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Articles

N-Substituted Pyrazino[2,3-c][1,2,6]thiadiazine 2,2-Dioxides. A New Class of Diuretics

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The synthesis and evaluation of a new class of diuretic agents derived from the pyrazino[2,3-c][1,2,6]thiadiazine 2,2-dioxide ring system are described. Preliminary structure–activity relationships indicate that the nature and location of the substituents at different positions of the heterocycle are crucial for activity. Thus, a novel synthetic methodology has been developed to selectively introduce the desired substituents at different positions. From the study of the pharmacological properties (dose–response curves, duration of action, and acute toxicity) of the most active compounds, 4-amino-1,7-diethyl-6-methylpyrazino[2,3-c][1,2,6]thiadiazine 2,2-dioxide (**9**) was selected for further investigation. Compound **9** ($C_{10}H_{15}N_5O_2S$) crystallizes in space group $P2_1/a$ with unit cell dimensions a = 16.482 (1), b = 9.3484 (3), c = 8.333 (3) Å, β = 103.003 (3)°, Z = 4.

Diuretics are among the most widely used therapeutic agents in the treatment of congestive heart failure and hypertension. From a structural point of view, they constitute an heterogeneous array of chemical compounds which includes, thiazides, (aryloxy)acetic acids, pteridines, and sulfamoylcarboxylic acids. Although a wide range of diuretics is currently available, research in this field is still active with particular emphasis on new agents with adjunctive renal and extrarenal effects and less prone to adverse biochemical effects observed in conventional diuretics.[1]

In this respect, and continuing with our ongoing research on heterocycles containing the NSO_2N residue,[2] we wish to report the synthesis and preliminary pharmacological results of a new substance class of diuretic agents derived from the pyrazino[2,3-c][1,2,6]thiadiazine 2,2-dioxide system.[3] This structure, in which the sulfamide moiety and the pteridine ring are combined represents a completely novel chemical entity in the diuretic field with potent activity and pharmacological profiles comparable to those of some of the commercially available drugs.

Chemistry

The compounds prepared for this study are listed in Table I. Their general synthetic route (outlined in Scheme I) involves formation of the parent 1*H*-pyrazino[2,3-c][1,2,6]thiadiazine 2,2-dioxide bearing different substituents at C-6 and C-7, and subsequent introduction of the substituent at N-1 (since previous results indicated that compounds unsubstituted at position 1 were devoid of diuretic activity).

The first step consists in the condensation of 3,4,5-triamino-2*H*-1,2,6-thiadiazine 1,1-dioxide (**1**)[4] with suitably

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(1) Hropot, M. New and Investigational Diuretics. *Drugs Today* **1988**, *24* (3), 193–210.
(2) Arán, V. J.; Goya, P.; Ochoa, C. Heterocycles Containing the Sulfamide Moiety. *Adv. Heterocycl. Chem.* **1988**, *44*, 81–267.
(3) Goya, P.; Páez, J. A.; Pfleiderer, W. Pyrazino[2,3-c][1,2,6]thiadiazine 2,2-Dioxides. Sulfur Dioxide Analogs of Pteridines. *J. Heterocycl. Chem.* **1984**, *21*, 861–864.
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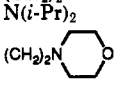
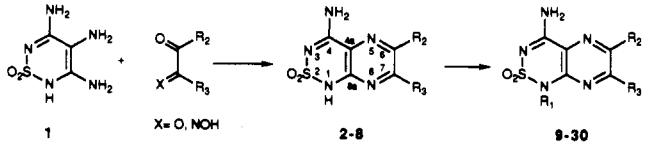
Table I. Physical and Analytical Data of 4-Aminopyrazino[2,3-c][1,2,6]thiadiazine Derivatives and Diuretic Screening[a] Data in Rats (Control/Drug Treatment Values)

compd	R1	R2	R3	method	mp °C	recrystn solvent	mol formula	anal.[b]	V[c]		Na+[d]	
2	H	Et	Me	-	273–275	H_2O	$C_8H_{11}N_5O_2S$	C,H,N	-		-	
3[e]	H	Me	Et	-	290–292	H_2O/EtOH	$C_8H_{11}N_5O_2S$	C,H,N,S	22.7/22.5	-	0.31/0.35	-
4	H	Me	*n*-Pr	-	211–213	H_2O/EtOH	$C_9H_{13}N_5O_2S$	C,H,N,S	-		-	
5	H	Et	Et	-	266–268	H_2O	$C_9H_{13}N_5O_2S$	C,H,N,S	-		-	
6	H	$CH_2(CH_2)_2CH_2$		-	>320	H_2O/EtOH	$C_9H_{11}N_5O_2S$	C,H,N	-		-	
7	H	CF_3	CF_3	-	332–334	H_2O/MeOH	$C_7H_3F_6N_5O_2S$	C,H,N	-		-	
8	H	*i*-Pr	*i*-Pr	-	238–239	H_2O/EtOH	$C_{11}H_{17}N_5O_2S$	C,H,N	-		-	
9[f]	Et	Me	Et	A	194–196	H_2O/EtOH	$C_{10}H_{15}N_5O_2S$	C,H,N,S	24.2/47.6***[g]	(2.0)[h]	0.35/2.88***	(8.2)
10[e]	Me	Me	Et	A	240–242	H_2O/MeOH	$C_9H_{13}N_5O_2S$	C,H,N,S	24.0/36.5**	(1.5)	0.52/2.02***	(3.9)
11[e]	Et	Et	Me	A	145–146	H_2O/MeOH	$C_{10}H_{15}N_5O_2S$	C,H,N,S	23.9/24.8	-	0.39/0.22	-
12[e]	Et	Me	*n*-Pr	A	142–144	H_2O/MeOH	$C_{11}H_{17}N_5O_2S$	C,H,N	21.7/23.5	-	0.42/0.65*	(1.5)
13[e]	Et	Et	Et	A	190–191	H_2O/EtOH	$C_{11}H_{17}N_5O_2S$	C,H,N,S	25.4/33.8***	(1.3)	0.48/0.95***	(2.0)
14[f]	Et	$CH_2(CH_2)_2CH_2$		A	198–200	H_2O/EtOH	$C_{11}H_{15}N_5O_2S$	C,H,N	26.7/19.4	-	0.38/0.23	-
15[f]	Et	CF_3	CF_3	A	217–219	H_2O/EtOH	$C_9H_7F_6N_5O_2S$	C,H,N,S	23.3/29.9***	(1.3)	0.51/0.66	-
16[e]	Et	*i*-Pr	*i*-Pr	A	157–158	H_2O/MeOH	$C_{13}H_{21}N_5O_2S$	C,H,N,S	25.8/26.1	-	0.61/0.37	-
17[f]	Et	Me	Me	A	167–169	H_2O/EtOH	$C_9H_{13}N_5O_2S$	C,H,N,S	27.7/36.4*	(1.3)	0.50/1.79***	(3.6)
18[e]	Et	H	Me	A	202–204	H_2O/MeOH	$C_8H_{11}N_5O_2S$	C,H,N	25.9/28.9	-	0.41/0.48	-
19[e]	Et	Br	Me	A	172–174	H_2O/MeOH	$C_8H_{10}BrN_5O_2S$	C,H,N,S,Br	23.9/25.4	-	0.42/0.43	-
20[e]	Et	H	Ph	A	246–248	H_2O/MeOH	$C_{13}H_{13}N_5O_2S$	C,H,N,S	25.3/30.5**	(1.2)	0.36/0.47*	(1.3)
21[e]	Et	Br	Ph	A	234–236	H_2O/EtOH	$C_{13}H_{12}N_5BrO_2S$	C,H,N,S,Br	26.1/26.6	-	0.45/0.37	-
22[f]	CH_2CO_2Et	H	Ph	B	190–192	H_2O/EtOH	$C_{15}H_{15}N_5O_4S$	C,H,N	24.7/27.6	-	0.71/0.36	-
23[e]	Et	Ph	H	B	265–267	H_2O/MeOH	$C_{13}H_{13}N_5O_2S$	C,H,N,S	24.2/26.9	-	0.36/0.29	-
24[e]	Et	Me	H	B	171–173	H_2O/EtOH	$C_8H_{11}N_5O_2S$	C,H,N,S	21.8/24.8	-	0.36/0.49	-
25[e]	*n*-Pr	Me	Et	C	158–160	H_2O/MeOH	$C_{11}H_{17}N_5O_2S$	C,H,N	22.8/35.1***	(1.5)	0.41/2.06***	(5.0)
26[f]	CH_2CO_2Et	Me	Me	C	186–188	H_2O/EtOH	$C_{11}H_{15}N_5O_4S$	C,H,N,S	23.9/24.2	-	0.26/0.23	-
27[e]	*n*-Pr	Me	Me	C	165–167	H_2O/EtOH	$C_{10}H_{15}N_5O_2S$	C,H,N,S	26.1/45.7***	(1.8)	0.44/2.68***	(6.1)
28[f]	$(CH_2)_2$-$N(i\text{-}Pr)_2$	Me	Me	D	161–162	H_2O/EtOH	$C_{15}H_{26}N_6O_2S$	C,H,N,S	19.8/16.6	-	0.20/0.38**	(1.9)
29[f]		Me	Me	D	260–262	H_2O/EtOH	$C_{13}H_{20}N_6O_3S$	C,H,N,S	21.7/23.5	-	0.17/0.43**	(2.5)
30[f]	$(CH_2)_2NMe_2$	Me	Me	D	218–220	H_2O/EtOH	$C_{11}H_{18}N_6O_2S$	C,H,N,S	23.9/22.6	-	0.28/0.26	-
31[b,e,i]	Et	Me	Et	-	164–166	H_2O/MeOH	$C_{11}H_{17}N_5O_2S$	C,H,N,S	22.8/31.6**	(1.4)	0.45/1.55***	(3.4)
32[c,e,j]	Me	Me	Me	-	269–271	H_2O/EtOH	$C_8H_{11}N_5O_2S$	C,H,N,S	24.3/37.9***	(1.6)	0.41/1.49***	(3.6)
hydrochlorothiazide[k]									24.1/39.5***	(1.6)	0.43/2.86***	(6.7)
triamterene[f]									23.9/37.2***	(1.6)	0.41/2.32***	(5.7)

