34}H_{31}ClN_6$·$(CO_2H)_2$	N,Cl,N;C[c]	61.6	0.95	
18	Ph	CH_3-c-$N(CH_2CH_2)_2N$	Cl	Ph	H	56.5	B[m]	227–231	95% EtOH	$C_{29}H_{29}ClN_6$·2HI	C,H,N[n]	61.3	1.02	
19	H	$(CH_3)_2N$	Cl	Ph	H	*h*						55.2	1.66	3.04
20	CH_3	$(CH_3)_2N$	Cl	Ph	H	*a*						105.3	1.25	
21	OCH_3	$(CH_3)_2N$	Cl	Ph	H	*a*						92.5	1.32	
22	$CH_2N(CH_3)_2$	$(CH_3)_2N$	Cl	Ph	H	*a*						94.6	1.22	
23	H	$(CH_3)_2N$	Br	Ph	H	*h*						68.7	2.98	7.84
24	H	$(CH_3)_2N$	Br	2-Py[q]	H	*h*						97.3	1.75	3.46
25	Ph	$(CH_3)_2N$	Br	Ph	H	51.2	A	180.5–181.5	EtOAc	$C_{26}H_{24}BrN_5$	C,H,Br,N	82.2	2.17	4.73
26	Ph	$(CH_3)_2N$	H	Ph	H	34.2	A	226	EtOH–EtOAc	$C_{26}H_{25}N_5$·HCl	C,H,Cl,N	90.1	1.48	
27	Ph	$(CH_3)_2N$	CH_3S	Ph	H	61.6	A	183–185	EtOAc	$C_{27}H_{27}N_5S$	C,H,N,S	88.2	2.29	6.60
28	Ph	$(CH_3)_2N$	CH_3SO_2	Ph	H	72.4	A	185–189.5	CH_2Cl_2–EtOAc	$C_{27}H_{27}N_5O_2S$	*e*	82.4	0.70	
29	Ph	$(CH_3)_2N$	I	Ph	H	59.0	A	194–196	EtOAc–hexane	$C_{26}H_{24}IN_5$	C,H,I,N	75.0	3.26	9.39
30	Ph	$(CH_3)_2N$	Cl	3-CH_3OPh	H	14.4	A	203–206	Et_2O–pentane	$C_{27}H_{26}ClN_5O$	H,Cl,N;C[d]	84.7	1.34	
31	3-CH_3OPh	$(CH_3)_2N$	Cl	Ph	H	24.7	A	139–140	Et_2O–pentane	$C_{27}H_{26}ClN_5O$	C,H,Cl,N	84.7	3.18	7.31
32	4-CH_3OPh	$(CH_3)_2N$	Cl	Ph	H	69.5	A	136–142	EtOAc–hexane	$C_{27}H_{26}ClN_5O$	C,H,Cl,N	84.7	2.77	5.57
33	2-ClPh	$(CH_3)_2N$	Cl	Ph	H	31.7	A	120–123	Et_2O–pentane	$C_{26}H_{23}Cl_2N_5$	H,Cl,N;C[o]	84.0	1.67	3.77
34	4-ClPh	$(CH_3)_2N$	Cl	Ph	H	30.0	A	165–167	Et_2O–pentane	$C_{26}H_{23}Cl_2N_5$	C,H,Cl,N	84.0	0.9	
35	3-ClPh	$(CH_3)_2N$	Cl	Ph	H	40.1	A	160	Et_2O–pentane	$C_{26}H_{23}Cl_2N_5$	C,H,Cl,N	84.0	1.86	3.20
36	2-CH_3OPh	$(CH_3)_2N$	Cl	Ph	H	72.9	A	194–196	EtOAc–hexane	$C_{27}H_{26}ClN_5O$	C,H,Cl,N[g]	84.7	2.69	5.03
37	Ph	$(CH_3)_2N$	Cl	Ph	CH_3	70.2	A	204–205	EtOAc	$C_{27}H_{26}ClN_5$	C,H,Cl,N	87.7	3.05	7.05
44	Ph	$(CH_3)_2N$	H	2-ClPh	H	21.0	A	178–180	EtOAc	$C_{26}H_{24}ClN_5$	C,H,Cl,N	90.5	1.62	

[a] Reference 1. [b] Decomposition. [c] C: calcd, 66.63; found, 66.18. [d] C: calcd, 68.71; found, 67.77. [e] EtOAc solvate, calcd for $C_{27}H_{27}N_5O_2S$: C, 66.78; H, 5.61; N, 14.42; S, 6.60. Found: C, 65.47: H, 5.85: N, 13.71; S, 6.37; EtOAc, 5.23. Analytical data recalculated for EtOAc: C, 66.07: H, 5.66; N, 14.47; S, 6.72. [f] See Experimental Section. [g] Sample contained 0.89% H_2O. Analytical data recalculated for H_2O was acceptable. [h] Reference 11. [i] Diuretic activity; using a two-stage test, compounds were administered orally to two groups of two male rats at a dose of 40 mg/kg. Urine was collected for 5 h and the ratio (T/C) of urine volume for treated animals to that for control animals was determined. If the ratio for the first stage (T/C 1) was 1.66 or greater, the test was repeated and if the product (T/C 1.2) of the ratios for the two stages was 3.34 or greater the compound was considered to be an active diuretic; see ref 12. [j] Reaction carried out at 95 °C for 2.25 h; chromatography on silica gel with 0.38% NH_4OH–3.75% MeOH–CH_2Cl_2. [k] Sample contained 7.77% MeOH. Analytical data recalculated for MeOH was acceptable. [l] Reaction carried out at 100 °C for 4.5 h, excess 1-phenylpiperazine was removed by distillation under reduced pressure, and the product was purified by silica gel chromatography with 2.5% MeOH–EtOAc. [m] Reaction carried out at 100 °C for 25 h and excess 1-methylpiperazine was removed by distillation under reduced pressure. [n] Sample contained 0.53% EtOH and 1.91% H_2O. Analytical data recalculated for EtOH and H_2O was acceptable. [o] C: calcd, 65.55; found, 64.85. [p] MeOH solvate, calcd for 1 MeOH: 6.21; found, 6.30. [q] 2-Pyridyl. [r] Screening dose expressed in μmol/kg.

