Eur J Med Chem (1995) 30, 343–351

Synthesis and pharmacology of pyrid-3-ylsulfonylcyanoguanidines as diuretics

B Masereel[1], L Dupont[2], D Laeckmann[1], JF Liégeois[1], B Pirotte[1], P de Tullio[1], J Delarge[1]

[1]*Department of Medicinal Chemistry, University of Liège, 3, rue Fusch, 4000 Liège;*
[2]*Department of Crystallography, University of Liège, Sart-Tilman, 4000 Liège, Belgium*

(Received 26 August 1994; accepted 28 November 1994)

sulfonylcyanoguanidine / torasemide / diuresis / $Na^+2Cl^-K^+$ cotransporter

Introduction

Furosemide **1** [1] and torasemide **2** (fig 1) are loop diuretics which inhibit the $Na^+2Cl^-K^+$ cotransporter of the thick ascending limb of Henle's loop [2, 3]. The pharmacomodulation of **2** leads to pyrid-3-ylsulfonyl ureas and thioureas, which have been described as high ceiling diuretics that act by blocking the same carrier [2, 4–7]. One of these products BM 20 **3** (fig 1), a sulfonylthiourea, has been found to be more diuretic than its parent [7, 8]. With the intention of discovering new diuretics, we have examined the bioisosteric replacement of the sulfonylurea or sulfonylthiourea function with the sulfonylcyanoguanidine moiety. This strategy has been successfully applied to the carboxamide side chain of penicillins [9] and to the gastric antisecretory *N'*-alkyl-*N*-imidazolylalkyl thioureas to give cimetidine [10]. Recently, *N'*-alkyl-*N*-substituted pyridylthioureas and pyridyl ureas were found to be as potent as pinacidil and its cyanoguanidine derivatives [11, 12]. The inhibitors [6, 13] of the $Na^+2Cl^-K^+$ cotransporter act *via* their anionic form with a chloride binding site on this carrier [14]. The presence of the electron-withdrawing group should therefore preserve the required acidity of the sulfonamide moiety.

Chemistry

All 4-alkylamino, 4-cycloalkylamino and 4-arylaminopyrid-3-ylsulfonamides **5** were prepared by the reaction of 4-chloropyrid-3-ylsulfonamide [15] **4** with the corresponding alkyl-, cycloalkyl- or arylamine (scheme 1). The sodium salt of **5** reacted with the required *N'*-alkyl-*N*-cyano-*S*-methylcarbamimidothioate (**7a** or **7b**) to obtain *N'*-alkyl-*N''*-{[4-(alkylamino)pyrid-3-yl]sulfonyl}-*N*-cyanoguanidines, *N'*-alkyl-*N*-cyano-*N''*-{[4-(cycloalkylamino)pyrid-3-yl]sulfonyl}guanidines or *N'*-alkyl-*N''*-{[4-(arylamino)pyrid-3-yl]sulfonyl}-*N*-cyanoguanidines **8**. The carbamimidothioates **7a–c** were synthesized from *N*-cyano-*S,S'*-dimethyldithioiminocarbonate **6** [16] and the appropriate amine. The *N*-cyano-*N''*-{[4-(cycloalkylamino)-pyrid-3-yl]sulfonyl}piperidinoamidines **8** were prepared by the reaction of the sodium salt of the sulfonamides **5** and the *N*-cyanomethylthiopiperidinoimine **7c**. All the synthesized molecules are listed in tables I and II.

Lipophilicity

The partition coefficient (log *P*) of a series of standards with a wide range of lipophilicity (table III)

