lowed for the preparation of **5**. Norketobemidone (23.3 g, 0.1 mol), 40 mL of 37% formaldehyde, sodium cyanoborohydride (10.0 g, 0.16 mol), and 300 mL of MeCN were stirred (**exothermic**) for 15 min. Glacial acetic acid was added to neutrality (wet litmus). The mixture was stirred for 45 min, maintaining neutrality, and then evaporated in vacuo. The residue was dissolved in 100 mL of distilled H_2O and made acidic with 6 N HCl. The mixture was basified with NH_4OH and extracted with three 100-mL portions of $CHCl_3$. The organic layer was washed with saturated NaCl, dried over $MgSO_4$, and evaporated in vacuo giving 21.2 g of a powder. Recrystallization from EtOAc afforded 17.1 g of **5**, mp 148–151 °C (lit.[14] mp 156–157 °C). The HBr salt was made, mp 191–193 °C (lit.[15] mp 194–196 °C).

A mixture of **5**·HBr (3.4 g, 10.4 mmol), 75 mL of EtOH, 1.4 g of KOH, and a solution of $NaBH_4$ (1.44 g, 38.1 mmol) in 20 mL of H_2O was maintained at 80 °C for 2 h. The mixture was evaporated in vacuo and acidified with 6 N HCl. Basification with NH_4OH and filtration gave 1.5 g of crude solid, mp 109–145 °C. Recrystallization from acetone gave 1.0 g of **9** (38%), mp 187–189 °C. A small sample was recrystallized from acetone for analysis: mp 188–190 °C; IR (KBr) 3250 cm^{-1} (OH), no carbonyl; ^1H NMR (Me_2SO-d_6) δ 3.2 (m, 1 H, C-H), 6.5–7.35 (m, 4 H, aromatic). Anal. ($C_{15}H_{23}NO_2$) C, H, N.

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Synthesis and Diuretic Profile of 3-(3-Amino-1,2,4-oxadiazol-5-yl)-5-chloro-2,6-pyrazinediamine, an Amiloride-Type Diuretic

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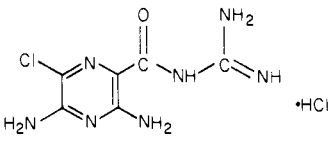
The synthesis of an analogue of amiloride in which the acylguanidine moiety has been replaced by a 1,2,4-oxadiazol-3-amine unit is described. This substance (**3**, CGS 4270) exhibited a diuretic profile similar to that of amiloride when evaluated in the rat and the dog. In the rat, combination with hydrochlorothiazide increased diuresis and saluresis and returned potassium levels to control values. A series of 5-aryl-1,2,4-oxadiazol-3-amines not directly related to amiloride was prepared, but these substances had no diuretic activity.

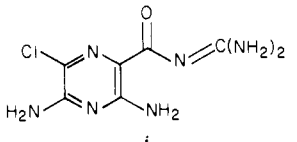
Amiloride (**1**)[1] is a clinically effective potassium sparing



1

diuretic which is marketed in a number of countries throughout the world. It is especially effective when used in combination with a thiazide diuretic, as the effects of the two agents are additive with respect to the excretion of water and sodium but antagonistic with respect to the excretion of potassium.[3] Of the many analogues of **1** which have been prepared and tested, the carboxamidoguanidine

(1) Formulation **1** has been routinely used for amiloride, but the major tautomeric form in Me_2SO is apparently **i**.[2]



i

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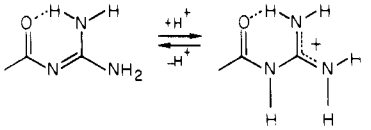
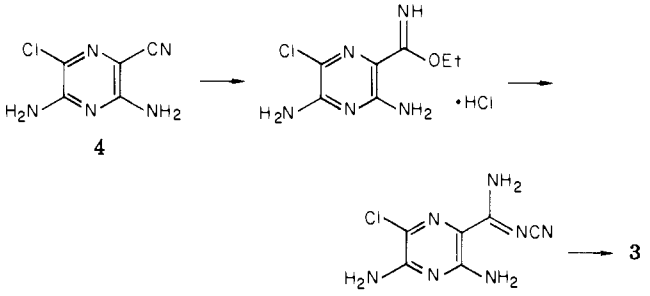
Scheme I

