s, 13-Me), 2.7–3.0 (4 H, m, 6-CH_2 and 21-CH_2), 3.22 (1 H, t, J = 3.5 Hz, 20-CH), 6.6–7.3 (3 H, m, A-ring CH's); MS, 314.1880 (M, $C_{20}H_{26}O_3$). Anal. ($C_{20}H_{26}O_3$) C, H. Fractional crystallization from methanol gave the (20*S*)-20,21-epoxide **3** (11%): mp 184–200 °C dec;[14] $[\alpha]_D$ +46.6° (*c* 0.9); IR (KBr) 3600, 3510 and 3240, 3420 (OH) cm^{-1}; ^1H NMR ($CDCl_3$/$(CD_3)_2CO$/D_2O) δ 0.94 (3 H, s, 13-Me), 2.7–3.0 (4 H, m, 6-CH_2 and 21-CH_2), 3.34 (1 H, t, J = 3.5 Hz, 20-CH), 6.6–7.3 (3 H, m, A-ring CH's); MS, 314.1876 (M, $C_{20}H_{26}O_3$). Anal. ($C_{20}H_{26}O_3$) C, H.

(20*S*)-3,21-Dihydroxy-17β,20-epoxy-19-norpregna-1,3,5(10)-triene (4). K_2CO_3 (1.31 g, 7.9 mmol) was added to a solution of the (20*R*)-20,21-epoxide **2** (1.01 g, 3.2 mmol) in methanol (69 mL). The resulting suspension was stirred overnight at room temperature during which time the suspended solid dissolved. Ethyl acetate was added to the methanol solution, and the resultant solution was washed with water, dried, and evaporated to afford a white solid (0.81 g, 80%), which on crystallization from ethyl acetate gave the (20*S*)-17β,20-epoxide **4** (0.63 g, 62%): mp 173–260 °C dec;[14] $[\alpha]_D$ +37.6° (*c* 0.9); IR (KBr) 3420, 3320 (OH) cm^{-1}; ^1H NMR ($(CD_3)_2CO$/D_2O) δ 0.87 (3 H, s, 13-Me), 2.6–3.0 (2 H, m, 6-CH_2), 3.17 (1 H, t, J = 5.5 Hz, 20-CH), 3.66 (2 H, m, 21-CH_2), 6.5–7.3 (3 H, m, A-ring CH's). At 400 MHz the multiplet at δ 3.66 is fully resolved into the expected 8 lines of the AB component of an ABX system (J_{AB} = 12 Hz); MS, 314.1885 (M, $C_{20}H_{26}O_3$). Anal. ($C_{20}H_{26}O_3$) C, H.

(20*R*)-3,21-Dihydroxy-17β,20-epoxy-19-norpregna-1,3,5(10)-triene (5). KO-*t*-Bu (1.0 g, 8.20 mmol) was added to a solution of the (20*S*)-20,21-epoxide **3** (0.93 g, 2.96 mmol) in *t*-BuOH (30 mL) at 30 °C. The reaction mixture was stirred overnight while the temperature was maintained at 30–40 °C after which it was diluted with water and extracted (2×) with ethyl acetate. The combined extracts were washed with water, dried, and evaporated to afford a white solid (0.83 g, 89%), which on crystallization from ethyl acetate (2×) gave the (20*R*)-17β,20-epoxide **5** (0.37 g, 40%): mp 185–189 °C[14]; $[\alpha]_D$ +29.9° (*c* 1.0); IR (KBr) 3160, 3400 (OH) cm^{-1}; ^1H NMR ($(CD_3)_2CO$/D_2O) δ 0.99 (3 H, s, 13-Me), 2.6–2.9 (2 H, m, 6-CH_2), 3.09 (1 H, q, J = 4 and 7 Hz, 20-CH), 3.90 (2 H, m (8 lines), J_{AB} = 12 Hz, 21-CH_2), 6.5–7.2 (3 H, m, A-ring CH's); MS 314.1876 (M, $C_{20}H_{26}O_3$). Anal. ($C_{20}H_{26}O_3$) C, H.