[a] See the Experimental Section for testing methodology. [b] Compounds gave satisfactory analyses (±0.4%). [c] Mean urinary output (mL/6 h/kg bw). [d] Mean sodium excretion (mequiv/6 h/kg bw). [e] Dose (mg/kg, po): 20. [f] Dose (mg/kg, po): 25. [g] *, **, and *** indicate a significant difference from their control groups, respectively, at $p < 0.05$, $p < 0.01$, and $p < 0.001$ (Student's *t*-test). [h] Ratio to the control is shown in parentheses; - indicates inactive. [i] Corresponding to 4-methylamino derivative. Dose (mg/kg, po): 10. [j] Reference 4. [k] Dose (mg/kg, po): 10.

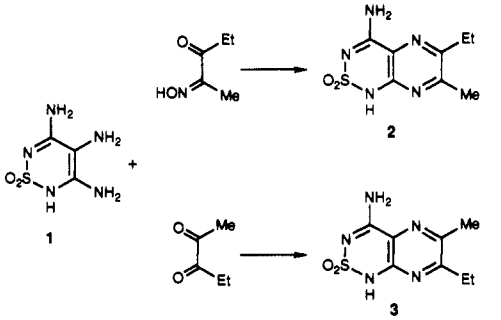
Scheme I



functionalized compounds. We have already described this reaction for symmetric 1,2-dicarbonyl compounds (leading to pyrazinothiadiazines bearing the same substituents at positions 6 and 7)[3] ($R_2 = R_3$); however, when unsymmetrical compounds are used, problems regarding selectivity arise. There was only one example of a regioselective reaction of 1 and phenylglyoxal[5] and thus, for the purpose of this study, we first had to find procedures that would allow the selective introduction of the desired substituents at 6 and 7. This was finally achieved by using either hydroxyimino or carbonyl compounds, as exemplified in the synthesis of isomers 2 and 3 (Scheme II).

Reaction of 1 with 2-(hydroxyimino)-3-pentanone[6] afforded only the 6-ethyl-7-methyl derivative 2 while the 7-ethyl-6-methyl isomer 3 was exclusively obtained when

Scheme II



using 2,3-pentanedione. This can be generalized and thus, compounds 4, 5, and 6 were obtained from 1 and the corresponding commercially available 1,2-dicarbonyl compounds.

This selective synthetic procedure is of the utmost importance for this study since, as it will be discussed later, *N*-ethylpyrazino[2,3-c][1,2,6]thiadiazine 2,2-dioxide 9 (R_1 = Et, R_2 = Me, R_3 = Et) is one of the most active compounds of this series while its positional isomer 11 (R_1 = Et, R_2 = Et, R_3 = Me) is completely devoid of diuretic properties.

Trifluoromethyl derivative 7 was prepared from 1 and 1,1,1,4,4,4-hexafluoro-2,3-butanedione, synthesized by a

(5) Alkorta, I.; Goya, P.; Páez, J. A.; Pfleiderer, W. Synthesis and Physicochemical Properties of 6- and 7-Monosubstituted Pyrazino[2,3-c][1,2,6]thiadiazine 2,2-Dioxides. *Pteridines* **1990**, *2*, 3–7.

(6) Dang Quoc Quan; Bernadou, J. Sur la Préparation et l'identification Structurale des α-hydroxyiminocétones Aliphatiques en C_5 de Type Monoisonitrosoacétone (MINA) et Diacétylmonoxime (DAM). *Bull. Soc. Chim.* **1973**, *4*, 1452–1454.