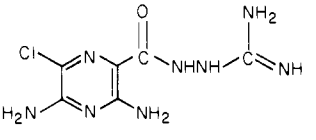


Scheme II



4

2 is apparently the only one where a significant structural departure did not result in loss of useful diuretic activity.[4,5]



2

Table I. Diuretic Activity in the Rat

dose, mg/kg po	oxadiazole analogue 3			amiloride (1)		
	% of load[a]	total Na^+, mequiv × 100	total K^+, mequiv × 100	% of load[a]	total Na^+, mequiv × 100	total K^+, mequiv × 100
5[b]	81.1[d]	24.7[e] ± 2.7	1.1[c] ± 0.2	94.0[d]	38.0[d] ± 6.1	1.5[c] ± 0.4
control	58.0	4.7 ± 0.6	3.2 ± 0.9	68.6	7.4 ± 2.2	5.1 ± 1.3
10	88.8[c]	33.8[e] ± 0.9	2.0[c] ± 0.3	96.8[c]	46.0[e] ± 1.7	1.7[d] ± 0.6
control	69.1	8.5 ± 1.6	7.1 ± 1.7	78.2	10.9 ± 0.5	8.3 ± 1.6
25	93.3[e]	33.6[e] ± 2.8	1.8[e] ± 0.2	82.1	33.6[c] ± 5.0	1.6[d] ± 0.8
control	64.0	4.7 ± 1.4	5.0 ± 0.4	78.2	10.9 ± 0.5	8.3 ± 1.6
50	103.0[e]	42.7[e] ± 4.4	1.9[c] ± 0.2	64.9	30.8[c] ± 7.2	1.1[c] ± 1.1
control	64.9	3.0 ± 0.8	5.3 ± 1.1	72.4	9.2 ± 1.5	6.3 ± 1.1
100	100.4[d]	41.6[e] ± 5.6	2.1[d] ± 0.5	59.7	20.3[c] ± 2.0	1.1[e] ± 0.3
control	64.0	4.7 ± 1.4	5.0 ± 0.4	69.9	13.5 ± 1.6	5.9 ± 0.8

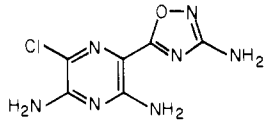
[a] Normotensive rats were fasted overnight. An oral load of 50 mL/kg of 0.2% NaCl was given to each of four rats along with the test agent. The rats were placed in metabolism cages and urine was collected for 3 h. Sodium and potassium content of the 3-h sample was measured by flame photometry. [b] Eight rats were used in this case. [c] $p < 0.05$. [d] $p < 0.01$. [e] $p < 0.001$.

Table II. Diuretic Activity in the Dog

dose, mg/kg po	oxadiazole analogue 3			amiloride (1)		
	urine vol[a]	mequiv of Na^+	mequiv of K^+	urine vol[a,b]	mequiv of Na^+	mequiv of K^+
5	91.0[d] ± 11.8	23.5[e] ± 1.9	1.8[d] ± 0.3	91.2 ± 24.7	24.9[d] ± 4.0	1.1[e] ± 0.1
control	45.2 ± 1.1	7.8 ± 1.0	4.8 ± 0.6	39.8 ± 3.0	6.4 ± 1.4	3.7 ± 0.4
10	76.8 ± 15.9	21.2[d] ± 2.6	1.6[d] ± 0.6	88.2 ± 29.4	27.9[d] ± 5.4	1.4[d] ± 0.4
control	41.3 ± 2.9	7.4 ± 1.4	5.4 ± 0.7	38.4 ± 2.8	6.1 ± 1.8	3.8 ± 0.5

[a] Six normotensive trained dogs (10.2-17.9 kg) were used. Each was given a control test with placebo prior to each test run. Immediately after drug administration, normal saline (100 mL) was injected subcutaneously. The urinary bladder was evacuated by catheterization at the onset and at 2, 4, and 6 h after drug administration. The dogs were kept in metabolism cages, total urine volume was recorded, and sodium and potassium content of an aliquot was determined by flame photometry. [b] Five dogs were used for the 10 mg/kg test runs. [c] $p < 0.05$. [d] $p < 0.01$. [e] $p < 0.001$.