Cell Culture Assay. HeLa S3 cells were cultivated in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 1% nonessential amino acids. GH3 cells were cultivated in Ham's F-10 medium supplemented with 15% donor horse serum and 2 mM L-glutamine. Both cell lines were grown in the presence of penicillin (50 IU mL^{-1}) and streptomycin (50 μg mL^{-1}). Compounds were assayed following the Cancer Chemotherapy National Service Center (CCNSC) protocol for KB cells.[15] Cell numbers were determined with use of a Coulter counter, and growth-inhibition curves were plotted from mean cell counts at 48 h. In all cases drugs were added in Me_2SO, which did not exceed a final concentration of 0.5%.

Inhibition of [^{3}H]Estradiol Binding. Immature rat uteri from estradiol benzoate treated rats (0.16 μg daily ×3) were homogenized in TED buffer (0.01 M Tris, 0.001 5 M EDTA, 0.000 5 M dithiothreitol, pH 7.4 at 25 °C). The homogenate was centrifuged at 100000*g* for 1 h (4 °C), and the cytosols were used immediately. Cytosol (150 μL) was incubated with different concentrations of competing ligands added in TED buffer (50 μL) and [^{3}H]estradiol (3.5×10^{-8} M, 50 μL) in TED buffer at 30 °C for 30 min. Parallel incubation of cytosol (150 μL), [^{3}H]estradiol (50 μL), and 50 μL TED or 50 μL of a solution of diethylstilbestrol in TED was used to determine the specific binding of [^{3}H]estradiol. All tubes were cooled in ice/water for 15 min, and then 200 μL of a suspension of dextran-coated charcoal (250 mg % Norit-A, 2.5 mg % dextran) in TED buffer was added and allowed to stand for 20 min in ice/water with occasional shaking. Tubes were centrifuged at 2000*g* for 5 min, and 200-μL samples of the supernatant were added to 5 mL Unisolve E (Koch-Light) and counted in a Phillips PW4700 liquid scintillation counter. Counting efficiency was 30–35%.

Reversibility of Drug–Estrogen-Receptor Binding. Cytosol aliquots (300 μL) were incubated with the test compound, at a level that displaced all specificity bound [^{3}H]estradiol, in TED buffer (100 μL) and [^{3}H]estradiol (35 nM, 100 μL) in TED buffer at 4 °C. After 16 h, dextran-coated charcoal suspension (400 μL) was added. Twenty minutes later, the tubes were centrifuged (2000*g*, 5 min), and an aliquot of the supernatant was counted for radioactivity to give a base-line level for binding. To the remaining supernatant, 20 μL of [^{3}H]estradiol (35 nM) was added, and the mixture was incubated for 20 h at 25 °C, then treated with dextran–charcoal, centrifuged as above, and the radioactivity in the supernatant counted. An increase in bound [^{3}H]estradiol after the second incubation was taken as an indication of the reversibility of binding of competing ligands. A parallel incubation containing 30 nM unlabeled estradiol to protect binding sites was included as a control on receptor denaturation during the prolonged incubations.

Acknowledgment. We thank the Cancer Research Campaign for a research studentship (to JCG) and the SERC for financial assistance in obtaining high-field ^1H NMR spectra.

Registry No. 1, 7678-95-7; **2**, 102651-47-8; **3**, 102651-48-9; 4, 102651-49-0; **5**, 102651-50-3.

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Synthesis and Biological Activity of 3-Amino-5-(3,5-diamino-6-chloropyrazin-2-yl)-1,2,4-oxadiazole: An Amiloride Prodrug

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The pyrazinyl-1,2,4-oxadiazoles **4a** and **4b** were synthesized by two different approaches. The corresponding *N*-methyloxadiazolium salts **13a** and **13b** were also prepared. These compounds were evaluated for their diuretic and saluretic activity in rats and dogs. All compounds exhibited electrolyte excretion profiles similar to amiloride 1. The facile conversion of **4a** to 1 was demonstrated chemically and in vivo in both rats and dogs.