Table II. 1-(Phenylmethyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine Intermediates



no.	R	R_1	R_2	R_3	yield, %	procedure	mp, °C	recrystn solvent	formula	analyses	ref[a]
4	H	Cl	2-Cl	H	71.5	D	213–214	EtOAc–hexane	$C_{23}H_{16}Cl_2N_4$	C,H,Cl,N	*h*
7	H	Cl	4-Cl	H	75.7	D	232–233	EtOAc–hexane	$C_{23}H_{16}Cl_2N_4$	C,H,Cl,N	*j*
14	H	Cl	H	H							*h*
38	H	Cl	3-OCH_3	H	45.7	D[g]	154	EtOAc–hexane	$C_{24}H_{19}ClN_4O$	C,H,Cl,N	*f*
39	3-OCH_3	Cl	H	H	43.9	E[g]	132–135	EtOAc–hexane	$C_{24}H_{19}ClN_4O$	C,H,Cl,N	
40	H	CH_3SO_2	H	H	73.3	*g*	222–224.5	MeOH–EtOAc	$C_{24}H_{20}N_4O_2S$	C,H,N,S	
41	H	CH_3S	H	H	65.6	D	168	EtOAc–hexane	$C_{24}H_{20}N_4S$	C,H,N,S	*h*
45	H	H	H	H	69.8	D	158	EtOAc–hexane	$C_{23}H_{18}N_4$	H,N;C[b]	*h*
46	H	H	2-Cl	H	65.2	D	155	EtOAc–hexane	$C_{23}H_{17}ClN_4$	C,H,Cl,N	*h*
47	4-Cl	Cl	H	H	69.3	E	212.5	EtOAc–hexane	$C_{23}H_{16}Cl_2N_4$	C,H,Cl,N	
48	3-Cl	Cl	H	H	40.7	E	147–148.5	EtOAc–hexane	$C_{23}H_{16}Cl_2N_4$	C,H,Cl,N	
49	2-Cl	Cl	H	H	42.5	E	207–208	EtOAc–hexane	$C_{23}H_{16}Cl_2N_4$	H,Cl,N;C[c]	
50	2-OCH_3	Cl	H	H	73.2	E	193–194	EtOAc–hexane	$C_{24}H_{19}ClN_4O$	C,H,Cl,N	
51	H	Cl	H	CH_3	38.9	D[d]	193–195	EtOAc–hexane	$C_{24}H_{19}ClN_4$	C,H,Cl,N	*i*
52	4-OCH_3	Cl	H	H	54.8	E[g]	183	EtOAc–hexane	$C_{24}H_{19}ClN_4O$	C,H,Cl,N	
53	H	Br	H	H	51.6	D	179–182	EtOAc	$C_{23}H_{17}BrN_4$	C,H,Br,N	*e*
54	H	I	H	H	30.0	D	201–202	EtOAc	$C_{23}H_{17}IN_4$	C,H,I,N	*g*

[a] Literature reference to starting material. [b] C: calcd, 78.83; found, 78.38. [c] C: calcd, 65.88; found, 65.35. [d] The crude product was mixed with acetic acid and refluxed for 2 h to complete the cyclization; see procedure E. [e] Reference 13. [f] Reference 9. [g] See Experimental Section. [h] Reference 3. [i] 7-Chloro-1,3-dihydro-3-methyl-5-phenyl-2*H*-1,4-benzodiazepine-2-thione, mp 246–248 °C was prepared by the reaction of 7-chloro-1,3-dihydro-3-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (ref, 14) with phosphorus pentasulfide in pyridine (ref 15). [j] 7-Chloro-5-(4-chlorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepine-2-thione, mp 253–254 °C was prepared by the reaction of 7-chloro-5-(4-chlorophenyl)-1,3-dihydro-2*H*-[1,4]benzodiazepin-2-one (ref 14) with phosphorus pentasulfide in pyridine.

The elimination product (**10**), a minor byproduct from this reaction, was prepared in 71% yield by the reaction of **9** with 1,5-diazabicyclo[5.4.0]undec-5-ene in warm toluene. Compound **11** was prepared by the reaction of **9** with anhydrous ammonia at 100 °C in a pressure reactor (procedure C).

The 6-phenyl-1-(phenylmethyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine intermediates listed in Table II were prepared either by the reaction of an appropriately substituted 1,3-dihydro-2*H*-1,4-benzodiazepine-2-thione with phenylacetic acid hydrazide in refluxing 1-butanol (procedure D)[3] or by the 1,1′-carbonyldiimidazole mediated acylation of 8-chloro-2-hydrazino-5-phenyl-3*H*-1,4-benzodiazepine with an appropriately substituted phenylacetic acid followed by cyclization with warm acetic acid (procedure E).[4] The intermediate, 1,3-dihydro-7-iodo-5-phenyl-2*H*-1,4-benzodiazepine-2-thione (**12**), was prepared from 5-iodo-3-phenylanthranil (**13**) by standard methods which are detailed in the Experimental Section.[5,6]