We reasoned that it should be possible to design an analogue of 1 in which the acylguanidine moiety[1] was replaced by a different functional unit. While in principle 1 is a conformationally mobile molecule, it seemed to us that various constraints on this mobility would be operative. In particular, the amide bond could be assumed to be essentially rigid, and hydrogen bonding between the carbonyl oxygen and a hydrogen atom attached to the guanidine group appeared likely. Smith et al.[2] have commented on the conformational integrity of the acylguanidine unit under both neutral and acidic conditions (Scheme I). It occurred to us that the cyclic structural elements shown in Scheme I were quite similar to a 1,2,4-oxadiazol-3-amine system and, accordingly, our synthetic goal was defined as **3**.



3

In their studies on the preparation and reaction of *N*-cyanoamidines, Huffman and Schaefer[6] described a sequence of reactions for the preparation of 5-phenyl-1,2,4-oxadiazol-3-amine from benzonitrile which we have successfully applied to the synthesis of **3**. Thus, treatment of the nitrile **4**[7] with ethanolic HCl at room temperature gave the iminoether hydrochloride (Scheme II), which was converted, without purification, to the *N*-cyanoamidine by treatment with cyanamide in methanol at room temperature in the presence of potassium carbonate. Finally, refluxing the *N*-cyanoamidine with hydroxylamine hydrochloride in ethanolic pyridine gave **3**.

Table III. Diuretic Activity of Hydrochlorothiazide Alone and in Combination with 3 in the Rat

drug	dose, mg/kg po	% of load[a]	total Na^+, mequiv × 100	total K^+, mequiv × 100
control		63.4	9.9 ± 1.7	7.7 ± 0.7
hydrochlorothiazide	50	83.9	40.0[d] ± 4.0	18.9[c] ± 2.0
hydrochlorothiazide + 3	50 / 10	96.9	65.1[b] ± 20.8	8.5 ± 0.4

[a] See footnote *a*, Table I. [b] $p < 0.05$. [c] $p < 0.01$. [d] $p < 0.001$.

Table IV. Diuretic Activity of 5[a] in the Rat

dose, mg/kg po	% of load[b]	total Na^+, mequiv × 100	total K^+, mequiv × 100
50	76.2	32.9[d] ± 2.5	1.7[c] ± 0.5
control	57.3	2.8 ± 0.5	6.2 ± 1.5

[a] NMR δ 0.86 (t, 3 H), 1.66 (m, 2 H), 3.13 (m, 5 H), 3.56 (t, 2 H), 6.28 (s, 2 H), 7.34 (s, 2 H); IR 3110, 3270, 3400 cm^{-1} (N-H); MS *m/e* (relative intensity) 283 (M^+, 52), 254 (100). Anal. ($C_{10}H_{14}ClN_7O$) C, H, N. [b] See footnote *a*, Table I. [c] $p < 0.05$. [d] $p < 0.001$.

The diuretic activity of **3** (CGS 4270) was determined in the rat and the dog; comparative data on 1 were also

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(6) K. R. Huffman and F. C. Schaefer, *J. Org. Chem.*, 28, 1812 (1963).
(7) J. H. Jones and E. J. Cragoe, *J. Med. Chem.*, 11, 322 (1968).

Table V. 5-Aryl-1,2,4-oxadiazol-3-amines and Intermediate *N*-Cyanoamidines

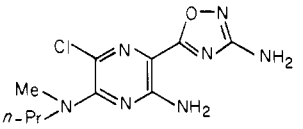
R	mp, °C	formula	anal.	mp, °C	formula	anal.
3-pyridyl	205-207	$C_7H_5N_4O$	C, H, N	224-225	$C_7H_6N_4$	C, H, N
2-thienyl	162-164	$C_6H_5N_3OS$	C, H, N	230-232	$C_6H_5N_3S$	C, H, N
3-thienyl	190-192	$C_6H_5N_3OS$	C, H, N	204-206	$C_6H_5N_3S$	C, H, N
3,4-Cl_2Ph	223-225	$C_8H_5Cl_2N_3O$	C, H, N	252-254	$C_8H_5Cl_2N_3$	C, H, N
3-NH_2-4-pyrazolyl	246-247	$C_5H_6N_6O$	C, H, N	242-243	$C_5H_6N_6$	C, H, N

obtained. As shown in Table I, the effects of the two agents in the rat are similar, the main difference being that **3** produced maximal effects at 50 mg/kg with similar responses at 100 mg/kg, while **1** produced maximal effects at 10 mg/kg with successively larger doses up to 100 mg/kg producing progressively smaller effects.