The unique electrolyte excretion profile elicited by amiloride (1)[1] in experimental animals[2] has thrust it to the forefront in medicinal research.[3] Accordingly, this clinically effective,[4] potassium-sparing diuretic and its closely

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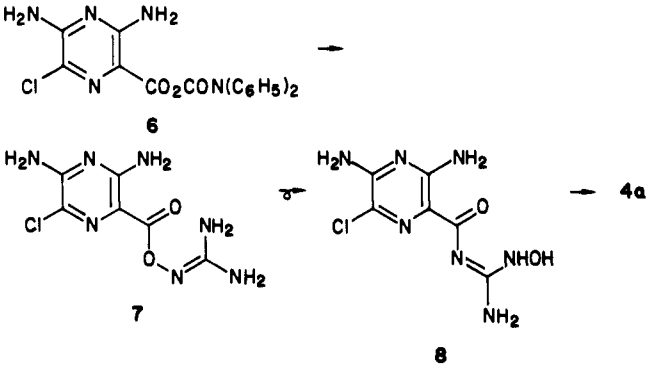
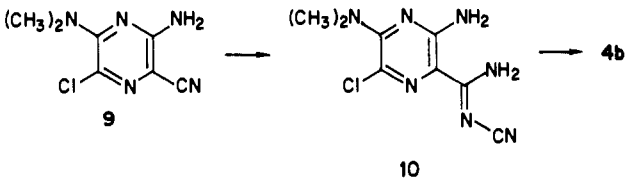
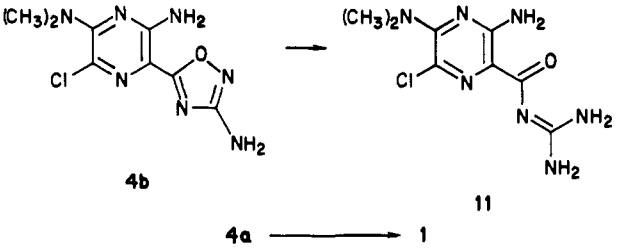
Scheme I



Scheme II

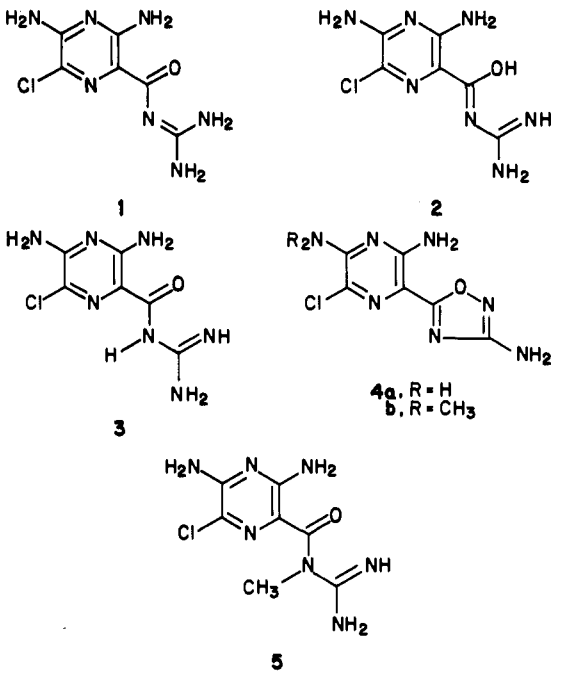


Scheme III



related analogues have been the subject of intense chemical[5] and biological[6] study for almost two decades. These efforts have established the pharmacological basis upon which amiloride manifests its diuretic activity[7] and have led to a stage where rational design of similar agents can reasonably be assumed as attainable.

Previous reports from these laboratories have detailed the optimal structure–activity relationships of amiloride (1),[5] including the seminal study[8] of the tautomeric and conformational dynamics of the acylguanidino side chain. In concert with the theoretical principles enumerated in the latter report, we designed amiloride analogues that would gauge the importance of conformation and tautomerism on the electrolyte excretion pattern of this class of compounds. Specifically, our goals were to investigate tautomeric structures exemplified by **2**, in which the guanidylimine bond is situated on the side chain terminus but still in conjugation and, hence, coplanar with the pyrazine ring, and to probe synthetic approaches to "unconjugated" acylguanidines as in **3**, where deviation of the side chain from the plane of the pyrazine ring seemed probable. Thus, our synthetic objectives were focused on the pyrazinyl-1,2,4-oxadiazoles **4** that represented cyclized forms of **2** and, therefore, appeared to meet the structural prerequisites for this tautomeric form. Additionally, we regarded the 1,2,4–oxadiazole ring in **4** as a valuable synthon that could subsequently be exploited chemically and, in principle, give access to the pyrazinyl-1-alkyl-1-acylguanidine **5**, a structural type forced to adopt the tautomeric form of **3**.