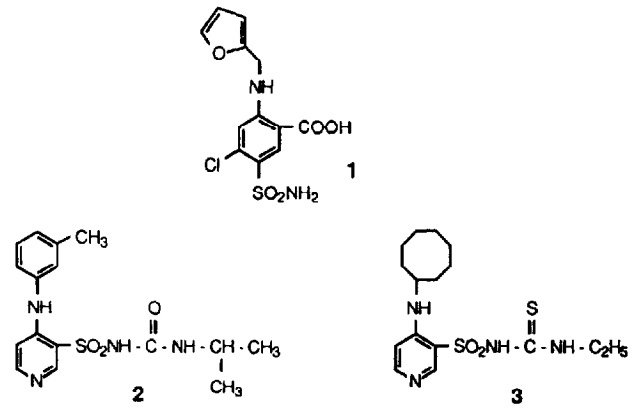
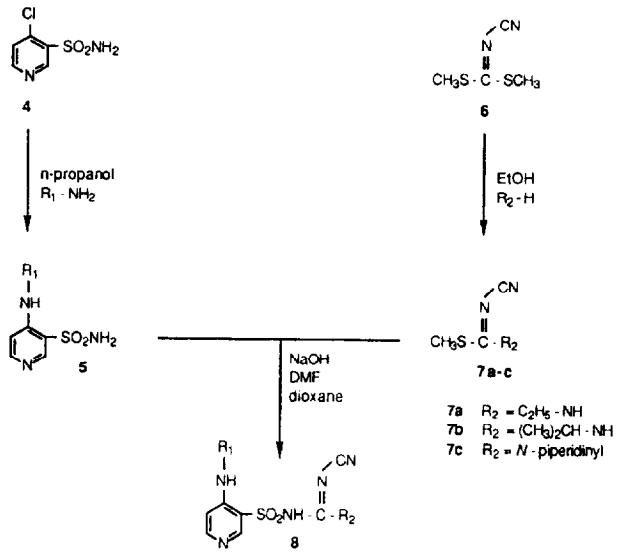


Fig 1. Structure of furosemide **1**, torasemide **2** and BM 20 **3**.



Scheme 1. Synthesis of sulfonylcyanoguanidines. R_1 = alkyl, cycloalkyl or aryl.

was determined by using the *n*-octanol/phosphate buffer pH 7.40 shake-flask system [17]. Each log *P* was correlated with the corresponding capacity factor (log *k'*) obtained by reversed-phase high-performance liquid chromatography (RP-HPLC). The log *P* of other compounds (tables I and II) was obtained by interpolation of the correlation curve (table III). As shown in table I, log *P* increases with the number of methylene groups in the R_1 cycloalkyl residue (Δ log *P* CH_2 = +0.5). The *para*-R_1-substituted compounds (table I) are more lipophilic than the *meta*-R_1-substituted derivatives (compare **27** with **28**, and **32** with **33**). At pH 7.40, the log *P* of sulfonylcyanoguanidines increases by about 0.3–0.7 as compared to the sulfonylureas counterparts and by 0.2–0.5 as compared to their previously described sulfonylthioureas bioisosters [18, 19].

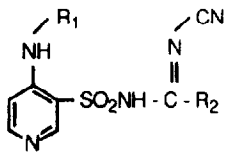
Results and discussion

The prepared substances were screened for their oral diuretic potency at 30 mg/kg in rats. Table I shows that eight compounds (**11–13**, **17**, **20–22** and **25**) induced a significantly ($P < 0.01$) higher urinary volume excretion than the control rats (22 ml/kg over 4 h). The log *P* of these compounds ranges from –0.43 to +2.15 and is not related to the activity. The lack of diuretic activity of the most hydrophobic compound **19** (log *P* = +3.73) is probably the consequence of a different tissue distribution or a steric hindrance of the cyclododecyl. The molecules bearing an R_2-ethylamino moiety are more active than their R_2-isopropylamino counterparts (compare **20–22**, and **16–18**). Six compounds (**28**, **29**, **31**, **32**, **34** and **37**), which have an aryl in the R_1 position (table II), exhibited diuretic properties but less than their parent **2**. As shown for the sulfonylureas [2], the substitution in *ortho* (**26**) or *para* (**27**, **33**) positions of the phenyl ring eliminates the biological response. The two most active derivatives (**21** and **28**) were selected for the study of their dose-dependent diuretic properties at doses ranging from 5 to 30 mg/kg. Compounds **1–3** were chosen as reference drugs. Dose-related increases in urine flow were observed with **1–3**, **21** and **28** after oral administration to rats (fig 2). The oral dose required to double the urinary volume excreted by control animals (OD2x) was calculated by regression analysis of the total urinary volume (fig 2). The OD2xs of **21** (11.3 mg/kg) and **28** (9.1 mg/kg) are higher than those of **3** (0.02 mg/kg) and **2** (1.6 mg/kg) but lower than that of **1** (14.1 mg/kg). Compounds **1**, **21** and **28** also produced dose-related increases in Na^+, K^+ and Cl^- excretion (fig 3). At 5 mg/kg, the urinary Na^+/K^+ ratio and the excretion of Na^+, K^+, Cl^- induced by both **21** and **28** are significantly higher ($P < 0.01$) than those produced by **1** (fig 3).