Results and Discussion

Results from the two-stage primary screen are presented in Table I. In this screen, all compounds were evaluated after oral administration to conscious rats. It should be noted, therefore, that the reported diuretic activity is dependent not only on differences in chemical structure but also on possible differences in absorption, plasma binding, distribution, biotransformation, excretion, and secondary effects on the kidney such as alterations in renal blood flow and glomerular filtration rate. We found that the aminoethyl side chain of **3** was necessary for activity. Neither the 1-phenylmethyl starting material (**14**) nor the elimination product (**10**) had any diuretic activity. The substituents on the amino group were also important; the N-unsubstituted and the *N,N*-dimethyl derivatives were active while the analogues with cyclic amino substituents were inactive (compare **3** and **11** with **15–18**). The β-aromatic substituent on the side chain (R, Table I) also appeared to be important for activity; the β-unsubstituted derivative (**19**) did not have statistically significant diuretic activity and compounds with other β-substituents were inactive (see **20–22**). It was found, however, that when the substituent at C-8 was bromine (**23**), the β-phenyl substituent was not necessary for significant diuretic activity. This was also true for the 8-bromo-6-(2-pyridyl) derivative (**24**). Further evaluation of substituent effects at C-8 demonstrated that the unsubstituted compound (**26**) was inactive and that soft[7] substituents appeared to favor activity. The methylthio-substituted derivative (**27**), for example, was active while the corresponding methylsulfonyl derivative (**28**) was inactive. The 8-iodo derivative (**29**) appeared to be the most active compound in the series. Substituents on the C-6 phenyl were generally detrimental

(3) Hester, J. B., Jr.; Rudzik, A. D.; Kamdar, B. V. *J. Med. Chem.* **1971**, *14*, 1078.
(4) Hester, J. B., Jr.; VonVoigtlander, P. F. *J. Med. Chem.* **1979**, *22*, 1390.
(5) For a review of 2-aminobenzophenone syntheses, see: Walsh, D. A. *Synthesis* **1980**, 677.
(6) For a review of 1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepine-2-thione syntheses, see: Hester, J. B. In *Antianxiety Agents*; Berger, J. G., Ed.; Wiley-Interscience: New York, 1986; Chapter 3.
(7) Pearson, R. G. *J. Am. Chem. Soc.* **1963**, *85*, 3533.

to activity (compare **30**, **6**, and **8** with **3**) while the effects of substituents on the β-phenyl moiety varied. Active compounds were obtained when the latter moiety carried methoxyl substituents at the 3- and 4-positions or a chloro substituent at the 2-position; the alternate substitution patterns, however, yielded inactive compounds (compare **31**, **32**, and **33** with **34**, **35**, and **36**). Finally, methyl substitution at C-4 was not detrimental to activity (compare **37** with **3**).

Results from secondary testing in which dose–response and electrolyte excretion analyses and comparisons to the standard diuretics hydrochlorothiazide, furosemide, and triamterene were carried out are presented in Table III. The dose–response data revealed that the 30 mg/kg dose provided either maximal or very near maximal activity for this series. Electrolyte excretion analyses revealed that, when compared to standard diuretics, active compounds of this series have little effect on potassium excretion at doses that cause significant diuresis and natriuresis. Hydrochlorothiazide and furosemide increase both sodium and potassium excretion while triamterene decreases potassium excretion at doses causing diuresis and natriuresis. The compounds of this series produce an increase in chloride excretion that parallels the increase in sodium excretion, but unlike triamterene they have no effect on bicarbonate excretion (data not shown). With regard to potency, the most active compounds of this series are considerably less potent than hydrochlorothiazide in the conscious rat. The latter compound has significant diuretic and saliuretic activity at 0.3 mg/kg while the most potent benzodiazepine (**29**) only begins to show significant activity at 10 mg/kg; the diuretic and natriuretic efficacy of the two compounds, however, appears to be similar.

To determine if diuresis associated with this series was mediated directly or indirectly, compound **3** was infused into one renal artery of anesthetized dogs; the second kidney served as a control. As shown in Table IV, urinary flow rate in the infused kidney was significantly greater after infusion of drug and significantly greater than urine flow in the control kidney either before or after infusion. Such a unilateral response implied that **3** had a direct renal effect. In contrast, GFR was not altered significantly by the infusion of **3**, suggesting that the diuresis produced by the drug resulted from a tubular effect. That is, the amount of filtered fluid excreted increased from <1% before infusion to 5% after infusion of the drug. Diuresis produced by **3** was associated with an increase in sodium excretion. In the infused kidney, it increased significantly ($P < 0.05$) from 37 (±13) to 105 (±29) μequiv/min while sodium excretion decreased insignificantly ($P > 0.05$) from 59 (±28) to 43 (±19) μequiv/min in the control kidney.

An investigation of the receptor-binding affinities for several members of this series demonstrated no high-affinity binding for the renal benzodiazepine receptors when tritiated flunitrazepam or RO-54864 was used as ligand.[8] It is thus not likely that this benzodiazepine receptor[2] is responsible for the observed diuretic activity.

More definitive studies designed to elucidate the site and mechanism of action for the lead compound (**3**) will be reported elsewhere.

Experimental Section

Chemistry. Melting points taken in a capillary tube are corrected. The structures of the compounds were supported by IR, UV, NMR, and mass spectra. IR spectra were determined in Nujol with a Digilab Model FTS14D spectrophotometer. UV spectra were determined in 95% EtOH with a Cary Model 14 spectrophotometer. NMR spectra were recorded on a Varian Model A60A or FT-80A spectrometer; chemical shifts were recorded in parts per million downfield from Me_4Si. Mass spectra were obtained on a Varian CH5 or CH7 spectrometer. The analytical results obtained were within ±0.4% of the theoretical values if not otherwise stated. The silica gel used for chromatography was obtained from E. Merck A.G., Darmstadt, Germany. Silica gel GF, 250 μ slides obtained from Analtech, Inc., Newark, DE, were used for TLC.