In this report we disclose the synthesis and biological activity of the pyrazinyl-1,2,4-oxadiazoles **4**[9] and their *N*-alkyl salts **13**[9] and discuss the ramifications of the reactivity of the 1,2,4-oxadiazole ring system on diuretic activity.[10]

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Table I. Comparative Oral Rat Diuretic Activity[a]

		diuretic effects							
		0–5 h				0–24 h			
compd	dose, mg/kg	Na^+, mequiv × 100	K^+, mequiv × 100	Cl^-, mequiv × 100	urine vol, mL	Na^+ mequiv × 100	K^+, mequiv × 100	Cl^- mequiv × 100	urine vol, mL
4a	3	64	7	36	24	184	79	152	47
	9	69	4	35	28	269	102	235	50
	27	67	4	32	26	292	79	258	46
	81	135	5	73	25	396	72	344	54
4b	3	64	2	40	28	202	97	170	52
	9	88	2	48	32	258	83	200	53
	27	107	1	63	31	341	82	270	56
	81	108	1	57	30	352	53	267	53
13a	3	17	3	28	25	281	139	203	48
	9	79	2	39	25	296	92	204	46
	27	104	4	49	27	389	89	277	51
	81	135	7	95	29	433	96	326	59
13b	3	55	2	37	26	185	114	153	48
	9	60	2	35	27	224	97	169	50
	27	100	6	56	29	299	62	209	52
	81	154	5	95	31	389	68	322	55
1	3	156	5	90	35	153	83	139	59
	9	162	6	96	33	207	67	176	64
	27	180	7	105	36	206	82	180	69
	81	158	6	93	28	458	47	328	66
placebo		13	17	15	24	93	127	94	43

[a] For testing protocol, see ref 19; female Sprague–Dawley rats weighing 160–170 g were used for the oral rat assay, three animals per cage, three cages per dose.

Chemistry. The pyrazinyl-1,2,4-oxadiazoles **4a** and **4b** could be prepared in either of two ways. In the first method (Scheme I), the mixed anhydride **6**[11] was reacted with hydroxyguanidine in 2-propanol to give initially the guanidyl ester **7**.[12] This material was then transformed to the *N*-acylhydroxyguanidine **8** by heating in DMF and subsequent precipitation with water.[13] Alternatively, extending the reaction time of the preparation of **7** in 2-propanol also effected O → N acyl transfer and afforded **8** directly, in comparable yield. Additional heating of **8** in 2-propanol containing sodium resulted in cyclization–dehydration to give **4a**. While in practice intermediates **7** and **8** were isolated, characterized, and tested,[14] Scheme I effectively represents a one-pot, three-step sequence of the preparation of **4a**.

The second method used to synthesize pyrazinyl-1,2,4-oxadiazoles is illustrated in Scheme II with the preparation of **4b**. The cyanopyrazine **9**[5d] was reacted with sodium methoxide in methanol at room temperature to give the corresponding imino ether. Without isolation, this intermediate was then converted to the crystalline cyanoamidine **10** by reaction with cyanamide. When **10** was then heated with hydroxylamine hydrochloride in THF in the presence of triethylamine, **4b** was obtained in virtually quantitative yield.

The structures of oxadiazoles **4a** and **4b** were verified spectroscopically (IR, ^1H NMR, ^{13}C NMR) and chemically.

Table II. Oral Dog Diuretic Assay[a] (5 mg/kg)

		0–5 h			
compd	no. of animals	Na^+, mequiv	K^+, mequiv	Cl^-, mequiv	urine vol, mL
4a	4	6.5	0.9	4.3	226
4b	4	7.2	0.6	3.0	266
1	>10	7.1	1.0	3.4	367
placebo	>10	3.2	1.0	2.5	225

[a] For testing protocol, see ref 20; mongrel dogs with an average weight of 14–16 kg were used.