Since deprotonation of the sulfonylurea side chain of **2** and its derivatives is required for interaction with a chloride site in their target [6, 13, 14], the lower potency of **21** and **28** could be caused by a higher pK_a value of their sulfonylcyanoguanidine function. To test this hypothesis, we determined the ionization constant of **21** and **28**. Due to their poor water solubility, the pK_a values were determined by $HClO_4$ titration of the sodium salt in water with ethanol as a cosolvent, and corrected [20]. The data in table IV show that the pK_a value (6.78) of the hydrosoluble compound **11** is not modified by the presence of ethanol. Similar results were obtained for **21** and **28** (table IV). These data reveal that the pK_a of **21** (6.84) is similar to that of **2** (6.68) [18] but lower than that of **3** (7.52) [18]. The acidity of the sulfonylcyanoguanidine function of **28** (pK_a = 6.00) is stronger than that of its parent **2**. At physiological pH (7.40), **21** and **28** are ionized to a greater extent than **3** and **2**, respectively, so that the acidity of the sulfonylcyanoguanidine moiety cannot explain the poor activity of **21** and **28**.

Furthermore, structural analogies between **2** and the most active sulfonylcyanoguanidine **28** were studied by the way of the molecular modeling software Sybil 6.03 [21]. Their crystallographic geometries were optimized with the program Mopac 5.0 [22]. Compound **2** crystallized in three different conformations, called α, β and γ, defined by the typical values

Table I. Physicochemical and biological properties of 4-alkylamino- and 4-cycloalkylaminopyrid-3-ylsulfonylcyanoguanidines.

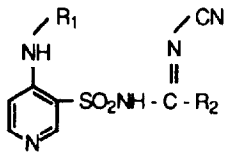


Compd	R_1	R_2	Formula [a]	mp,°C [b]	Yield (%)	log P [c]	Diuresis [d]
1	Furosemide		$C_{12}H_{11}N_2O_5SCl$	205-207		-0.92	48.8 ± 2.0 *
2	Torasemide		$C_{16}H_{20}N_4O_3S$	163-164	81	+0.47	88.4 ± 2.5 *
3	BM 20		$C_{16}H_{26}N_4O_2S_2$	196-198	77	+0.96	104.6 ± 3.2 *
9	CH_3	$(CH_3)_2CHNH$	$C_{11}H_{16}N_6O_2S$	219-221	31	- 1.43	24.7 ± 1.5
10	CH_3CH_2	$(CH_3)_2CHNH$	$C_{12}H_{18}N_6O_2S$	201-203	47	- 1.02	15.1 ± 2.5
11	$CH_3(CH_2)_2$	$(CH_3)_2CHNH$	$C_{13}H_{20}N_6O_2S$	199-201	72	- 0.43	37.5 ± 2.1 *
12	$CH_3(CH_2)_3$	$(CH_3)_2CHNH$	$C_{14}H_{22}N_6O_2S$	182-184	57	+0.27	34.4 ± 2.0 *
13	$(CH_3)_2CHCH_2$	$(CH_3)_2CHNH$	$C_{14}H_{22}N_6O_2S$	192-194	63	+0.10	37.1 ± 1.8 *
14	c-C_3H_5	$(CH_3)_2CHNH$	$C_{13}H_{18}N_6O_2S$	201-203	68	- 0.77	21.6 ± 1.3
15	c-C_5H_9	$(CH_3)_2CHNH$	$C_{15}H_{22}N_6O_2S$	215-217	70	+0.20	25.2 ± 1.8
16	c-C_6H_{11}	$(CH_3)_2CHNH$	$C_{16}H_{24}N_6O_2S$	231-233	69	+0.75	25.8 ± 1.6
17	c-C_7H_{13}	$(CH_3)_2CHNH$	$C_{17}H_{26}N_6O_2S$	229-231	40	+1.29	34.3 ± 1.8 *
18	c-C_8H_{15}	$(CH_3)_2CHNH$	$C_{18}H_{28}N_6O_2S$	234-236	68	+1.80	19.7 ± 1.5
19	c-$C_{12}H_{23}$	$(CH_3)_2CHNH$	$C_{22}H_{36}N_6O_2S$	250-252	65	+3.73	23.9 ± 1.6
20	c-C_6H_{11}	C_2H_5NH	$C_{15}H_{22}N_6O_2S$	230-232	52	+0.16	32.7 ± 1.9 *
21	c-C_7H_{13}	C_2H_5NH	$C_{16}H_{24}N_6O_2S$	227-229	70	+0.75	54.6 ± 2.1 *
22	c-C_8H_{15}	C_2H_5NH	$C_{17}H_{26}N_6O_2S$	229-231	58	+1.27	43.8 ± 1.9 *
23	c-C_6H_{11}	$(CH_2)_5$=N	$C_{18}H_{26}N_6O_2S$	210-212	59	+1.07	20.6 ± 1.4
24	c-C_7H_{13}	$(CH_2)_5$=N	$C_{19}H_{28}N_6O_2S$	208-210	63	+1.65	22.3 ± 1.5
25	c-C_8H_{15}	$(CH_2)_5$=N	$C_{20}H_{30}N_6O_2S$	199-201	52	+2.15	30.7 ± 1.7 *

