responses was determined.

In 90–100% of Robidoux Swiss male mice weighing 18–22 g, intraperitoneal injections of 25 mg/kg of 3-mercaptopropionic acid induced tonic-extensor seizures within 3–7 min. Test substances were dissolved in saline and administered by the oral route 30 min prior to injection of the convulsant agent. The dose required to prevent tonic-extensor seizures in 50% of the animals (ED_{50}) was calculated using regression analysis and based upon at least three doses with ten mice per level.

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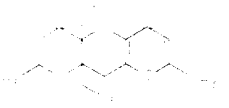
(26) Note Added in Proof: subsequent examination of the ^{13}C NMR spectrum of **10b** in Me_2SO-d_6 indicates that, in solution at least, it actually exists in the hemiketal form, i. Presumably **10a** also exists as this cyclized structure.



i = **10b**

No deduction concerning the stereochemistry could be made, but only one isomer is present, as judged from the spectrum.

Notes

Synthesis and Evaluation for Diuretic Activity of 1-Substituted 6-Chloro-5-sulfamylindolines

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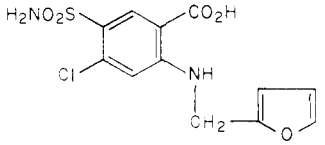
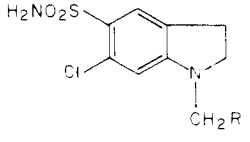
The synthesis of a series of 1-substituted 6-chloro-5-sulfamylindolines is described. In the Lipschitz test for diuretic activity, two of the compounds showed significant excretion of urine and sodium and were approximately equivalent in potency to chlorothiazide but with a later onset of activity.

Furosemide is a potent, high-ceiling diuretic, one of the most widely used in medicine today. We thought it would be of interest to synthesize and evaluate for diuretic activity a series of 1-substituted 6-chloro-5-sulfamylindolines (**7–9**) which can be regarded as nonacidic ring-closed



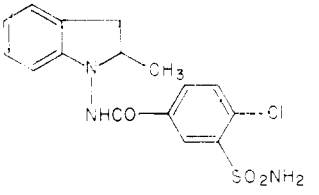
furosemide



7–9

analogues of furosemide. Recent reports of indolines[1,2] and isoindolines[3] with diuretic activity lent encouragement to this investigation.

One of the more promising indolines with diuretic properties demonstrated in clinical trials is indapamide.[2] As part of this present work on 1-substituted 6-chloro-5-sulfamylindolines, an analogue, **12**, structurally related to indapamide was also synthesized and tested.



indapamide

Chemistry. The starting material required for the

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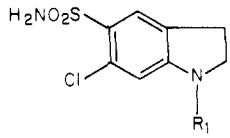
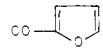
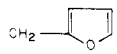
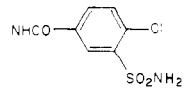
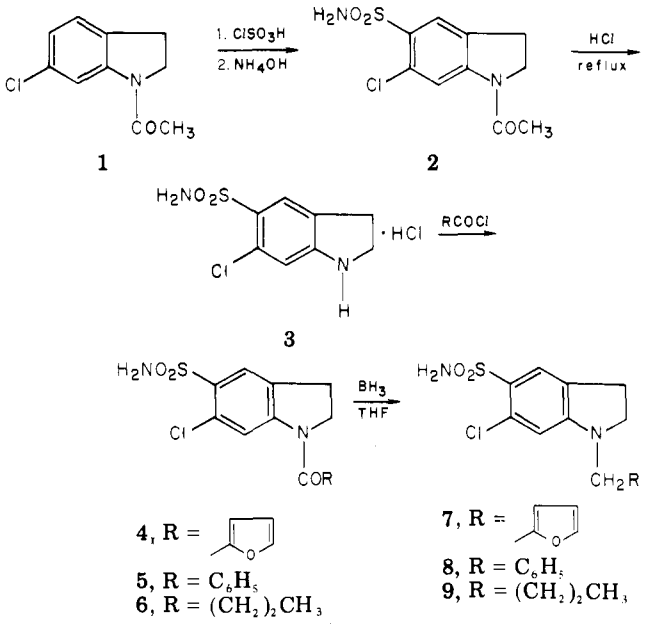
Table I. 1-Substituted 6-Chloro-5-sulfamylindolines