2-[8-Chloro-6-(4-chlorophenyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-1-yl]-*N*,*N*-dimethyl-2-phenylethanamine (8). Procedure A. A stirred solution of **7** (2.10 g, 0.005 mol) in warm DMF (35 mL) was cooled in an ice bath and treated with *N*,*N*,*N*′,*N*′-tetramethyldiaminomethane (0.819 mL, 0.006 mol) and then dropwise with acetyl chloride (0.5 mL, 0.007 04 mol). The cloudy mixture was kept in the ice bath for 4 h 40 min and poured into a mixture of saturated $NaHCO_3$ and ice. This mixture was extracted with CH_2Cl_2. The extract was washed with dilute NaCl, dried (Na_2SO_4), and concentrated in vacuo with xylene to help remove residual DMF. The residue was chromatographed on silica gel with MeOH. The product thus obtained was dissolved in EtOAc, decolorized with activated carbon, and crystallized from EtOAc–hexane to give 1.80 g, mp 120–123 °C dec, and 0.18 g, mp 122.5–126 °C dec, of **8**. The analytical sample was crystallized from CH_2Cl_2–Et_2O and had mp 118.5 °C dec.

8-Chloro-1-[2-(4-morpholinyl)-1-phenylethyl]-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (15). Procedure B. A stirred mixture of **9** (2.0 g, 0.0034 mol) and morpholine (10 mL) was warmed, under N_2, at 110 °C for 3 h, cooled, and mixed with ice-cold, saturated $NaHCO_3$ (30 mL). The resulting precipitate was collected by filtration, washed with water, dried in vacuo, and chromatographed on silica gel (100 g) with 3.75% MeOH–CH_2Cl_2. The first compound eluted from the column was the elimination product (**10**), which amounted to 0.09 g (6.6%). The second compound eluted from the column amounted to 1.24 g. This was recrystallized from MeOH–H_2O to give 0.91 g of **15**, mp 120–121 °C, which was a MeOH solvate.

2-(8-Chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-1-yl)-2-phenylethanamine Hydriodide (11). Procedure C. A mixture of 4.0 g (6.85 mmol) of **9** and 200 mL of freshly distilled NH_3 was sealed in a pressure reaction vessel and heated in an oil bath at 100 °C for 18 h. The cooled reaction mixture was concentrated under a N_2 stream and the residue was treated with 400 mL of 1:1 EtOAc–CH_2Cl_2. The suspended solid was collected on a filter and recrystallized from CH_3CN to yield 1.28 g of **11**, mp 270–273 °C.

8-Chloro-6-(3-methoxyphenyl)-1-(phenylmethyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (38). Procedure D. A stirred solution of 7-chloro-1,3-dihydro-5-(*m*-methoxyphenyl)-2*H*-1,4-benzodiazepine-2-thione[9] (3.16 g, 0.01 mol), phenyl acetic acid hydrazide (4.51 g, 0.03 mol), and 1-butanol (150 mL) was refluxed for 16 h while N_2 was bubbled through the mixture. The reaction mixture was concentrated under vacuum, and the residue was mixed with iced H_2O. The product was collected by filtration and dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed successively with dilute HCl and brine, dried (Na_2SO_4), and concentrated in vacuo. Crystallization of this material from EtOAc–hexane gave 1.35 g, mp 154 °C, and 0.54 g, mp 150–153 °C, of **38**.

8-Chloro-1-[(3-methoxyphenyl)methyl]-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (39). Procedure E. A mechanically stirred solution of 3-methoxyphenylacetic acid (1.66 g, 0.01 mol) in THF (30 mL), under N_2, was cooled in an ice bath and treated with 1,1′-carbonyldiimidazole (CDI). After 1 h, 7-chloro-2-hydrazino-5-phenyl-3*H*-1,4-benzodiazepine[10] (2.56 g, 0.009 mol) was added and the reaction was allowed to continue at 0 °C for 4–6 h and at ambient temperature for 16 h. The precipitate was collected by filtration, washed with THF, and dried in vacuo. A stirred mixture of this product in acetic acid (50 mL) was refluxed, under N_2, for 2–3 h and then concentrated in vacuo.

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The residue was mixed with CH_2Cl_2 and H_2O. The CH_2Cl_2 solution was washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. This material was crystallized from EtOAc–hexane to give 1.08 g, mp 133–134 °C, and 0.56 g, mp 135–137 °C, of **39**. The analytical sample had mp 132–135 °C.

2-[8-Chloro-6-(2-chlorophenyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-1-yl]-*N*,*N*-dimethyl-2-phenylethanamine (6), 8-Chloro-6-(2-chlorophenyl)-*N*,*N*-dimethyl-1-(phenylmethyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine-4-methanamine Sesquiethanedioate (5). A stirred solution of **4** (2.10 g, 0.005 mol) in warm DMF (25 mL) was cooled in an ice bath, under N_2, and treated with *N*,*N*,*N'*,*N'*-tetramethyldiaminomethane (0.819 mL, 0.006 mol) and then dropwise with acetyl chloride (0.48 mL, 0.0065 mol); a white precipitate formed in the yellow solution. The mixture was kept in the ice bath for 5 h during which time the solid slowly dissolved. It was then mixed with ice and saturated $NaHCO_3$ and extracted with CH_2Cl_2. The extract was washed with dilute NaCl, dried (Na_2SO_4), and concentrated in vacuo with xylene to help remove last traces of DMF. The residue was chromatographed on silica gel with MeOH. The product appeared to be two overlapping spots by TLC with MeOH on silica gel; no separation was obtained. It was crystallized from EtOAc–hexane to give 0.975 g of **6**, mp 110.5–115 °C dec. The analytical sample was recrystallized from Et_2O–petroleum ether and had mp 122–128 °C dec. The filtrates from the crystallization of **6** were acidified to pH 2–3 with an EtOAc solution of oxalic acid. The resulting crystalline salt was recrystallized from MeOH–EtOAc to give 0.817 g (26.7%) of **5**, mp 153–157.5 °C dec. The analytical sample was recrystallized from EtOAc–hexane and had mp 160–161.5 °C dec. Anal. ($C_{26}H_{23}Cl_2N_5 \cdot C_3H_3O_6$) C, H, Cl, N.