Thus, when **4a** and **4b** were hydrogenated[15] (Pd/C, EtOH, 50 psi), there were obtained amiloride **1** and the 5-dimethylamino analogue **11**, respectively, identical in all respects with the corresponding authentic compounds (Scheme III).

The obvious synthetic routes to **5** were investigated and failed (e.g., regioselective N-alkylation of **1** and protected forms thereof; acylation of *N*-alkylguanidine with **6** under a variety of conditions). However, preliminary CNDO/2 calculations[16] on **4b** and a related series of pyrazinyl-1,2,4-oxadiazoles indicated that the oxadiazole ring nitrogen atoms are the sites most susceptible to electrophilic attack; owing to the approximations of the calculational method, a reliable distinction between the reactivities of the two ring nitrogen atoms could not be made. Nevertheless, this prediction, coupled with our observation that **4a** can be chemically reduced to amiloride **1**, led us to propose the synthesis of **5** via **12** (Scheme IV). It remained to alkylate the oxadiazole ring of **4** regioselectively. In the event, treatment of either **4a** or **4b** in DMF with excess iodomethane did not afford the desired **12a** and **12b** but the 2-*N*-methyl-1,2,4-oxadiazole salts **13a** and **13b** whose structures were established spectroscopically and by chemical reduction to the amiloride analogues **14a** and **14b**. The unambiguous structure assignment of **13b** was secured by X-ray crystallographic analysis.[17]

(10) After this work was completed, a report appeared on the synthesis and diuretic profile of **4a**. No rationale for the observed biological activity was proposed. Cf.: Watthey, J. W. H.; Desai, M.; Rutledge, R.; Dotson, R. *J. Med. Chem.* **1980**, *23*, 690.

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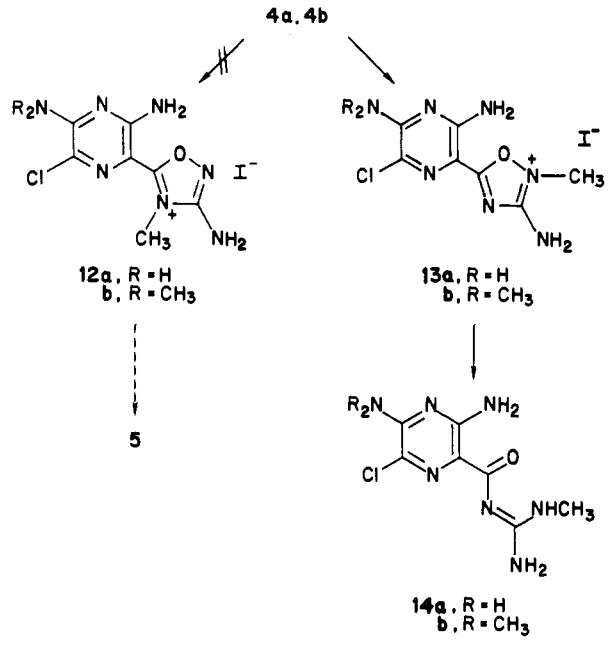
(16) Bock, M. G.; Smith, G., unpublished results.

Table III. Urinary Levels (mg) of Amiloride From Dogs Dosed With Pyrazinyl-1,2,4-oxadiazole **4a** or Amiloride[a]

		pyrazinyl-1,2,4-oxadiazole (**4a**)			amiloride (**1**)		
no. of animals	dose, mg/kg	0–6 h	6–24 h	% rec	0–6 h	6–24 h	% rec
6	0.1	0.20 ± 0.08[b]	0.19 ± 0.07	36.2 ± 4.2	0.30 ± 0.11	0.22 ± 0.07	41.4 ± 15.0
6	0.3	0.35 ± 0.08	0.42 ± 0.15	24.2 ± 6.0	0.68 ± 0.16	0.41 ± 0.13	29.3 ± 3.4
6	1.0	0.74 ± 0.51	2.02 ± 1.08	22.4 ± 9.6	3.31 ± 1.42	2.19 ± 0.44	40.0 ± 6.8