compd	R_1	yield,[a] %	mp, °C	recrystn solvent[b]	formula[c]
2	$COCH_3$	48	258-260	A	$C_{10}H_{11}ClN_2O_3S$
3	H	54	225 dec	B	$C_8H_9ClN_2O_2S \cdot HCl$
4		66	273-275	C	$C_{13}H_{11}ClN_2O_4S$
5	COC_6H_5	72	285-287	C	$C_{15}H_{13}ClN_2O_3S$
6	$COCH_2CH_2CH_3$	91	260-261	D	$C_{12}H_{15}ClNO_3S$
7		68	154-156	D	$C_{13}H_{13}ClN_2O_3S$
8	$CH_2C_6H_5$	90	163-165	D	$C_{15}H_{15}ClN_2O_2S$
9	$(CH_2)_3CH_3$	86	155-156	D	$C_{12}H_{17}ClN_2O_2S$
10	N=O	80	183-185	E	$C_8H_8ClN_3O_3S$
11	NH_2	65	235-237 dec	E	$C_8H_{10}ClN_3O_2S \cdot HCl$
12		45	177-179	F	$C_{15}H_{24}Cl_2N_4O_5S$

[a] Yield of analytically pure product; no effort was made to optimize yields. [b] A = CH_3OH, B = CH_3OH–ether, C = DMF–CH_3OH, D = DMF–H_2O, E = 2-PrOH, F = EtOAc. [c] All compounds were analyzed for C, H, and N within ±0.40% of the calculated values.

Scheme I



synthesis was 1-acetyl-6-chloroindoline (1), prepared from indoline in four steps by a literature procedure.[4] It was necessary to have an acyl substituent on the indoline nitrogen atom to serve both as a protective group and to direct subsequent attack by an electrophile to the 5 position of the molecule.[5] Accordingly, reaction of 1 with chlorosulfonic acid as reagent and solvent introduced the chlorosulfonyl group at the desired C_5 position. This intermediate was not isolated but was converted directly to the corresponding sulfonamide 2 by slow addition of the reaction solution to ammonium hydroxide at low temperature. The acetyl group was then removed by hydrolysis in refluxing hydrochloric acid to provide the parent structure of this series, 6-chloro-5-indolinesulfonamide (3). The primary target compounds 7–9 bearing 1-alkyl and 1-aralkyl substituents were prepared by acylation of 3, followed by reduction of the resulting amides 4–6 with diborane. This synthetic sequence is outlined in Scheme I (see Table I for physical data).

The preparation of the 6-chloro-5-sulfamyl analogue 12 related to indapamide was straightforward and is shown in Scheme II.

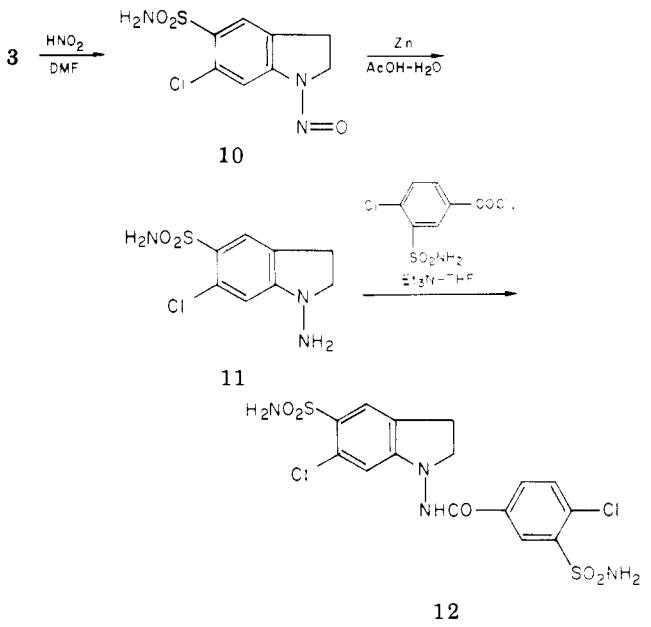
Table II. Results of Diuretic Screening

	ratio of drug/urea[a]					
	0-5 h			5-24 h		
compd	vol	Na^+	K^+	vol	Na^+	K^+
2	0.53	0.63	0.53	0.72	1.09	1.10
3	0.62	0.95	0.76	0.94	1.02	0.83
4	0.31	0.46	0.38	0.87	1.20	1.23
5	0.83	0.79	0.41	0.85	1.08	1.10
6	0.54	1.04	0.53	0.96	1.30	1.17
7	0.15	0.29	0.24	1.00	1.09	1.29
8	0.77	1.00	0.73	1.80	1.30	1.20
9	0.70	0.98	0.78	0.90	1.15	1.06
12	0.99	1.47	1.09	1.26	1.24	1.18
vehicle control	0.70	0.50	0.50	0.60	0.90	1.10
chlorothiazide	1.30	1.90	1.50	1.00	1.10	1.00
furosemide	2.75	9.63	1.12	1.08	0.96	1.31

[a] All compounds and reference drugs were tested at 50 mg/kg po.