2-(8-Chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-1-yl)-*N*,*N*,*N*-trimethyl-2-phenylethanaminium Iodide (9). An ice-cold solution of 10.0 g (0.0226 mol) of **3** in 175 mL of MeOH, under N_2, was treated with 16.90 mL (0.272 mol) of methyl iodide during 18 min. The solution was stirred at ambient temperature for 6.5 h during which time a solid crystallized. The solid was collected on a filter, washed with a small amount of MeOH, and dried to yield 9.82 g (68.1%) of **9**, mp 216–217 °C dec. The combined filtrates were concentrated in vacuo and crystallized from MeOH to yield 3.76 g (26%), mp 212–215 °C dec, of additional product. The analytical sample was recrystallized from MeOH and had mp 217–218 °C. Anal. Calcd for $C_{27}H_{27}ClIN_5 \cdot 2CH_4O$: C, 53.75; H, 5.44; N, 10.80; CH_3OH, 9.89. Found: C, 54.06; H, 5.00; N, 11.61; CH_3OH, 9.03.

8-Chloro-6-phenyl-1-(1-phenylethenyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (10). A stirred mixture of 0.50 g (0.856 mmol) of **9**, 0.30 mL (0.30 g, 1.99 mmol) of 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU), and 5 mL of toluene was heated under N_2 in an oil bath at 85 °C for 2 h and allowed to cool. The reaction mixture was diluted with EtOAc, washed successively with 2 mL of 1 N HCl, H_2O, aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was recrystallized from EtOAc to yield 0.24 g (70.7%) of **10**, mp 217–219.5 °C. The analytical sample had mp 218–219.5 °C. Anal. ($C_{24}H_{17}ClN_4$) C, H, Cl, N.

8-(Methylsulfonyl)-6-phenyl-1-(phenylmethyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (40). A stirred solution of **41** (0.793 g, 0.002 mol) in CH_2Cl_2 (9 mL) was cooled in an ice bath, under N_2, and treated dropwise during 45 min with a solution of *m*-chloroperbenzoic acid (0.893 g of 80–90% pure material) in CH_2Cl_2 (14 mL). The mixture was allowed to warm to ambient temperature during 1 h and stand for an additional 7 h. It was then diluted with CH_2Cl_2, washed successively with saturated $NaHCO_3$ and dilute NaCl, dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica gel with 2% MeOH–$CHCl_3$. The product thus obtained was crystallized from MeOH–EtOAc to give 0.628 g of **40**, mp 221–223.5 °C.

5-Iodo-3-phenylanthranil (13). A mechanically stirred solution of NaOH (178.8 g, 4.47 mol) in MeOH (650 mL) was cooled to 18 °C, under N_2, and treated during 1 h with a solution of *p*-iodonitrobenzene (70 g, 0.281 mol) in THF (600 mL). This addition was followed by the addition of phenylacetonitrile (34.5 g, 0.295 mol) during 30 min. Both additions were made in a dropwise fashion at a rate so as to maintain the temperature between 20 and 25 °C. Stirring was continued at 20–25 °C for 1 h. Ice water (3.6 L) was added and the stirring continued in an ice bath for 30 min. The resulting suspension was filtered and the solid was washed with water until neutral, slurried in methanol, filtered, and dried in vacuo to yield 67.8 g (75.2%) of **13**, mp 114–115 °C. The analytical sample was recrystallized from $CHCl_3$–CH_3OH and had mp 118–120 °C. Anal. Calcd for $C_{13}H_8NIO$: C, 48.62; H, 2.51; N, 4.36; I, 39.52. Found: C, 48.11; H, 2.48; N, 4.26; I, 39.15.

2-Amino-5-iodobenzophenone (42). A mechanically stirred mixture of **13** (85 g, 0.265 mol) and acetic acid (850 mL) was heated to 55 °C, under N_2. A total of 29.7 g (0.521 mol) of iron powder and 320 mL of water were added portionwise at 15-min intervals during 2 h. After an additional 30 min the reaction mixture was poured into ice water, stirred (20 min), and filtered. The solid was washed with CH_2Cl_2 and the filtrate was extracted with CH_2Cl_2. The combined CH_2Cl_2 solution was washed with dilute NaOH and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was crystallized from MeOH to yield 60.4 g (70.6%) of **42**, mp 110–112 °C. The analytical sample was recrystallized from EtOH and had mp 111–112 °C. Anal. ($C_{13}H_{10}NIO$) C, H, I, N.

1,3-Dihydro-7-iodo-5-phenyl-2*H*-1,4-benzodiazepin-2-one (43). Bromoacetyl bromide (50.1 g, 0.248 mol) was added dropwise to a stirred solution of **42** (40.0 g, 0.124 mol) in toluene (800 mL), under N_2. After the addition was complete, the reaction mixture was heated at 80 °C for 4 h. The mixture was allowed to cool to ambient temperature, treated with 20% aqueous NaOH (500 mL), and stirred for 15 min. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined, washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was crystallized from MeOH to yield 47.9 g (87%) of the α-bromoacetamide, mp 124–126 °C.