[a] Catheterized mongrel dogs with an average weight of 14–16 kg were dosed orally. Urine was collected, and 1-mL aliquots were partitioned between saturated sodium carbonate solution (2 mL) and ethyl acetate (25 mL). The phases were shaken (20 min) and centrifuged (2 min). The organic phase (20 mL) was combined with 0.5 N HCl (2 mL) and agitated (10 min). After centrifugation (5 min) the organic phase was separated and the aqueous phase was analyzed for amiloride spectrophotofluorometrically (Amico-Bouman spectrophotofluorometer with excitation at 365 nm and emission at 420 nm). [b] Mean ± SD.

Scheme IV



Results and Discussion

The diuretic and saluretic activity of the pyrazine-1,2,4-oxadiazoles **4a** and **4b** and their *N*-alkyl salts **13a** and **13b** was determined in the rat and the dog. These data are collated in Tables I and II where they are compared with that of amiloride (**1**).

A perusal of the data in Table I indicates that all four pyrazinyl-1,2,4-oxadiazoles have a salidiuretic pattern in the rat that parallels that of amiloride. While there may be some dose–response differences among the group in the 0–5-h testing interval, the antikaliuretic–diuretic profile characteristic of **1** is evident for all compounds. Significantly, these dose–response differences disappear in the 0–24-h time interval as the electrolyte excretion profile of the pyrazinyl-1,2,4-oxadiazoles and amiloride become virtually indistinguishable.

A similar result was obtained in the dog assay (Table II). Analysis of compounds **4a** and **4b** showed that they had essentially the same natriuretic effect as **1** in this testing protocol.

The facility with which the 1,2,4-oxadiazole ring in **4a** and **4b** was chemically reduced to give **1** and **11**, respectively, provided a clue to why these compounds elicit salidiuretic responses similar to **1**. Consequently, compound **4a** was examined further. In order to establish that the 1,2,4-oxadiazole ring in **4a** was a determinant in its amiloride-like activity, the pooled rat urines from the assay of **4a** were sampled. Qualitative analysis (TLC) showed no trace of compound **4a**; instead, the major component was identified as **1**.[18] This result was verified quantitatively in the dog. As can be seen in Table III, when dogs were dosed with either **4a** or **1**, there was no significant difference in the percent of **1** recovered for the 0.1 and 0.3 mg/kg treatment groups. At 1 mg/kg, **1** was absorbed to a greater extent than **4a**, accounting for the difference in percent recovery.

The implications of these results are manifold. The 3-amino-1,2,4-oxadiazole ring in **4** represents a sequestered form of a 1-acylguanidine. As such, it is readily prepared and can substantially simplify synthetic operations relative to the polar, basic acylguanidines. Moreover, the transformation of the 3-amino-1,2,4-oxadiazole ring to 1-acylguanidines is a facile process, as has been demonstrated by chemical means and in vivo. Pyrazinoylguanidines, like **1**,[21] may, therefore, be delivered in protected or unprotected form with equal efficacy.

Conclusion

The pyrazinyl-1,2,4-oxadiazoles **4a** and **4b** and their *N*-alkyloxadiazolium salts **13a** and **13b** have electrolyte excretion profiles similar to that of amiloride (**1**). Analysis of the pooled urine samples from the oral rat and oral dog