Table II. ^{13}C NMR Chemical Shifts (δ Values) of Compounds **9–31** in DMSO-d_6

compd	C-4	C-7	C-8a	C-6	C-4a	N-R	other signals
9	158.8	158.3	146.6	144.5	119.3	37.6, 13.9	27.5, 10.7, 20.3
10	158.9	161.9	147.3	144.7	119.7	28.0	27.7, 10.9, 20.4
11	159.0	157.7	146.3	148.9	119.8	37.5, 14.0	26.3, 11.6, 22.1
12	158.8	160.8	146.6	144.7	119.5	37.6, 13.7	36.1, 19.8, 14.0, 20.4
13	159.1	161.6	146.6	148.8	119.7	37.8, 14.1	27.2, 11.4, 25.9, 12.1
14	158.8	158.1	146.5	145.3	120.5	37.5, 13.9	32.2, 30.6, 21.9, 21.6
15	158.8	141.6	148.4	129.9	124.5	38.9, 13.1	120.4, 119.6
16	158.7	163.8	146.2	151.4	120.0	37.6, 13.9	30.6, 29.5, 22.1, 21.8
17	158.9	158.2	146.5	145.0	119.7	37.5, 14.0	22.5, 20.8
18	158.8	159.3	148.0	136.9	120.8	37.7, 13.9	22.0
19	157.6	159.6	147.3	130.3	121.0	38.1, 13.8	24.5
20	158.1	154.2	147.9	133.5	121.6	37.6, 13.6	134.5, 131.2, 129.0, 127.5
21	157.3	157.6	147.2	128.1	121.8	38.3, 13.8	136.3, 130.4, 129.7, 128.2
22	158.1	154.1	147.7	134.6	121.9	43.4, 167.6, 61.1, 14.1	134.1, 131.8, 129.2, 127.8
23	158.6	145.8	147.5	143.8	122.3	38.0, 13.9	134.6, 129.7, 128.9, 126.7
24	158.7	148.5	146.9	145.9	122.2	37.7, 13.9	20.2
25	158.9	161.8	146.9	144.6	119.3	43.8, 21.6, 11.1	27.5, 10.5, 20.2
26	158.7	158.3	146.3	146.0	119.8	43.0, 167.8, 61.8, 14.0	22.4, 20.8
27	159.0	158.2	146.8	145.1	119.7	43.8, 21.6, 11.1	22.6, 20.8
28	158.8	158.2	146.6	145.1	119.5	43.7, 43.0, 49.1, 20.6	22.5, 20.8
29	159.1	158.1	146.9	145.3	119.9	66.2, 56.4, 53.3, 39.3	22.4, 20.8
30	159.0	158.2	146.8	145.2	119.8	57.1, 45.3, 39.8	22.5, 20.8
31	156.7	161.3	146.2	144.5	119.8	37.6, 13.9	27.9, 27.5, 10.7, 20.3

literature procedure,[7] and the 6,7-diisopropyl derivative **8** from **1** and 2,5-dimethyl-3,4-hexanedione.[8] The rest of the N-unsubstituted pyrazinothiadiazines were synthesized according to reported procedures.[3,5]

In order to selectively obtain the final N-1-substituted derivatives different alkylation procedures were used. When substituents are present at position C-7, reaction of the NH compounds with alkyl sulfates generally yields exclusively N-1 derivatives, as in pyrazinothiadiazines **9–21**. In case of **22**, ethyl bromoacetate was used as alkylating agent. With 7-unsubstituted compounds, alkyl halides are preferred over alkyl sulfates as in case of **23** and **24**. In the cases **25–27**, *N*-silyl derivatives were alkylated using appropriate alkyl halides. Liquid–liquid phase transfer conditions had to be used for derivatives **28–30**. This method had previously failed for 1,2,6-thiadiazine 1,1-dioxides.[2] Finally the 4-(methylamino) derivative **31** was prepared by treatment of **9** with methyl iodide.

The structures of all the newly synthesized compounds were established on the basis of analytical and NMR data and X-ray diffraction analysis. The positions of the 6 and 7 substituents can be distinguished by ^{13}C NMR spectra, examining the long range coupling constants and taking into account that C-7 appears always at lower field than C-6 (see Table II).

A view of the final X-ray model of compound **9** with the atomic numbering is shown in Figure 1.[9] The thiadiazine ring presents the conventional envelope conformation with the sulfur atom at the flap, 0.629 (2) Å out of the plane.[2] Molecules are held together by hydrogen bonds.

Pharmacological Results and Discussion

As already mentioned, pyrazinothiadiazines must be N-substituted in order to show diuretic properties. The lack of activity of the NH compounds might be related to their strong acid character as a result of the SO_2 group inside the ring system (pK_a values in the range of 3–4, which are about 6 units lower than those of the corresponding pteridines).[3] Thus, all the N-substituted (**9–32**) compounds here synthesized were screened for diuretic and natriuretic effects in comparison to reference diuretics hydrochlorothiazide and triamterene. Target compounds were evaluated after a single administration (20–25 mg/kg, po) to water-loaded rats, and the results of this primary screen are summarized in Table I.

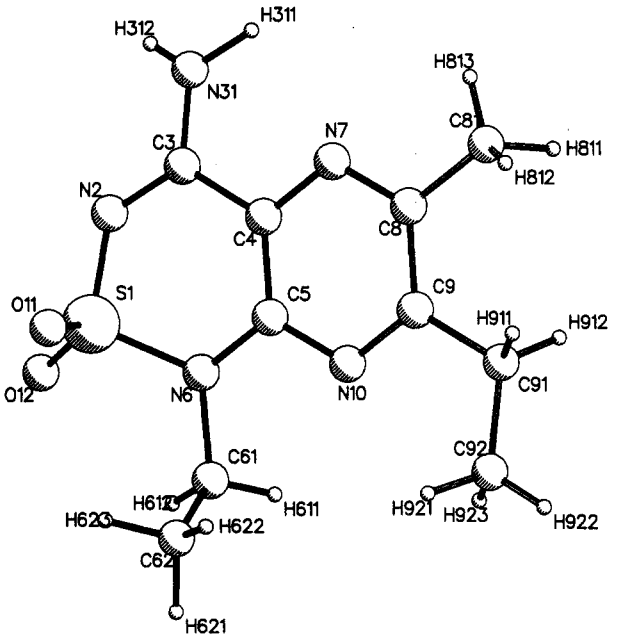


Figure 1. Perspective drawing of the final X-ray model.

Analysis of these data indicates that very few structural variations are allowed and that only compounds with lower alkyl groups have interesting activity. Structural requirements for diuretic activity within this series are specific and include simple alkyl groups at positions 1, 6, and 7 of the heterocycle. The relative positions of these groups are also critical for activity as exemplified in the case of **9** and **11**. Other simple variants such as hydrogen, aryl or halogen (at 6 or 7), or (dimethylamino)alkyl or ethoxy-

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(8) Leonard, N. J.; Laitinen, H. A.; Mottus, E. H. The Behavior of 1,2-Diketones at the Dropping Mercury Electrode. *J. Am. Chem. Soc.* **1953**, *75*, 3300–3303.

(9) Motherwell, W. D. S.; Clegg, W. *Pluto. A Program for Plotting Crystal and Molecular Stuctures*; University of Cambridge, England, 1978.