Ammonia (2150 mL) was condensed into a flask fitted with a cold finger condenser and a mechanical stirrer with the reaction vessel immersed in a dry ice–acetone bath. The α-bromoacetamide (40 g, 0.09 mol) obtained in the previous reaction was added and the dry ice bath removed, allowing the reaction mixture to reflux. After 2 h the ammonia was allowed to evaporate under a slow stream of N_2. The residue was combined with CH_2Cl_2 (450 mL) and H_2O (250 mL) and stirred for 15 min. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was combined with EtOH (500 mL) and refluxed with portions of EtOH being removed by distillation and replaced with fresh EtOH periodically during the reaction. After 2.5 h the reaction mixture was concentrated and crystallized to yield 26.3 g (80.7%) of **43**, mp 220–225 °C. The analytical sample was recrystallized from EtOH and had mp 223–225 °C. Anal. ($C_{15}H_{11}IN_2O$) C, H, I, N.

1,3-Dihydro-7-iodo-5-phenyl-2*H*-1,4-benzodiazepine-2-thione (12). A stirred mixture of phosphorus pentasulfide (1.2 g, 0.0054 mol) and pyridine (50 mL) was placed in a preheated oil bath (130 °C), under N_2. After the phosphorus pentasulfide had dissolved, **43** (2.0 g, 0.0055 mol) was added, and the mixture was quickly heated to reflux. After refluxing 45 min, the reaction mixture was cooled in an ice bath and concentrated in vacuo. The residue was combined with a small amount of CH_2Cl_2 and ice-cold aqueous $NaHCO_3$ (50 mL) and stirred in an ice bath for 45 min. The resulting suspension was filtered and the solid was washed with CH_2Cl_2 and H_2O and dried in vacuo to yield 1.53 g of **12**, mp 248–250 °C, which was sufficiently pure for subsequent reactions.

Biology. Diuretic Screen. Diuretic activity was assessed by using two tests: a two-stage primary test[16] in which urinary volume only was measured to declare agents active or inactive and a

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Table III. Dose–Response Relationships for Effects of Selected 1-(2-Aminoethyl)-6-aryl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepines on Urine Volume and Na^+, K^+, and Cl^- Excretion in Conscious Rats