In the dog, the diuretic activity of the two agents was determined at 5 and 10 mg/kg po (Table II). Again, the diuretic effects of the two agents were similar, although the effects of **3** were somewhat reduced at the higher dose.

Finally, an assessment was made of the effects of a combination of **3** and hydrochlorothiazide in the rat. As shown in Table III, the addition of a 10 mg/kg po dose of **3** to a 50 mg/kg po dose of hydrochlorothiazide resulted in increased diuresis and naturesis, while potassium loss was prevented.

Some information on the effect of alkyl substitution on N-6 of the pyrazine ring of **3** was obtained from evaluation of the methylpropylamino analogue **5**. As indicated in



5

Table IV, this substance had similar but weaker activity in comparison to **3** when tested at 50 mg/kg po in the rat. Analytical data are also included in Table IV.

We prepared a number of 5-aryl-1,2,4-oxadiazol-3-amines not directly related to **1** by the method described for the preparation of **3**. These substances and the intermediate *N*-cyanoamidines are listed in Table V. None of these substances exhibited significant diuretic activity.

Conclusion

3-(3-Amino-1,2,4-oxadiazol-5-yl)-5-chloro-2,6-pyrazinediamine (**3**, CGS 4270) has been identified as a diuretic with an overall profile similar to that of amiloride (**1**).

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. NMR spectra are obtained either on a Varian A-60A or XL-100 instrument, IR spectra on a Perkin-Elmer 137 spectrophotometer, and mass spectra on an A.E.I. MS902 spectrometer.

N^{α}-Cyano-3,5-diamino-6-chloropyrazine-2-carboxamidine. 3,5-Diamino-6-chloro-2-pyrazinecarbonitrile (**4**; 10 g)[7] was added to a solution of hydrogen chloride (500 g) in ethanol (4 L) at 0 °C. The solution was stirred for 20 min at 0 °C and then at room temperature for 72 h. The solution was evaporated and the residue triturated with ether (25 mL) to give 15 g of the iminoether hydrochloride: mp 240–245 °C dec; IR no absorption at 2180 cm^{-1}, characteristic of **4**.

Cyanamide (4 g) was added to a solution of the iminoether hydrochloride (15 g) and anhydrous potassium carbonate (9.5 g) in methanol (800 mL). The reaction mixture was stirred at room temperature for 30 h and evaporated. The residue was stirred with water (200 mL) and filtered. The solid was washed with water (50 mL), dried, and then boiled with methanol (100 mL) to give the *N*-cyanoamidine (8.0 g, 64% from **4**): NMR δ 7.47 (s, 4 H), 8.00 (s, 2 H); IR 2165 (C≡N), 3180, 3220, 3300 cm^{-1} (N-H); MS *m/e* (relative intensity) 211 (M^+, 100), 176 (56), 170 (45). Anal. ($C_6H_6ClN_7$) C, H, N.

3-(3-Amino-1,2,4-oxadiazol-5-yl)-5-chloro-2,6-pyrazinediamine. Hydroxylamine hydrochloride (6.0 g) was added to a solution of N^{α}-cyano-3,5-diamino-6-chloropyrazine-2-carboxamidine (6.0 g) in methanol (600 mL) and pyridine (84 mL). The solution was refluxed for 24 h and evaporated, and the residue was stirred with water (250 mL) and filtered. The solid was washed with water (70 mL), dried, and then boiled with methanol (100 mL) to give the oxadiazole **3** (CGS 4270; 4.5 g, 70%): NMR δ 6.26 (s, 2 H), 7.32 (s, 4 H); IR 3100, 3280, 3420 cm^{-1} (N-H); MS *m/e* (relative intensity) 227 (M^+, 100), 171 (77). Anal. ($C_6H_6ClN_7O$) C, H, N.

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