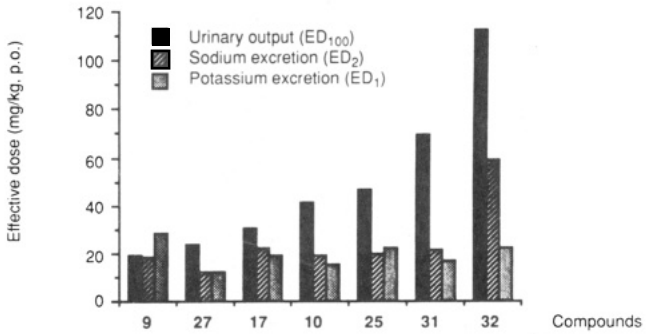

Figure 2. Diuretic (ED_{100}), natriuretic (ED_2), and kaliuretic (ED_1) activity of pyrazinothiadiazines selected in the primary screening. Compounds were administered orally (10–40 mg/kg) to water-loaded rats, and urine was collected over the ensuing 6-h period.

carbonyl (at N-1) always result in a loss of activity (compounds **18–24** and **28–30**). A methyl at position 6 seems to be optimal for activity.

Compounds **9, 10, 17, 25, 27, 31,** and **32** (all with a methyl at 6) caused an important increase of urine volume and resulted in higher sodium excretion than reference diuretics when administered orally to fasted, water-loaded rats. These derivatives were considered sufficiently interesting to undergo further testing and so their activities were evaluated in the same experimental model at different dose levels in order to establish dose–response relationships. To compare the relative potencies of these selected derivatives we considered ED_{100} values for diuresis and ED_2 and ED_1 values for natriuresis and kaliuresis, respectively. Results depicted in Figure 2 show that compound **9** exhibits the most favorable combination of diuretic and natriuretic effects with less potassium excretion. On the basis of these findings and of the preliminary data of toxicological effects, we selected this compound for further testing. Comparative pharmacological evaluation of compound **9** was carried out in rodents in comparison to furosemide, hydrochlorothiazide, and triamterene as reference diuretics. According to the results gathered in Table III and summarized in Table V, **9** showed significant diuretic activity at doses ranging from 5 to 160 mg/kg po. Diuresis is accompanied by an important excretion of electrolytes with reasonable Na^+/K^+ ratios. Comparison of effective doses for salidiuretic activity in rats clearly indicates that **9** has a high-ceiling diuretic activity with marked natriureis and chloruresis, its potency being comparable to that of furosemide.

Onset and duration of action have also been evaluated for **9** and standard diuretics and results are shown in Table IV. The onset of activity of **9** was rapid with a maximum of activity at 1–2 h postdosing, and a duration of 4 h at the dose of 100 mg/kg po. This profile is similar to that of hydrochlorothiazide but it clearly differs from that of furosemide which exhibits a rapid onset and less sustained activity.

Acute toxicity of **9** in mice is comparable to furosemide, the compound being more toxic than hydrochlorothiazide but less toxic than triamterene (Table V).

In summary, as a result of this study, a new chemical class of diuretics (derivatives of pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide), not closely related to any of the known marketed agents, has been found.

Structural requirements for diuretic activity within this series are specific and include simple alkyl groups at positions 1, 6, and 7 of the heterocycle. Other simple variants such as hydrogen, aryl or halogen (at 6 or 7), or (dimethylamino)alkyl or ethoxycarbonyl (at N-1) always result in a loss of activity. A methyl group at position 6 seems to be optimal for activity.

Regarding the chemistry of this heterocycle a synthetic methodology has been developed to selectively introduce at the 6 and 7 positions specific substituents, conditional for activity.

Several compounds **9, 10, 17, 25, 27,** and **32** were found to possess marked diuretic and natriuretic activity. Specifically, compound **9** combines the efficacy of furosemide with a less abrupt onset of action and it could offer a suitable alternative to other diuretics in the treatment of mild to moderate hypertension. Therefore, this compound is currently undergoing extensive pharmacological evaluation.

Experimental Section

Chemistry. Melting points were determined with a Reichert-Jung Thermovar micro melting point apparatus and are uncorrected. ^1H NMR spectra (300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded on a Varian XL-300 spectrometer and are reported in ppm on the δ scale. Elemental analyses were performed on a Heraeus CHN-O-Rapid analyzer. Column chromatography was carried out on silica gel (Merck, particle size 70–230 mesh).

4-Amino-6-ethyl-7-methyl-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-Dioxide (2). A suspension of **1** (3.0 g, 16.9 mmol) in methanol (250 mL) and concentrated hydrochloric acid (1 mL) was treated with 2-(hydroxyimino)-3-pentanone (2.3 g, 20.0 mmol) and the mixture was refluxed for 10 h. The reaction mixture was evaporated to dryness, and water was added to the residue. The precipitate was filtered and recrystallized from water to give **2** (3.3 g, 81%): ^1H NMR (DMSO-d_6) δ 11.91 (br s, 1 H, NH_2), 8.50 (br s, 1 H, NH_2), 8.23 (br s, 1 H, NH_2), 2.80 (q, 2 H, CH_2), 2.53 (s, 3 H, CH_3), 1.25 (t, 3 H, CH_3).

Reaction of 3,4,5-Triamino-2*H*-1,2,6-thiadiazine 1,1-Dioxide (1) with 1,2-Dicarbonyl Compounds. 4-Amino-7-ethyl-6-methyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (3). To a suspension of **1** (8.0 g, 45.2 mmol) in acetic acid (300 mL) was added dropwise 2,3-pentanedione (5.5 g, 54.9 mmol), and the mixture was stirred at 100 °C for 6 h. The suspension was concentrated (40 mL), and the precipitate was filtered and recrystallized from water/ethanol to give **3** (7.0 g, 64%): ^1H NMR (DMSO-d_6) δ 8.47 (br s, 1 H, NH_2), 8.31 (br s, 1 H, NH_2), 2.83 (q, 2 H, CH_2), 2.51 (s, 3 H, CH_3), 1.22 (t, 3 H, CH_3).

In a similar manner were compounds **4–8** obtained.

4-Amino-6-methyl-7-propyl-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (4): from **1** (5.0 g, 28.2 mmol), acetic acid (100 mL), and 2,3-hexanedione (3.6 g, 31.5 mmol). Recrystallization from water/ethanol yielded **4** (5.8 g, 85%): ^1H NMR (DMSO-d_6) δ 11.91 (br s, 1 H, NH_2), 8.48 (br s, 1 H, NH_2), 8.33 (br s, 1 H, NH_2), 2.80 (t, 2 H, CH_2), 2.51 (s, 3 H, CH_3), 1.69 (m, 2 H, CH_2), 0.98 (t, 2 H, CH_3).

4-Amino-6,7-diethyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (5): from **1** (4.0 g, 22.6 mmol), acetic acid (100 mL), and 3,4-hexanedione (2.8 g, 24.5 mmol). Recrystallization from water yielded **5** (3.6 g, 62%): ^1H NMR (DMSO-d_6) δ 11.93 (br s, 1 H, NH), 8.50 (br s, 1 H, NH_2), 8.23 (br s, 1 H, NH_2), 2.85 (q, 2 H, CH_2), 2.82 (q, 2 H, CH_2), 1.26 (t, 3 H, CH_3), 1.22 (t, 3 H, CH_3).