no.	urinary excretion	dose, mg/kg[a]						
		0[f]	0.3	1.0	3.0	10	30	100
3	dose[j]	0	0.679	2.26	6.79	22.6	67.9	226
	V[b]	4.35	4.15	4.47	5.36	6.70[e]	12.00[e]	9.54[e]
	Na^+[c]	0.66	0.74	0.81	0.82	1.15[e]	1.80[e]	1.51[e]
	K^+[c]	0.27	0.25	0.25	0.22	0.22[e]	0.27	0.27
	Cl^-[c]	0.72	0.73	0.81	0.89[e]	1.16[e]	1.72[e]	1.26[e]
	Na^+/K^+[d]	2.46	2.91	3.25[e]	3.69[e]	5.32[e]	6.74[e]	5.57[e]
11	dose[j]	0	0.554	1.85	5.54	18.5	55.4	185
	V	4.09	4.34	4.45	3.92	6.21[e]	10.47[e]	8.43[e]
	Na^+	0.68	0.77	0.76	0.69	1.08[e]	2.01[e]	1.63[e]
	K^+	0.26	0.28	0.32	0.22	0.27	0.28	0.27
	Cl^-	0.68	0.81	0.79	0.73	1.09[e]	1.72[e]	1.57[e]
	Na^+/K^+	2.66	2.74	2.39	3.10	3.96[e]	7.30[e]	5.97[e]
23	dose[j]	0	0.515	1.72	5.15	17.2	51.5	172
	V	4.17	3.99	4.38	5.97	5.90	8.44[e]	13.00[e]
	Na^+	0.55	0.66	0.65	0.78	0.68	1.00[e]	1.57[e]
	K^+	0.25	0.25	0.23	0.23	0.21	0.16[e]	0.27
	Cl^-	0.61	0.66	0.65	0.82	0.74	0.94[e]	1.42[e]
	Na^+/K^+	2.24	2.67	2.81	3.43[e]	3.22[e]	6.21[e]	5.87[e]
24	dose[j]	0	0.729	2.43	7.29	24.3	72.9	243
	V	4.15	5.94[e]	4.69	5.53[e]	5.63[e]	8.05[e]	11.02[e]
	Na^+	0.71	0.74	0.60	0.79	0.77	1.03[e]	1.26[e]
	K^+	0.33	0.38	0.32	0.39	0.33	0.37	0.41
	Cl^-	0.73	0.81	0.70	0.84	0.81	1.05[e]	1.21[e]
	Na^+/K^+	2.13	1.92	1.85	2.02	2.34	2.77[e]	3.05[e]
25	dose[j]	0	0.617	2.06	6.17	20.6	61.7	206
	V	3.79	4.29	3.59	3.40	5.10	8.50[e]	7.74[e]
	Na^+	0.56	0.63	0.64	0.53	0.74	1.18[e]	1.16[e]
	K^+	0.21	0.24	0.21	0.17	0.17	0.17	0.15[e]
	Cl^-	0.61	0.72	0.62	0.60	0.78	1.16[e]	1.01[e]
	Na^+/K^+	2.67	2.67	2.99	3.13	4.24[e]	7.07[e]	7.85[e]
27	dose[j]	0	0.661	2.20	6.61	22.0	66.1	220
	V	4.26	3.92	5.12	5.20	6.83[e]	10.39[e]	14.14[e]
	Na^+	0.76	0.88	0.94	0.91	1.39[e]	1.87[e]	2.52[e]
	K^+	0.31	0.31	0.31	0.36	0.23[e]	0.25	0.28
	Cl^-	0.76	0.76	0.89	0.90	1.28[e]	1.69[e]	2.17[e]
	Na^+/K^+	2.46	2.87	3.07	2.53	6.04[e]	7.60[e]	9.10[e]
29	dose[j]	0	0.562	1.87	5.62	18.7	56.2	187
	V	5.16	5.09	4.80	4.86	10.80[e]	12.90[e]	14.57[e]
	Na^+	0.69	0.75	0.65	0.66	1.64[e]	1.98[e]	2.09[e]
	K^+	0.23	0.18	0.25	0.23	0.19	0.24	0.24
	Cl^-	0.73	0.80	0.70	0.76	1.50[e]	1.73[e]	1.82[e]
	Na^+/K^+	2.99	4.14[e]	2.58	2.89	8.59[e]	8.29[e]	8.76[e]
31	dose[j]	0	0.636	2.12	6.36	21.2	63.6	212
	V	4.03	3.37	3.74	4.29	5.99[e]	12.21[e]	12.03[e]
	Na^+	0.82	0.52[e]	0.64	0.79	1.13	1.99[e]	1.76[e]
	K^+	0.20	0.22	0.21	0.17	0.17	0.24	0.30
	Cl^-	0.80	0.59	0.72	0.78	1.11	1.99[e]	1.80[e]
	Na^+/K^+	4.06	2.37[e]	3.04	4.68	6.81[e]	8.29[e]	5.92
32	dose[j]	0	0.636	2.12	6.36	21.2	63.6	212
	V	5.16	4.13[e]	4.81	4.98	5.74	10.47[e]	12.25[e]
	Na^+	0.69	0.67	0.66	0.68	0.93[e]	1.58[e]	1.91[e]
	K^+	0.23	0.23	0.25	0.23	0.19[e]	0.18[e]	0.26
	Cl^-	0.73	0.71	0.71	0.75	0.93[e]	1.55[e]	1.65[e]
	Na^+/K^+	2.99	2.89	2.63	2.98	5.03[e]	8.71[e]	7.49[e]
33	dose[j]	0	0.630	2.10	6.30	21.0	63.0	210
	V	3.72	5.36[e]	4.54	4.67	5.37[e]	6.73[e]	9.85[e]
	Na^+	0.71	1.00[e]	0.93[e]	0.81	1.03[e]	1.22[e]	1.76[e]
	K^+	0.19	0.25	0.24	0.22	0.21	0.16	0.22
	Cl^-	0.70	1.08[e]	0.93[e]	0.84	1.13[e]	1.21[e]	1.69[e]
	Na^+/K^+	3.74	4.04	3.84	3.63	5.12	7.84[e]	7.80[e]
36	dose[j]	0	0.636	2.12	6.36	21.2	63.6	212
	V	5.16	4.80	5.08	5.38	4.92	5.19	4.56
	Na^+	0.75	0.63	0.60	0.68	0.72	0.61	0.60
	K^+	0.21	0.17	0.23	0.24	0.22	0.23	0.26
	Cl^-	0.75	0.69	0.69	0.80	0.71	0.76	0.69
	Na^+/K^+	3.61	3.75	2.60	2.87	3.29	2.63	2.29[e]
37	dose[j]	0	0.658	2.19	6.58	21.9	65.8	219
	V	3.72	3.97	4.39	4.96[e]	7.53[e]	9.56[e]	10.66[e]
	Na^+	0.71	0.74	0.82	0.86	1.33[e]	1.65[e]	2.04[e]
	K^+	0.19	0.24	0.19	0.24	0.22	0.24	0.21
	Cl^-	0.70	0.82	0.86	0.91[e]	1.33[e]	1.64[e]	1.90[e]
	Na^+/K^+	3.74	3.11	4.29	3.55	6.06[e]	6.93[e]	9.87[e]
g	dose[j]	0	1.01	3.36	10.1	33.6	101	336
	V	3.87	7.18[e]	8.87[e]	8.48[e]	9.24[e]	10.03[e]	10.61[e]

Table III (Continued)

no.	urinary excretion	dose, mg/kg[a] 0[f]	0.3	1.0	3.0	10	30	100
	Na^+	0.77	1.32[e]	1.59[e]	1.66[e]	1.86[e]	1.86[e]	1.90[e]
	K^+	0.21	0.26	0.32[e]	0.34[e]	0.31[e]	0.30[e]	0.36[e]
	Cl^-	0.84	1.42[e]	1.77[e]	1.78[e]	1.91[e]	2.03[e]	2.09[e]
	Na^+/K^+	3.71	5.16[e]	4.89	4.83	5.93[e]	6.10[e]	5.26[e]
h	dose[j]	0	0.907	3.02	9.07	30.2	90.7	302
	V	3.87	4.45	4.70	5.23[e]	8.48[e]	18.30[e]	27.80[e]
	Na^+	0.77	0.82	0.83	0.86	1.13[e]	2.37[e]	3.52[e]
	K^+	0.21	0.22	0.21	0.27	0.34[e]	0.46[e]	0.67[e]
	Cl^-	0.84	0.85	0.93	1.05[e]	1.41[e]	2.88[e]	4.08[e]
	Na^+/K^+	3.71	3.80	3.90	3.22	3.35	5.13[e]	5.24[e]
i	dose[j]	0	1.18	3.95	11.8	39.5	118	395
	V	2.98	2.22	3.50	4.32	6.45[e]	9.28[e]	7.01[e]
	Na^+	0.38	0.40	0.60[e]	0.83[e]	1.00[e]	1.41[e]	0.88[e]
	K^+	0.18	0.12[e]	0.10[e]	0.10[e]	0.08[e]	0.04[e]	0.06[e]
	Cl^-	0.51	0.47	0.68	0.74[e]	0.81[e]	1.15[e]	0.70
	Na^+/K^+	2.1	3.4[e]	5.9[e]	8.3[e]	13.1[e]	32.2[e]	15.4[e]