[a]C, H, N, S analyses were within ±0.4% of the theoretical values; [b]all compounds were crystallized from ethanol; [c]values are means of three determinations obtained by the RP-HPLC method; [d]diuresis (ml/kg over 4 h; mean ± SD) induced in rats after oral administration of 30 mg/kg. *Statistically different ($P < 0.01$) from control (22.1 ± 1.8 ml/kg over 4 h).

of the torsional angles ϕ_1, ϕ_2, ϕ_3 and ϕ_4 (table V) [23, 24]. All of the crystallized sulfonylureas related to **2** adopt one of these three conformations [25–31]. X-ray crystallographic data for the bioisoster **28** revealed a new conformation (δ), characterized by the following theoretical values: $\phi_1 = +90°$, $\phi_2 = +90°$, $\phi_3 = 0°$ and $\phi_4 = 180°$ [32]. This conformation was confirmed by optimization (table V). Figure 4 shows the superimposition of the β and γ conformations of **2** with the δ conformer of **28**. Like **2** [23, 24], **28** adopts a zwitterionic structure in the crystal due to the transfer of the sulfonamide proton to the nitrogen atom of the pyridine ring. The side chain of **28** is then in an anionic form, which favours interaction with the Na^+ $2Cl^-$ K^+ cotransporter [6, 13, 14]. Two intramolecular H-bonds stabilize the sulfonylcyanoguanidine side chain: $N_{10}H...O_8$ (as observed for **2** [23, 24]) and $N_6H...O_9$. Theoretically, δ only differs from the γ conformer of torasemide by the value of ϕ_3 (table V). A conformational analysis was carried out by rotating the

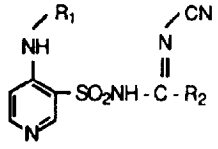
Table II. Physicochemical and biological properties of 4-arylaminopyrid-3-ylsulfonylcyanoguanidines.



Compd	R_1	R_2	Formula [a]	mp,°C [b]	Yield (%)	log P [c]	Diuresis [d]
26	2-$CH_3C_6H_4$	$(CH_3)_2CHNH$	$C_{17}H_{20}N_6O_2S$	197-199	37	+0.67	20.8 ± 1.6
27	4-$CH_3C_6H_4$	$(CH_3)_2CHNH$	$C_{17}H_{20}N_6O_2S$	209-211	59	+0.91	26.3 ± 2.1
28	3-$CH_3C_6H_4$	$(CH_3)_2CHNH$	$C_{17}H_{20}N_6O_2S$	187-189	37	+0.86	69.1 ± 2.5 *
29	3-$C_2H_5C_6H_4$	$(CH_3)_2CHNH$	$C_{18}H_{22}N_6O_2S$	174-176	74	+1.40	34.7 ± 1.4 *
30	3-$CF_3C_6H_4$	$(CH_3)_2CHNH$	$C_{17}H_{17}N_6O_2SF_3$	195-197	64	+1.54	29.4 ± 1.8
31	3-FC_6H_4	$(CH_3)_2CHNH$	$C_{16}H_{17}N_6O_2SF$	197-199	68	+0.59	45.4 ± 1.4 *
32	3-ClC_6H_4	$(CH_3)_2CHNH$	$C_{16}H_{17}N_6O_2SCl$	188-190	25	+1.14	47.2 ± 1.8 *
33	4-ClC_6H_4	$(CH_3)_2CHNH$	$C_{16}H_{17}N_6O_2SCl$	203-205	67	+1.37	28.1 ± 1.2
34	3-BrC_6H_4	$(CH_3)_2CHNH$	$C_{16}H_{17}N_6O_2SBr$	173-175	36	+1.27	48.5 ± 2.0 *
35	3-IC_6H_4	$(CH_3)_2CHNH$	$C_{16}H_{17}N_6O_2SI$	190-192	36	+1.51	26.3 ± 1.5
36	3-CF_3,4-ClC_6H_3	$(CH_3)_2CHNH$	$C_{17}H_{16}N_6O_2SF_3Cl$	195-197	48	+2.27	22.9 ± 1.7
37	$C_6H_5CH_2$	$(CH_3)_2CHNH$	$C_{17}H_{20}N_6O_2S$	212-214	65	+0.36	42.7 ± 1.8 *