(17) (a) The single-crystal analysis of structure **13b** was carried out by an X-ray diffraction experiment on a specimen grown in dimethylformamide using a fully automated Syntex $P2_1$ diffractometer with $2\theta/\omega$ scan at 50 kV/20 mA up to a maximum 2θ of 115°. The unit cell parameters are a = 11.719 (2) Å, b = 6.771 (1) Å, c = 12.313 (2) Å, β = 108.21 (1)°, and V = 928.1 (3) Å^3 in the noncentrosymmetric, monoclinic space group $P2_1$ (Z = 2). Of a total of 1391 symmetry-independent reflections, 1156 were considered observed at the level $I \geq 3\sigma(I)$. Initial phasing was carried out by MULTAN[17b] and expanded to a complete structure by difference electron density syntheses. Refinement was carried out by minimizing the function $\sum w(|F_o| - |F_c|)^2$ where $w = 1/[\sigma(F_o)]^2$ using full-matrix least squares and anisotropic temperature factors for non-hydrogen atoms. The final unweighted residual index R ($=\sum||F_o| - |F_c||/\sum|F_o|$) was 0.065. Data reduction, least-squares analyses, electron density syntheses, and other related programs were performed with the SDP[17c] software system on a PDP 11/60 computer; (b) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M. *MULTAN, A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*; University of York, York, England, and University of Louvaine, and Louvaine, Belgium, 1980. (c) Okaya, Y.; Frenz, B.; Brice, M.; Corfield, P.; Hodgson, K.; Rohrer, D.; Linn, E. Enraf-Nonius Structure Determination Package Plus, Version 1.1A; An Integrated Set of Computer Programs Written for Use on PDP-11 Series of Computers, Jan 19, 1984.

(18) Similar results were obtained with compounds **4b**, **13a**, and **13b**, indicating that these compounds undergo reductive ring opening and dealkylation as well.

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(21) The pyrazinyl-1,2,4-oxadiazole **4b** had an estimated LD_{50} of 90 mg/kg compared with an LD_{50} of 56 mg/kg for **1**.

assays indicated that **4a** was converted to **1** in vivo. Compound **4a** (and by inference **4b**, **13**, and **13b**) may, therefore, be considered to be a prodrug of amiloride (**1**).

Experimental Section

Melting points were determined in open capillary tubes on a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian EM 390 spectrometer, and ^{13}C NMR spectra were recorded on a Varian CFT-20 spectrometer. Chemical shifts are reported in δ values and ppm, respectively, relative to Me_4Si as internal standard. IR spectra were obtained on a Perkin-Elmer 297 spectrophotometer.

2-(3-Amino-1,2,4-oxadiazol-5-yl)-6-chloro-3,5-diaminopyrazine (4a). A suspension of 3,5-diamino-6-chloro-*N*-[(hydroxyimino)aminomethylene]pyrazine-2-carboxamide (23.82 g, 0.97 mol) in 200 mL of 2-propanol containing 2.3 g (0.1 mol) of sodium was refluxed on a steam bath for 12 h. The reaction mixture was cooled and filtered to give the crude product in quantitative yield. Recrystallization from DMF–H_2O afforded the analytical sample as a bright yellow solid: mp 278 °C; IR (KBr, partial) 3460, 3140, 1600, 1400, 1250, 1050, 880, 760 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 6.2 (2 H, br s), 7.35 (4 H, br s); ^{13}C NMR (Me_2SO-d_6) 107.27, 120.13, 152.73, 153.22, 167.70, 170.35 ppm. Anal. ($C_6H_6ClN_7O$) C, H, N.

***N*$^{\alpha}$-Cyano-3-amino-5-(dimethylamino)-6-chloropyrazine-2-carboxamidine (10).** To a solution of 800 mL of methanol containing 2.48 g (0.046 mol) of sodium methoxide was added 46.1 g (0.233 mol) of 3-amino-5-(dimethylamino)-6-chloropyrazinecarbonitrile in one portion. The resulting reaction mixture was stirred at room temperature for 30 h, filtered, and neutralized with 2.63 mL (0.046 mol) of acetic acid. The filtrate was concentrated to approximately 200 mL, treated with cyanamide (10.51 g, 0.25 mol), and allowed to stand at room temperature. After 5 h, the product (22.1 g) was collected and recrystallized from methanol to give the analytical sample as a bright yellow solid: mp 223–224 °C; IR (KBr, partial) 3400, 3150, 2190, 1600, 1550, 810, 775 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 3.15 (6 H, s), 7.5 (4 H, br s); ^{13}C NMR (Me_2SO-d_6) 40.54, 111.31, 115.76, 119.63, 152.61, 153.48, 165.11 ppm. Anal. ($C_8H_{10}ClN_7$) C, H, N.