Pharmacology. Compounds were screened for diuretic activity according to a modification of the Lipschitz method by Kagawa and Kalm.[6] Groups of six female Wistar rats (150–200 g) were deprived of food for 16 h prior to dosing. Test compounds were prepared in 1% saline and administered in a volume of 15 mL/kg orally. After dosing, each animal was placed in a separate metabolic cage. Water was permitted ad libitum. Urine was collected from 0 to 5 h after dosing and during the following 5–24 h. Each experiment consisted of a vehicle control, a positive control group treated with urea (1000 mg/kg), and the test compound (50 mg/kg). Urine samples were analyzed for sodium and potassium using a flame photometer

Scheme II



(IL Model 343). Sodium and potassium values were normalized as mean milliequivalents (mequiv) and urine volume (diuresis) as the mean milliliters over the 0–5- and 5–24-h test period. The mean values are expressed in a ratio to the sodium, potassium, and diuresis values obtained for the urea-treated group. This ratio is called the "drug to urea ratio". A drug to urea ratio equal to or greater than 1.00 for diuresis and/or sodium is considered indicative of diuretic activity.

The compounds tested for diuretic activity and the results obtained are shown in Table II. The parent structure (**3**) as well as the amides **2** and **4–6** was inactive over the initial 0–5-h test period, but all showed indications of natriuresis during the next 5–24 h. The furosemide analogue **7** was also inactive over the first 5 h but exhibited enhanced urine and Na^+ excretion from 5 to 24 h after dosing. Essentially the same result was obtained for the 1-benzyl compound **8** and for the 1-*n*-butyl analogue **9** which has the same substituent on nitrogen as the diuretic bumetanide.[7]

The indapamide analogue **12** proved to be the most interesting of the series. It showed the best, albeit borderline, volume clearance during the first 5 h but was accompanied by significant excretion of electrolytes. However, it, together with **8**, displayed marked diuresis including good Na^+ excretion from 5 to 24 h after dosing. Both test compounds over the 5–24-h period were roughly equivalent in potency to chlorothiazide during its earlier peak of activity from 0 to 5 h.

These results indicate that, unlike the thiazides and diuretics of the furosemide type which have a rapid onset and a short duration of action, the active compounds of the present series reveal their diuretic potential much later in the test period. This may be due to a slower rate of absorption and distribution or to the intermediacy of an active metabolite.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, IL. The structures of all compounds were confirmed by their IR (Perkin-Elmer 457) and NMR (Jeolco $C_{60}HL$) spectra.

1-Acetyl-6-chloro-5-indolinesulfonamide (2). To 40 mL of chlorosulfonic acid cooled to 0 °C under N_2 was added in portions 15.0 g (0.077 mol) of 1-acetyl-6-chloroindoline[4] with good stirring. A moderate exotherm ensued which was controlled by the rate of addition. The stirred solution was then cautiously heated to 75 °C and kept at that temperature for 4 h. After cooling to 0 °C, the solution was transferred to an addition funnel and added slowly, with good stirring, to 300 mL of concentrated NH_4OH cooled to −10 °C in an ice–MeOH bath. The addition was very exothermic and the temperature was kept below 0 °C by the rate of addition (1 h required). When the addition was completed, the reaction was stirred at ambient temperature for 1 h and then filtered. The cake was washed repeatedly with H_2O and then dried to afford 12.6 g of solid with mp 233–243 °C. Recrystallization from CH_3OH (charcoal) gave 10.1 g (48%) of pure white crystals, mp 258–260 °C.