4-Amino-6,7,8,9-tetrahydro-1*H*-[1,2,6]thiadiazino[3,4-*b*]-quinoxaline 2,2-Dioxide (6). This compound was prepared starting from **1** (4.0 g, 22.6 mmol), acetic acid (100 mL), and 1,2-cyclohexanedione (3.0 g, 26.7 mmol). Recrystallization from water yielded **6** (2.8 g, 49%): ^1H NMR (DMSO-d_6) δ 11.92 (br s, 1 H, NH), 8.47 (br s, 1 H, NH_2), 8.32 (br s, 1 H, NH_2), 2.87 (m, 4 H, $2CH_2$), 1.85 (m, 4 H, $2CH_2$).

4-Amino-6,7-bis(trifluoromethyl)-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (7): from **1** (4.0 g, 22.6 mmol), acetic acid (125 mL), and 1,1,1,4,4,4-hexafluoro-2,3-butanedione (4.4 g, 22.6 mmol). Recrystallization from water yielded **7** (3.9

Table III. Dose–Response Relationships for Effects of Compound 9 and Reference Diuretics on Urine Output and Na^+, K^+, and Cl^- Excretion in Water-Loaded Rats[a]

compound		dose, mg/kg po							
		0[b]	2.5	5.0	10	20	40	80	160
9	dose, μmol/kg	0	9.28	18.56	37.13	74.26	148.52	297.03	594.07
	V[c]	21.96	20.52	25.06	32.60***[d]	42.25***	59.12***	62.99***	74.26***
	Na^+[e]	0.43	0.45	0.80***	1.21***	1.70***	3.63***	5.10***	6.14***
	K^+[e]	0.49	0.46	0.52	0.72**	0.63	1.25***	2.06***	2.35***
	Cl^-[e]	1.02	1.21	1.83***	2.67***	3.99***	6.37***	8.04***	9.28***
	Na^+/K^+[f]	1.01	1.03	1.49***	1.68***	2.43***	2.95***	2.60***	2.66***
hydrochlorothiazide	dose, μmol/kg	0	8.40	16.79	33.59	67.18	134.35	268.71	–
	V	24.30	32.50**	38.07***	39.01***	42.94***	44.48***	47.06***	[g]
	Na^+	0.32	2.14***	3.03***	2.86***	3.05***	2.78***	3.19***	[g]
	K^+	0.53	1.26***	1.23***	1.14***	1.43***	1.64***	1.57***	[g]
	Cl^-	0.78	4.42***	4.88***	4.44***	4.67***	3.94***	4.88***	[g]
	Na^+/K^+	0.67	1.81***	2.67***	2.64***	2.20***	1.79***	2.11***	[g]
furosemide	dose, μmol/kg	0	7.56	15.12	30.23	60.46	120.93	241.86	483.72
	V	24.01	18.13	22.43	30.40**	46.33***	63.28***	75.55***	86.58***
	Na^+	0.39	0.46	0.55	1.33***	2.91***	4.86***	6.34***	7.67***
	K^+	0.47	0.44	0.60	0.86**	1.38***	1.50***	1.85***	2.52***
	Cl^-	0.58	0.79	1.30***	2.25***	4.85***	7.00***	8.76***	11.34***
	Na^+/K^+	0.85	1.23*	0.91	1.69***	2.10***	3.29***	3.49***	3.07***
triamterene	dose, μmol/kg	0	9.87	19.74	39.48	78.97	157.94	315.88	631.76
	V	24.94	31.39**	30.19**	31.12***	32.87***	38.15***	44.92***	52.00***
	Na^+	0.58	1.69***	2.28***	2.61***	2.42***	2.88***	3.37***	3.32***
	K^+	0.50	0.32*	0.19***	0.18***	0.16***	0.18***	0.14***	0.21***
	Cl^-	0.88	1.34***	1.55***	1.89***	1.83***	2.83***	2.80***	3.05***
	Na^+/K^+	1.26	5.75***	14.25***	15.40***	17.81***	16.80***	25.22***	18.79***

[a] See the Experimental Section for testing methodology. [b] Control. [c] Mean urine output (mL/6 h/kg bw). [d] Statistically significant from control * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's t-test). [e] Mean excretion (mequiv/6 h/kg bw). [f] Ratio of Na^+ to K^+ excretion. [g] Not determined.

Table IV. Onset and Duration of Action of Compound 9 and Reference Diuretics in Water-Loaded Rats[a]

time after dosing (h)	urine volume (mL)[b]					urinary sodium (mequiv)[b]				
	C[c]	9[d]	FUR[e]	HCTZ[f]	TR[g]	C[c]	9[d]	FUR[e]	HCTZ[f]	TR[g]
1	0.93	1.84***[h]	10.33***	1.38	0.48	0.03	0.15***	1.03***	0.19*	0.04
2	3.46	8.70***	8.19*	4.97	3.20	0.04	0.67***	0.61***	0.39***	0.16***
3	1.40	5.52***	3.82**	3.37*	2.73***	0.02	0.56***	0.26***	0.17***	0.19***
4	0.82	2.71***	2.43	2.92**	1.78**	0.01	0.27***	0.12***	0.16***	0.15***
6	1.56	1.99	3.42***	3.42**	4.36***	0.02	0.16***	0.15***	0.24***	0.46***

[a] See the Experimental Section for testing methodology. [b] Results are expressed as the mean values for six couples of rats. [c] Vehicle group. [d] Compound 9 group: 100 mg/kg po. [e] Furosemide group: 84 mg/kg po. [f] Hydrochlorothiazide group: 10 mg/kg po. [g] Triamterene group: 125 mg/kg po. [h] *, **, and *** indicate a significant difference from their control groups respectively at $p < 0.05$, 0.01 and 0.001 (Student's t-test).

Table V. Comparison of Pharmacological Activities of Compound 9 and Reference Diuretics

compound	salidiuretic activity in rats				acute toxicity in mice DL_{50} (mg/kg, po)
	effective doses (mg/kg, po)			postdosing time interval of maximum activity (h)	
	$ED_{50(DIUR)}$[a]	$ED_{200(DIUR)}$[a]	$ED_{2(Na^+)}$[b]		
9	9.17	87.61	14.62	1–2	1840
furosemide	12.10	70.59	12.95	0–1	2383
hydrochlorothiazide	4.86	[c]	1.57	1–2	>6000
triamterene	34.51	[c]	3.80	4–6	344

[a] Doses required to produce an increase of 50% and 200%, respectively, in urine production (mL/kg bw/6 h) vs control groups. [b] Doses required to produce an excretion of 2 mequiv of Na^+/kg bw/6 h vs control groups. [c] Not determined.

g, 52%): ^{13}C NMR (DMSO-d_6) δ 156.0 (C-4), 149.5 (C-8a), 142.7 (C-7), 130.2 (C-6), 123.2 (C-4a), 120.4 (CF_3), 118.7 (CF_3).

4-Amino-6,7-diisopropyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (8): from 1 (4.0 g, 22.6 mmol), acetic acid (125 mL), and 2,5-dimethyl-3,4-hexanedione (3.3 g, 23.2 mmol). Recrystallization from water/ethanol yielded 8 (2.8 g, 44%): ^1H NMR (DMSO-d_6) δ 8.48 (br s, 1 H, NH_2), 8.19 (br s, 1 H, NH_2), 3.40 (m, 1 H, CH), 3.35 (m, 1 H, CH), 1.24 (d, 6 H, $2CH_3$), 1.22 (d, 6 H, $2CH_3$).