[a]C, H, N, S analyses were within ±0.4% of the theoretical values; [b]all compounds were crystallized from ethanol; [c]values are means of three determinations obtained by the RP-HPLC method; [d]diuresis (ml/kg over 4 h; mean ± SD) induced in rats after oral administration of 30 mg/kg. *Statistically different ($P < 0.01$) from control (22.1 ± 1.8 ml/kg over 4 h).

Table III. Correlation between the logarithm of the partition coefficient (log P) determined by the shake-flask method and the logarithm of the capacity factor (log k') obtained by RP-HPLC.



Compd	R_1	R_2	log P [a]	log k' [b]
12	$CH_3(CH_2)_3$	$(CH_3)_2CHNH$	+ 0.329	- 0.1121
21	c-C_7H_{13}	C_2H_5NH	+ 0.691	+ 0.0091
23	c-C_6H_{11}	$(CH_2)_5$=N	+ 1.068	+ 0.0928
34	3-BrC_6H_4	$(CH_3)_2CHNH$	+ 1.229	+ 0.1435
28	3-$CH_3C_6H_4$	c-$C_6H_{11}NH$	+ 2.060	+ 0.3363

log P = (3.903 x log k') + 0.709; n = 5, r = 0.997

[a]Partition coefficient in *n*-octanol/phosphate buffer pH 7.40; values are means of three determinations; [b]capacity factor (log $(t_r - t_0)/t_0$) obtained by RP-HPLC.

Legend	Compound	OD2x (mg/kg)
●	furosemide (1)	14.1
○	torasemide (2)	1.6
▲	BM 20 (3)	0.02
△	**21**	11.3
□	**28**	9.1

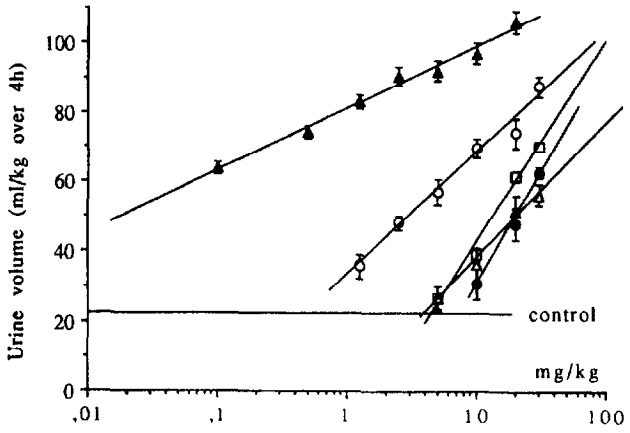


Fig 2. Oral doses (OD2x) that double the urinary volume excreted by control rats over 4 h and dose–response curves of furosemide (●), torasemide (○), BM 20 (▲), **21** (△) and **28** (□). Each point represents the mean ± SD of 9 rats.

single bonds S_3-N_4, N_4-C_5 and C_5-N_6 of **28**. This study led to two conformers as stable as δ, which are similar to the β and γ conformers of **2** (table V). For steric reasons, **28** cannot adopt the α conformation. This suggests that neither the α nor the δ conformation is responsible for the activity, since both compounds are active. The activity of both **2** and **28** must therefore be due to the β or γ conformation that can be present in both molecules. The weak diuretic potency of **28**, compared with **2**, probably lies in the difficulty of adopting the β or γ conformation.

Experimental protocols

Chemistry

All compounds were synthesized from 4-chloropyrid-3-ylsulfonamide [15]. Elemental analyses for C, H, N and S were performed on a Carlo Erba EA 1108 analyzer. Melting points were determined in open capillary with a Büchi Tottoli apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Brucker 80 MHz using tetramethylsilane as an internal standard and chemical shifts are expressed in part per million (δ). IR spectra were determined with a Perkin Elmer 1750 as KBr pellets. All reactions were routinely checked by TLC on silica gel 60F 254.