6-Chloro-5-indolinesulfonamide Hydrochloride (3). A stirred mixture of 55.0 g (0.20 mol) of **2** and 220 mL of concentrated HCl was slowly and cautiously heated to reflux and then refluxed for 1.5 h. The hot solution was stirred for 5 min with 5 g of Darco-G-60 charcoal, filtered, and then kept in a −10 °C cooling bath overnight. The product was collected, washed several times with ether, and dried to yield 31.9 g, mp 225–227 °C dec. Recrystallization by dissolving the salt in hot methanol and adding an equal volume of ether furnished pure **3**, mp 225 °C dec.

The free base, which was used in subsequent acylation reactions, was liberated with aqueous $NaHCO_3$ and recrystallized from CH_3OH, mp 200–202 °C.

6-Chloro-1-(2-furoyl)-5-indolinesulfonamide (4). To a stirred solution of 3.50 g (0.0150 mol) of **3** (base) in 60 mL of acetone was added 5.53 g (0.040 mol) of anhydrous K_2CO_3. Then 2.60 g (0.020 mol) of 2-furoyl chloride in 5 mL of acetone was added over a 10-min period, with exclusion of moisture. After the mixture was stirred overnight, 100 mL of water was added to dissolve the salts and to maximize precipitation of the product. The product was filtered, washed well with H_2O, and dried to give 3.8 g of amide, mp 265–270 °C. This was dissolved in 20 mL of DMF on the steam bath and diluted while hot with 20 mL of methanol to furnish 3.2 g (66% yield) of pure **4**, mp 273–275 °C. By following a similar procedure, the amides **5** and **6** were prepared.

6-Chloro-1-(2-furfuryl)-5-indolinesulfonamide (7). To a stirred solution of 3.26 g (0.010 mol) of **4** in 100 mL of THF was added 50 mL of 1.0 M diborane in THF over a 15-min period, with exclusion of moisture. The reaction was then slowly heated to reflux and kept there for 2 h. After the mixture was cooled to 15 °C, 50 mL of 3 N HCl was slowly added. The THF was then removed by distillation at aspirator pressure. A dilute NaOH solution was added to the neutralization point, and 0.5 g of Na_2CO_3 was added to make the system alkaline. The precipitated product was filtered, washed well with H_2O, and dried to afford 2.86 g of product, mp 145–153 °C. This was dissolved in 25 mL of DMF on the steam bath and diluted while hot with 20 mL of H_2O to give 2.20 g (68% yield) of pure **7**, mp 154–156 °C. By following a similar procedure, amides **5** and **6** were reduced to amines **8** and **9**.

6-Chloro-1-nitroso-5-indolinesulfonamide (10). A stirred solution of 4.64 g (0.020 mol) of **3** in 40 mL of DMF was cooled to 0 °C, and 1.9 g (0.027 mol) of $NaNO_2$ was added in one portion. Then 25 mL of 2 N HCl was added dropwise over 45 min, while the reaction temperature was kept below 5 °C. The resulting mixture was stirred at ambient temperature for 30 min, when 25 mL of ice H_2O was added. The product was filtered, washed well with H_2O, and dried to afford 4.8 g, mp 180–184 °C. Recrystallization from 2-propanol gave 3.90 g (80% yield) of pure **10**, mp 183–185 °C.

1-Amino-6-chloro-5-indolinesulfonamide Hydrochloride (11). To a stirred suspension of 14.1 g of Zn dust in 315 mL of 2:1 AcOH–H_2O was added 15.7 g (0.060 mol) of **10**, while the temperature was kept between 10 and 20 °C. The mixture was then heated at 40 °C for 1 h, when an additional 9.4 g of Zn dust was added. After 0.5 h, the mixture was cooled to room temperature and filtered, and the cake was washed with 100 mL of 1 N HCl. The combined filtrate and washings were basified with NH_4OH to precipitate the product which was filtered, washed well with water, and dried to give 16.5 g, mp 211–215 °C. The base was recrystallized from CH_3OH to yield 12.6 g (76%), mp 215–218 °C.

The purified base was dissolved in hot EtOH and ethereal HCl was added to the turbidity point. After the mixture was cooled, the salt was collected and recrystallized from 2-propanol to give 12.3 g (65% overall yield), mp 235–237 °C dec.