General Procedures for the Alkylation of Pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxides. Example of Method A of Table I. 4-Amino-1,7-diethyl-6-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (9). Diethyl sulfate (5 mL) was added dropwise to a solution of 3 (4.0 g, 16.5 mmol) in water (50 mL) and potassium carbonate (4.0 g, 28.9 mmol). The reaction mixture was stirred at room temperature for 24 h. The precipitate was filtered, washed with potassium carbonate solution, and recrystallized from water to give 9 (2.67 g, 60%): ^1H NMR (DMSO-d_6) δ 8.65 (br s, 1 H, NH_2), 8.50 (br s, 1 H, NH_2), 4.03 (q, 2 H, NCH_2), 2.89 (q, 2 H, CH_2), 2.52 (s, 3 H, CH_3), 1.30 (t, 3 H, CH_3), 1.26 (t, 3 H, CH_3).

Method A was followed to prepare compounds 9–21.

4-Amino-7-ethyl-1,6-dimethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (10): from 3 (1.0 g, 4.14 mmol) and dimethyl sulfate (1 mL). Recrystallization from water/ethanol yielded 10 (0.64 g, 61%): ^1H NMR (DMSO-d_6) δ 8.71 (br s, 1 H, NH_2), 8.58 (br s, 1 H, NH_2), 3.37 (s, 3 H, CH_3), 2.88 (q, 2 H, CH_2), 2.52 (s, 3 H, CH_3), 1.25 (t, 3 H, CH_3).

4-Amino-1,6-diethyl-7-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (11): from 2 (2.0 g, 8.29 mmol) and diethyl sulfate (2 mL). Recrystallization from water/methanol yielded 11 (1.4 g, 63%): ^1H NMR (DMSO-d_6) δ 8.69

(br s, 1 H, NH_2), 8.42 (br s, 1 H, NH_2), 4.02 (q, 2 H, CH_2), 2.83 (q, 2 H, CH_2), 2.58 (s, 3 H, CH_3), 1.28 (t, 3 H, CH_3), 1.26 (t, 3 H, CH_3).

4-Amino-1-ethyl-6-methyl-7-propylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (12): from **4** (2.0 g, 7.83 mmol) and diethyl sulfate (2 mL). Recrystallization from water/methanol yielded **12** (1.5 g, 68%): ^{1}H NMR (DMSO-d_6) δ 8.66 (br s, 1 H, NH_2), 8.52 (br s, 1 H, NH_2), 4.02 (q, 2 H, CH_2), 2.81 (t, 2 H, CH_2), 2.52 (s, 3 H, CH_3), 1.76 (m, 2 H, CH_2), 1.29 (t, 3 H, CH_3), 0.98 (t, 3 H, CH_3).

4-Amino-1,6,7-triethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (13): from **5** (3.0 g, 11.75 mmol) and diethyl sulfate (3 mL). Recrystallization from water/methanol yielded **13** (2.3 g, 69%): ^{1}H NMR (DMSO-d_6) δ 8.65 (br s, 1 H, NH_2), 8.36 (br s, 1 H, NH_2), 4.03 (q, 2 H, CH_2), 2.89 (q, 2 H, CH_2), 2.82 (q, 2 H, CH_2), 1.29 (t, 3 H, CH_3), 1.26 (t, 3 H, CH_3), 1.25 (t, 3 H, CH_3).

4-Amino-1-ethyl-6,7,8,9-tetrahydro[1,2,6]thiadiazino[3,4-*b*]quinoxaline 2,2-dioxide (14): from **6** (3.0 g, 11.8 mmol) and diethyl sulfate (3 mL). Recrystallization from water/methanol yielded **14** (1.9 g, 57%): ^{1}H NMR (DMSO-d_6) δ 8.67 (br s, 1 H, NH_2), 8.52 (br s, 1 H, NH_2), 3.97 (q, 2 H, CH_2), 2.90 (m, 4 H, CH_2), 1.87 (m, 4 H, CH_2), 1.27 (t, 3 H, CH_3).

4-Amino-1-ethyl-6,7-bis(trifluoromethyl)pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (15): from **7** (2.5 g, 7.46 mmol) and diethyl sulfate (3 mL). Recrystallization from water/methanol yielded **15** (1.8 g, 66%): ^{1}H NMR (DMSO-d_6) δ 9.26 (br s, 1 H, NH_2), 8.99 (br s, 1 H, NH_2), 4.06 (q, 2 H, CH_2), 1.32 (t, 3 H, CH_3).

4-Amino-1-ethyl-6,7-diisopropylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (16): from **8** (2.0 g, 7.1 mmol) and diethyl sulfate (2 mL). Recrystallization from water/methanol yielded **16** (1.7 g, 75%): ^{1}H NMR (DMSO-d_6) δ 8.73 (br s, 1 H, NH_2), 8.45 (br s, 1 H, NH_2), 4.02 (q, 2 H, CH_2), 3.43 (m, 1 H, CH), 3.36 (m, 1 H, CH), 1.30 (t, 3 H, CH_3), 1.25 (d, 6 H, $2CH_3$), 1.23 (d, 6 H, $2CH_3$).

4-Amino-1-ethyl-6,7-dimethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (17): from 4-amino-6,7-dimethyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[3] (4.0 g, 16.5 mmol) and diethyl sulfate (4 mL). Recrystallization from water/ethanol yielded **17** (2.8 g, 66%): ^{1}H NMR (DMSO-d_6) δ 8.66 (br s, 1 H, NH_2), 8.55 (br s, 1 H, NH_2), 4.00 (q, 2 H, CH_2), 2.55 (s, 3 H, CH_3), 2.50 (s, 3 H, CH_3), 1.27 (t, 3 H, CH_3).

4-Amino-1-ethyl-7-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (18): from 4-amino-7-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[5] (1.5 g, 7.0 mmol) and diethyl sulfate (1.5 mL). Recrystallization from water/ethanol yielded **18** (0.9 g, 53%): ^{1}H NMR (DMSO-d_6) δ 8.70 (br s, 2 H, NH_2), 8.31 (s, 1 H, CH), 4.02 (q, 2 H, CH_2), 2.57 (s, 3 H, CH_3), 1.28 (t, 3 H, CH_3).

4-Amino-6-bromo-1-ethyl-7-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (19): from 4-amino-6-bromo-7-methyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[5] (2.0 g, 6.8 mmol) and diethyl sulfate (2.0 mL). Recrystallization from water/methanol yielded **19** (0.8 g, 37%): ^{1}H NMR (DMSO-d_6) δ 8.81 (br s, 1 H, NH_2), 8.68 (br s, 1 H, NH_2), 4.01 (q, 2 H, CH_2), 2.66 (s, 3 H, CH_3), 1.28 (t, 3 H, CH_3).

4-Amino-1-ethyl-7-phenylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (20): from 4-amino-7-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[6] (2.0 g, 7.3 mmol) and diethyl sulfate (2.0 mL). Recrystallization from water/methanol yielded **20** (1.2 g, 54%): ^{1}H NMR (DMSO-d_6) δ 9.00 (s, 1 H, CH), 8.74 (br s, 2 H, NH_2), 8.26 (m, 2 H, Ph), 7.60 (m, 3 H, Ph), 4.16 (q, 2 H, CH_2), 1.39 (t, 3 H, CH_3).