[a] Compounds were administered orally to two groups of two male rats (housed in metabolism cages) per dose and urine was collected for 5 h; see ref 12 and the Experimental Section for details. [b] Mean urine volume (mL). [c] Mean excretion (mequiv). [d] Ratio of Na^+ to K^+ excretion. [e] Statistically significant from control ($P < 0.05$). [f] Control. [g] Hydrochlorothiazide. [h] Furosemide. [i] Triamterene. [j] Dose expressed as μmol/kg.

Table IV. Effect of Compound 3 on Urinary Volume and Glomerular Filtration Rate (GFR) When Infused (10 mg/kg per h) into One Renal Artery of Anesthetized Dogs

	before infusion[a]	after infusion[b]
infused kidney		
urine volume[c]	0.2 (±0.1)	0.9 (±0.2)[d]
GFR[e]	23 (±3)	18 (±2)
control kidney		
urine volume[c]	0.4 (±0.1)	0.3 (±0.1)
GFR[e]	24 (±4)	23 (±4)

[a] Before infusion values were obtained from a 30-min collection just prior to the start of drug infusion. [b] After infusion values were obtained from a 30-min collection period beginning 30 min after the start of drug infusion. [c] Values are means ±SEM in mL/min, $N = 5$. [d] Significantly different ($P < 0.05$) from urine volume before infusion and from urine volume before or after infusion in the control kidney. [e] GFR = inulin (I) clearance in mL/min = (I concentration in urine × urine volume)/I concentration in plasma.

secondary test to carry out dose–response and electrolyte-excretion analyses on all active compounds. The primary test was conducted in male Sprague–Dawley rats weighing approximately 160 g. The rats were deprived of food 24 h and water $1^1/_2$ h before test time. During testing both food and water were withheld. Testing was initiated by simultaneous hydration and oral administration of test agent or standard. This was accomplished by gavage with 25 mL/kg of normal saline (0.9%) containing (carboxymethyl)-cellulose (0.5%) and test agent or standard either dissolved or suspended. Rats were placed in metabolism cages (two rats/cage), and urine was collected over the ensuing 5 h. Rats in 10 cages (20 rats) served as controls and those in two cages received hydrochlorothiazide as the test standard while animals in the remaining cages (up to 44) received test agents. Each test agent was given to four animals in two cages. In all instances, the dose of test agent or standard was 40 mg/kg. The ratio (denoted T/C) of urine volume in treated animals to urine volume in control rats was determined. Criteria for declaring test substances active or inactive were established for the two-stage test as described by Roseberry and Gehan.[17] If the T/C in stage 1 was <1.66, the test agent was declared inactive; if it was 1.66 or greater, the test agent was retested in stage 2. When the product of T/C for stage 1 and stage 2 was 3.34 or greater, the test agent was declared active. The respective decision values for stages 1 and 2 were determined from cumulative values obtained in control and hydrochlorothiazide-treated rats and were subject to periodic reevaluation. With this two-stage procedure, the expected probability of a test agent with a true T/C of 2.25 being declared active was 0.99 while the probability of a test agent with a true T/C of 1.5 of being declared active was less than 0.01.

Secondary Evaluation. Secondary testing was carried out on all agents declared active in the primary test. These agents were subjected to dose–response analyses using a wide range of doses (0.3–100 mg/kg) and tested for effects on urinary Na, K, Cl, and HCO_3 excretion. Test agents were given to three cages of rats (two rats/cage). In all other respects, i.e., test animals, route of administration, hydration, and collection periods, secondary testing was carried out identically to primary testing. Urinary electrolytes were determined on a Technicon Auto Analyzer: Na and K using flame photometry and Cl and HCO_3 colorimetrically.

Follow-Up Study in Dogs. Beagle dogs weighing between 8 and 16 kg were anesthetized with sodium pentabarbital (30 mg/kg), and a tube was placed in the trachea to insure an unobstructed airway. Glomerular filtration rate was determined from the renal clearance of inulin by using standard clearance techniques. A solution containing 0.9% NaCl and 0.75% inulin was given at a rate of 0.2 mL/kg per min by way of a femoral venous cannula. A prime of the same solution of 5 mL/kg was given initially. For collection of urine, right and left ureters were exposed through a ventral midline incision and cannulated (PE 160). Blood samples were withdrawn into heparinized syringes from a femoral arterial cannula. One renal artery (usually the left) was approached from a retroperitoneal flank incision and a 22-gauge fish-hook-shaped needle was inserted into the artery. In this manner, test agent could be given unilaterally with the opposite kidney serving as the control. Once inserted, saline was continuously pumped through the needle at a rate of 0.3 mL/min to which drug was added to obtain "after infusion" values. Preparations were left unperturbed for 30–60 min in order to stabilize before experimentation was initiated. Data were analyzed statistically by using Student's *t* test for compared comparisons with $P < 0.05$ as the criterion for significance.

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(17) Roseberry, T. D.; Gehan, E. A. *Biometrics* **1964**, *20*, 73.