1-(4-Chloro-3-sulfamylbenzamido)-6-chloro-5-indolinesulfonamide (12). To a stirred solution of 2.84 g (0.010 mol) of 11 and 3.03 g (0.030 mol) of triethylamine in 100 mL of THF was added 3.75 g (0.015 mol) of 4-chloro-3-sulfamylbenzoyl chloride[8] in portions over a 30-min period. After the mixture was stirred overnight, the precipitate salt was removed by filtration and the filtrate was evaporated to dryness. The residual solid was triturated with Na_2CO_3 and with H_2O and then dried. Recrystallization from EtOAc furnished 2.5 g (45%) of pure **12**, mp 177–179 °C.

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Phosphonate Analogue of 2′-Deoxy-5-fluorouridylic Acid

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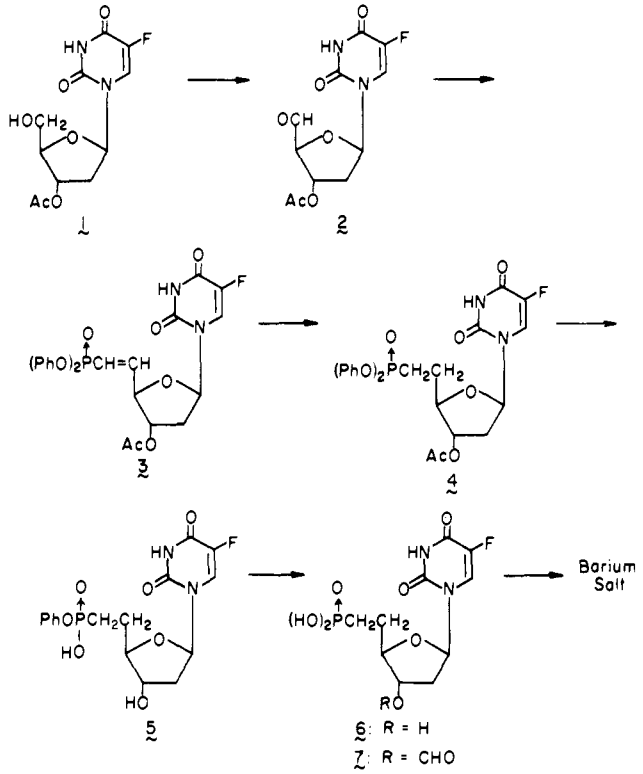
The phosphonate analogue (**6**) of 2′-deoxy-5-fluorouridylic acid has been prepared via a Pfitzner–Moffatt oxidation and Wittig reaction. This compound was found to inhibit thymidylate synthetase from three sources and to be cytotoxic to H.Ep.-2 cells in culture.

It is widely accepted that 5-fluorouracil (FU) exerts its cytotoxic action and anticancer effects by its inhibition, after conversion to 2′-deoxy-5-fluorouridylic acid (FdURP), of thymidylate synthetase.[1] Further, resistance to FU is thought to be due to loss of the anabolizing enzymes that carry out its conversion to FdURP.[1,2] Efforts to enhance the activity of FU and to circumvent the resistance problem by the use of 2′-deoxy-5-fluorouridine have been essentially negative, probably because it is rapidly degraded to FU, but attempts to overcome this phosphorolysis have so far met with little success.[3]

Another approach to overcoming this problem involves the preparation of a metabolically stable derivative of FU that will penetrate cells and inhibit thymidylate synthesis. A logical candidate would appear to be the phosphonate analogue of FdURP (**6**). This compound should be sterically very similar to FdURP; yet, it should not be dephosphorylated because of the stability of the C–P bond. However, since nucleotides are not phosphorolyzed, the glycosyl bond should also be metabolically stable. In fact, Moffatt and co-workers have shown that phosphonates of this type are substrates for the phosphodiesterase of snake venom[4] and that uridine cyclic 2′,3′-phosphonate binds more tightly to ribonuclease A than the cyclic phosphate.[5]

The recently established antitumor activity of PALA[6] clearly indicates that a phosphonate is capable of penetrating mammalian cells sufficiently to cause cell death by the inhibition of a specific enzyme[7] and provided impetus for the present work.

The synthesis of this phosphonate derivative was based on the work of Jones and Moffatt.[4] 3′-*O*-Acetyl-2′-deoxy-5-fluorouridine (**1**)[8] was oxidized to the corresponding aldehyde **2** by treatment with Me_2SO, DCC, pyridine, and trifluoroacetic acid. The aldehyde was



allowed to react with diphenyl (triphenylphosphoranylidene)methylphosphonate[9,10] and the resultant olefin **3**, predominantly the trans isomer (ca. 7:3, NMR), reduced

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