4-Amino-6-bromo-1-ethyl-7-phenylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (21): from 4-amino-6-bromo-7-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[5] (2.0 g, 5.6 mmol) and diethyl sulfate (2.0 mL). Recrystallization from water/methanol yielded **21** (1.1 g, 51%): ^{1}H NMR (DMSO-d_6) δ 8.93 (br s, 1 H, NH_2), 8.80 (br s, 1 H, NH_2), 7.80 (m, 2 H, Ph), 7.55 (m, 3 H, Ph), 4.02 (q, 2 H, CH_2), 1.29 (t, 3 H, CH_3).

Example of Method B of Table I. 4-Amino-1-[(ethoxycarbonyl)methyl]-7-phenylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (22). To a solution of 4-amino-7-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[5] (2.0 g, 7.3 mmol) in acetone (125 mL) were added potassium carbonate (0.5 g, 3.6 mmol), potassium iodide (catalytic amounts), and ethyl bromoacetate (1 mL). The reaction mixture was refluxed for 24 h and evaporated to dryness, and water was added to the residue. The precipitate was filtered and recrystallized from ethanol/water to give **22** (1.9 g, 72%): ^{1}H NMR (acetone-d_6) δ 9.00 (s, 1 H, CH), 8.23 (m, 2 H, Ph), 7.56 (m, 3 H, Ph), 4.82 (s, 2 H, CH_2), 4.18 (q, 2 H, CH_2), 1.20 (t, 3 H, CH_3).

Method B was followed to prepare compounds **23** and **24**.

4-Amino-1-ethyl-6-phenylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (23): from 4-amino-6-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[5] (3.0 g, 10.9 mmol), acetone (300 mL), potassium carbonate (0.75 g, 5.4 mmol), and ethyl iodide (3 mL). Recrystallization from water/methanol yielded **23** (1.9 g, 57%): ^{1}H NMR (DMSO-d_6) δ 9.31 (s, 1 H, CH), 8.86 (br s, 2 H, NH_2), 8.30 (m, 2 H, Ph), 7.50 (m, 3 H, Ph), 4.10 (q, 2 H, CH_2), 1.34 (t, 3 H, CH_3).

4-Amino-1-ethyl-6-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (24): from 4-amino-6-methyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[5] (2.0 g, 9.4 mmol), acetone (200 mL), potassium carbonate (0.65 g, 4.7 mmol), and ethyl iodide (3 mL). Recrystallization from water/methanol yielded **24** (1.2 g, 60%): ^{1}H NMR (DMSO-d_6) δ 8.77 (br s, 1 H, NH_2), 8.63 (s, 2 H, NH_2, CH), 4.02 (q, 2 H, CH_2), 2.53 (s, 3 H, CH_3), 1.27 (t, 3 H, CH_3).

Example of Method C of Table I. 4-Amino-7-ethyl-6-methyl-1-propylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (25). A mixture of **3** (2.0 g, 8.3 mmol) in hexamethyldisilazane (50 mL) and trimethylsilyl chloride (catalytic amounts) under argon was refluxed for 7 h. The reaction mixture was evaporated to dryness in vacuo, and the residue was treated with dichloromethane (50 mL), propyl iodide (1.1 mL), and potassium carbonate (0.6 g, 4.3 mmol) and refluxed for 4 h. The reaction mixture was evaporated to dryness, and water was added to the residue. The precipitate was filtered and recrystallized from water to give **25** (1.1 g, 47%): ^{1}H NMR (DMSO-d_6) δ 8.65 (br s, 1 H, NH_2), 8.51 (br s, 1 H, NH_2), 3.91 (t, 2 H, CH_2), 2.55 (s, 3 H, CH_3), 2.51 (s, 3 H, CH_3), 1.72 (m, 2 H, CH_2), 0.89 (t, 3 H, CH_3).

Compounds **26** and **27** were prepared following method C from 4-amino-6,7-dimethyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide and ethyl bromoacetate and propyl iodide, respectively.

4-Amino-1-[(ethoxycarbonyl)methyl]-6,7-dimethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (26): yield (73%); ^{1}H NMR (DMSO-d_6) δ 8.82 (br s, 1 H, NH_2), 8.69 (br s, 1 H, NH_2), 4.65 (s, 2 H, CH_2), 4.14 (q, 2 H, NCH_2), 2.53 (s, 3 H, CH_3), 2.52 (s, 3 H, CH_3), 1.17 (t, 3 H, CH_3).

4-Amino-6,7-dimethyl-1-propylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (27): yield (41%); ^{1}H NMR (DMSO-d_6) δ 8.65 (br s, 1 H, NH_2), 8.51 (br s, 1 H, NH_2), 3.91 (t, 2 H, CH_2), 2.55 (s, 3 H, CH_3), 2.51 (s, 3 H, CH_3), 1.72 (m, 2 H, CH_2), 0.89 (t, 3 H, CH_3).

Example of Method D of Table I. 4-Amino-1-[2-(*N*,*N*-diisopropylamino)ethyl]-6,7-dimethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (28). A mixture of 4-amino-6,7-dimethyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (3.0, 13.2 mmol), water (150 mL), potassium carbonate (8.0 g), dichloromethane (125 mL), tetrabutylammonium bromide (0.3 g, 0.9 mmol), and 2-(diisopropylamino)ethyl chloride hydrochloride (2.65 g, 13.2 mmol) was stirred at room temperature for 24 h. The organic layer was separated, evaporated to dryness and recrystallized from ethanol/water to give **28** (3.3 g, 70%): ^{1}H NMR (DMSO-d_6) δ 8.66 (br s, 1 H, NH_2), 8.53 (br s, 1 H, NH_2), 3.85 (m, 2 H, N-CH_2), 2.96 (m, 2 H, 2CH), 2.66 (m, 2 H, CH_2), 2.55 (s, 3 H, CH_3), 2.51 (s, 3 H, CH_3), 0.98 (d, 6 H, CH_3).

Compounds **29** and **30** were prepared following essentially method D from 4-amino-6,7-dimethyl-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide and 2-(dimethylamino)ethyl chloride hydrochloride and 4-(2-chloroethyl)morpholine hydrochloride, respectively.

4-Amino-6,7-dimethyl-1-(2-morpholinoethyl)pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (29): yield 42% (recrystallized from water/ethanol); ^{1}H NMR (DMSO-d_6) δ 8.68 (br s, 1 H, NH_2), 8.54 (br s, 1 H, NH_2), 4.07 (t, 2 H, CH_2), 3.48 (m, 4 H, $2CH_2$), 2.60 (t, 2 H, CH_2), 2.55 (s, 3 H, CH_3), 2.52 (s, 3 H, CH_3), 2.43 (m, 4 H, CH_2).