2-(3-Amino-1,2,4-oxadiazol-5-yl)-3-amino-6-chloro-5-(dimethylamino)pyrazine (4b). To a solution of tetrahydrofuran (250 mL) containing 50 mL of methanol and 12.36 g (51.57 mmol) of **10** was added hydroxylamine hydrochloride (6.65 g, 103.13 mmol) and triethylamine (21.56 mL, 154.71 mmol). The resulting reaction mixture was protected from moisture and heated to reflux for 6 h. The reaction mixture was cooled, poured into water (1 L), and filtered. Concentration of the filtrate afforded more solid, which was combined with the initial precipitate to give 12.64 g (96%) of **4b** in analytically pure form: mp 210–211 °C; IR (KBr, partial) 1635, 1580, 1540, 1390, 1180, 910, 780 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 3.16 (6 H, s), 6.34 (2 H, s), 7.45 (2 H, s); ^{13}C NMR (Me_2SO-d_6) 40.6, 108.5, 120.7, 151.4, 153.3, 167.8, 170.1 ppm. Anal. ($C_8H_{10}ClN_7O$) C, H, N, Cl.

2-Methyl-3-amino-5-(6-chloro-3,5-diaminopyrazin-2-yl)-1,2,4-oxadiazolium Iodide (13a). The pyrazine–oxadiazole **4a** (2.03 g, 8.92 mmol) was dissolved in 20 mL of dry DMF with warming. The resulting solution was protected from moisture, treated with 10 mL of iodomethane, and allowed to stand at 40 °C overnight. The product, **13a**, was collected as a yellow solid in 94% yield: mp 211–213 °C dec; IR (KBr, partial) 3325, 3155, 1655, 1610, 1520, 1420, 1260 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 3.89 (3 H, s), 7.55 (2 H, br s), 8.10 (2 H, br s), 9.3 (2 H, br s); ^{13}C NMR (Me_2SO-d_6) 39.75, 102.94, 123.67, 154.13, 155.98, 161.82, 169.0 ppm. Anal. ($C_7H_9ClIN_7O\cdot{}^1/_4DMF$) C, H, N.

2-Methyl-3-amino-5-[3-amino-5-(dimethylamino)-6-chloropyrazin-2-yl]-1,2,4-oxadiazolium Iodide (13b). This compound was prepared in the same way as **13a** in 88% yield: mp 300 °C (from EtOH); IR (KBr, partial) 3075, 1670, 1520, 1465, 1395, 1180, 950 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 3.28 (6 H, s), 3.88 (3 H, s), 7.53 (2 H, br s), 9.3 (2 H, br s); ^{13}C NMR (Me_2SO-d_6) 36.43, 41.01, 103.51, 122.67, 153.56, 153.71, 161.59, 168.69 ppm. Anal. ($C_9H_{13}ClIN_7O$) C, H, N.

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Registry No. **4a**, 73631-29-5; **4b**, 80776-64-3; **8**, 32205-65-5; **9**, 14340-29-5; **10**, 80776-63-2; **13a**, 102652-56-2; **13b**, 80783-75-1.

Supplementary Material Available: Structure of **13b** and listings of fractional coordinates and temperature factors, bond distances, and bond angles of **13b** (4 pages). Ordering information is given on any current masthead page.

Pyrazole Derivatives. 5. Synthesis and Antineoplastic Activity of 3-(2-Chloroethyl)-3,4-dihydro-4-oxopyrazolo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxamide and Related Compounds

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Two pyrazolotetrazine derivatives were synthesized as the analogous prodrugs of the light-sensitive antineoplastic agents dacarbazine and BIC. Both the pyrazole derivatives are stable under oridinary light illumination. Biological evaluation of these pyrazoles revealed that the compound containing a 2-chloroethyl function (**6a**) demonstrated good antineoplastic activity in experimental animals, but the one containing a methyl function (**6b**) was inactive. The inactivity of compound **6b** may suggest that compound **6a** and related imidazotetrazines may simply act as biological alkylating agents per se rather than as prodrugs. The information could also imply that the postulated dealkylation mechanism for the triazene derivatives should be reexamined.

The antineoplastic activity displayed by dacarbazine[1] [4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide, DTIC, DIc, **1a**] in experimental animals[2] and in clinical trials[3] is well-known. It has been suggested that a meta-

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