4-Amino-1-[2-(*N*,*N*-dimethylamino)ethyl]-6,7-dimethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (30): yield 41% (recrystallized from water/ethanol); ^{1}H NMR (DMSO-d_6) δ 8.69

(br s, 1 H, NH_2), 8.68 (br s, 1 H, NH_2), 4.04 (t, 2 H, NCH_2), 2.56 (t, 5 H, CH_2, CH_3), 2.51 (s, 3 H, CH_3), 2.18 (s, 6 H, CH_3).

1,7-Diethyl-4-(methylamino)-6-methylpyrazino[2,3-c][1,2,6]thiadiazine 2,2-Dioxide (31). A solution of **9** (1.0 g, 3.7 mmol) in acetone (60 mL) was treated with potassium carbonate (0.26 g, 1.85 mmol) and methyl iodide (2 mL). The reaction mixture was refluxed for 24 h and evaporated to dryness, and water was added to the residue. The precipitate was filtered and purified on silica gel using dichloromethane as eluent to afford **31** (0.7 g, 67%): ^{1}H NMR (DMSO-d_6) δ 9.08 (q, 1 H, NH), 4.03 (q, 2 H, NH_2), 2.91 (d, 3 H, CH_3), 2.89 (q, 2 H, CH_2), 2.53 (s, 3 H, CH_3), 1.30 (t, 3 H, CH_3), 1.26 (t, 3 H, CH_3).

X-ray Analysis of 9. A prismatic single crystal of dimensions 0.2 × 0.3 × 0.3 mm was used to collect 2126 independent reflections (1836 considered as observed with $I > 2\sigma(I)$ criterion) up to θ = 65° with graphite monochromated Cu Kα radiation on a PW1100 diffractometer by using an $\omega/2\theta$ scan mode. No crystal decomposition was observed. Unit cell parameters were refined using 50 reflections measured for both positive and negative Bragg angles. The structure was solved using the Patterson function. All hydrogen atoms were located on a difference map. Anisotropic (isotropic fixed for H atoms) refinement was carried out with XRAY76.[10] Coordinates for H atoms were also refined. An empirical weighting scheme was used to avoid dependences in $\langle w\Delta^2F\rangle$ vs $\langle F_o\rangle$ and vs $\langle \sin\theta/\lambda\rangle$. Final disagreement indices are $R = 0.053$ and $R_w = 0.053$ and no peak in the final difference map exceeded 0.25 eÅ^{-3}. Fractional coordinates and thermal parameters have been deposited as supplementary material.

Pharmacological Methods. Diuretic Activity in Water-Loaded Rats. Diuretic and saluretic activity was assessed in male rats (Sprague–Dawley, SPF, HC/CFY strain, 175–200 g) according to the method reported by Lipschitz et al.[11] with minor modifications. A primary screening for compounds was performed as follows. Groups of eight rats deprived of food and water for 18 h prior to and during the experiment, were dosed by gavage with the vehicle (1% carboxymethyl cellulose with 0.1% Tween 80 in water), test compounds at 20–25 mg/kg, or reference diuretics (hydrochlorothiazide, 10 mg/kg, and triamterene, 25 mg/kg), in a volume of 0.5 mL/100 g body weight (bw). Each group was immediately hydrated with distilled water (2.5 mL/100 g bw, po). The rats were placed individually in plastic metabolic cages, and urine was collected for 6 h after dosing. The cumulative urine volume and urinary electrolyte outputs were determined (Na^+ and K^+ by flame photometry, and Cl^- colorimetrically). For each product, mean diuresis and natriuresis were quantitated and the ratio between the effects observed in treated animals and those of control rats were calculated. Ratios equal or greater than 1.5 and 2 for urine volume and Na^+ excretion, respectively, were taken as criteria for activity. A secondary test was carried out on all agents found active in the primary test. These selected agents were submitted to a dose–response study at three dose levels (10, 20, and 40 mg/kg, po), and tested for effects on diuresis as well as electrolyte excretion using the methodology mentioned above. The order of activity was established taking into account ED_{100}, ED_2, and ED_1 values, defined as doses required to produce an increase of 100% in urine production (mL/kg bw) and an excretion of 2 mequiv of Na^+/kg bw and 1 mequiv of K^+/kg bw, respectively, during the 6-h collection period. Oral activity for the most active compound selected, in comparison to that of standard diuretics, was evaluated using a wide range of doses. In addition, onset and duration of diuretic and saluretic effects were established in rats (two/metabolic cage) using the protocol described, except that each parameter was determined at 0–1-, 1–2-, 2–3-, 3–4-, and 4–6-h intervals after oral dosing. Dose–response relationships were calculated by linear regression analysis.[12] Nonpaired Student t-test was used to determine a significant difference in the urine volume and electrolyte outputs between groups.

Acute Lethal Toxicity. LD_{50} values were determined according to the method of Litchfield and Wilcoxon[13] from the 15-days mortality in male Swiss (SPF, HC/CFLP strain, 20–25 g) mice.

Supplementary Material Available: Tables listing atomic parameters, bond distances, bond angles, torsional angles, ring planes and atomic contact, for compound **9** (17 pages). Ordering information is given on any current masterhead page.

Registry No. 1, 61403-61-0; 2, 141957-13-3; 3, 141957-14-4; 4, 141957-15-5; 5, 141957-16-6; 6, 141957-17-7; 7, 141957-18-8; 8, 141957-19-9; 9, 141957-20-2; 10, 141957-21-3; 11, 141957-22-4; 12, 141957-23-5; 13, 141957-24-6; 14, 141957-25-7; 15, 141957-26-8; 16, 141957-27-9; 17, 141957-28-0; 18, 141957-29-1; 19, 141957-30-4; 20, 141957-31-5; 21, 141957-32-6; 22, 141957-33-7; 23, 141957-34-8; 24, 141957-35-9; 25, 141957-36-0; 26, 141957-37-1; 27, 141957-38-2; 28, 141957-39-3; 29, 141957-40-6; 30, 141957-41-7; 31, 141957-42-8; 32, 93290-56-3; $CH_3CH_2C(O)C(CH_3)$=NOH, 32818-79-4; $CH_3CH_2C(O)C(O)CH_3$, 600-14-6; $CH_3(CH_2)_2C(O)C(O)CH_3$, 3848-24-6; $CH_3CH_2C(O)C(O)CH_2CH_3$, 4437-51-8; $CF_3C(O)C(O)CF_3$, 685-24-5; $C(CH_3)_2C(O)C(O)C(CH_3)_2$, 4388-87-8; (i-Pr)$_2$N-$(CH_2)_2$Cl·HCl, 4261-68-1; $(Me)_2N(CH_2)_2Cl$·HCl, 4584-46-7; 1,2-cyclohexanedione, 765-87-7; 4-amino-6,7-dimethyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, 93290-53-0; 4-amino-7-methyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, 131619-60-8; 4-amino-6-bromo-7-methyl-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide, 132992-66-6; 4-amino-7-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, 132992-63-3; 4-amino-6-bromo-7-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, 132992-65-5; 4-amino-6-phenyl-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide, 132992-62-2; 4-amino-6-methyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, 132992-64-4; 4-(2-chloroethyl)morpholine hydrochloride, 3647-69-6; hydrochlorothiazide, 58-93-5; triamterene, 396-01-0; furosemide, 54-31-9.

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