General procedure for the synthesis of sulfonamides 5

The sulfonamides **5** have been described previously and prepared by the following procedure [6]. 4-Chloropyrid-3-ylsulfonamide **4** (2 g, 10.4 mmol) was heated under reflux with an excess of the appropriate cycloalkylamine (15 mmol) in *n*-propanol (20 ml). At the end of the reaction (3–5 h), the solvent was evaporated under reduced pressure and the residue dissolved in water (100 ml) and 2.5 N NaOH (10 ml). The solution was extracted three times with diethyl ether (100 ml) and adjusted to pH 7 with dilute hydrochloric acid. The precipitated compounds were collected by filtration, washed with water and dried. The reaction of alkylamines with **4** was performed in a hermetically closed autoclave.

N-Cyano-N'-ethyl-S-methylcarbamimidothioate 7a

An aqueous solution of 70% ethylamine (5.0 ml, 88.3 mmol) was added to a solution of *N*-cyano-*S,S'*-dimethyldithioiminocarbonate [16] **6** (5.45 g, 37.3 mmol) in EtOH (20 ml). After 1 h stirring at room temperature, the solution was evaporated under reduced pressure. The residue was dissolved in boiling ethanol (5 ml) and diluted with water (20 ml). After cooling, the precipitate was collected by filtration, washed with water and dried to afford 4.81 g of **7a** (yield: 90%). Mp: 161–163°C; IR (KBr) 2170 cm^{-1} (C≡N st); ^{1}H-NMR (DMSO-d_6) δ 0.99 (3H, t, CH_3CH_2), 2.43 (3H, s, CH_3S), 3.26 (2H, m, CH_2), 8.18 (1H, br s, NH). Anal $C_5H_9N_3S$ (C, H, N, S).

N-Cyano-N'-isopropyl-S-methylcarbamimidothioate 7b

The title compound was obtained from isopropylamine (3.5 ml, 85.3 mmol) and *N*-cyano-*S,S'*-dimethyldithioiminocarbonate [16] **6** (5.45 g, 37.3 mmol) following the procedure described for **7a**. The reaction gave 5.35 g of **7b** (yield: 91%). Mp: 114–116°C; IR (KBr) 2167 cm^{-1} (C≡N st); ^{1}H-NMR (DMSO-d_6) δ 1.06 (6H, 1s, $(CH_3)_2CH$), 2.46 (3H, s, CH_3S), 4.00 (1H, m, CH), 7.97 (1H, br s, NH). Anal $C_6H_{11}N_3S$ (C, H, N, S).

N-Cyano-methylthiopiperidinoimine 7c

The title compound was obtained from piperidine (4.0 ml, 40.4 mmol) and *N*-cyano-*S,S'*-dimethyldithioiminocarbonate [16] **6** (5.45 g, 37.3 mmol) following the procedure described for **7a**. The reaction gave 5.33 g of **7c** (yield: 78%). Mp: 57–59°C; IR (KBr) 2164 cm^{-1} (C≡N st); ^{1}H-NMR (DMSO-d_6) δ 1.51 (6H, m, $-(CH_2)_3-$), 2.58 (3H, s, CH_3S), 3.67 (4H, m, $-N<(CH_2)_2$). Anal $C_8H_{13}N_3S$ (C, H, N, S).

N-Cyano-N'-isopropyl-N''-{[4-(propylamino)pyrid-3-yl]sulfonyl}guanidine 11

The 4-propylaminopyrid-3-ylsulfonamide **5** (0.72 g, 3.34 mmol) was dissolved in one equivalent of 0.25 N NaOH (13.4 ml) and then evaporated under reduced pressure. N-Cyano-*N'*-isopropyl-*S*-methylcarbamimidothioate **7b** (0.56 g, 3.56 mmol) was added to the sodium salt of **5** suspended in a mixture of *N,N'*-dimethylformamide (3 ml) and dioxane (3 ml), and refluxed for 6 h. After evaporation of solvents under reduced pressure the residue was dissolved in water (50 ml) and 2.5 N NaOH (5 ml). The solution was extracted three times with diethyl ether (50 ml) and adjusted to pH 7 with dilute hydrochloric acid. The precipitate was collected by filtration, washed with water, dried and recrystallized in ethanol to afford 0.78 g

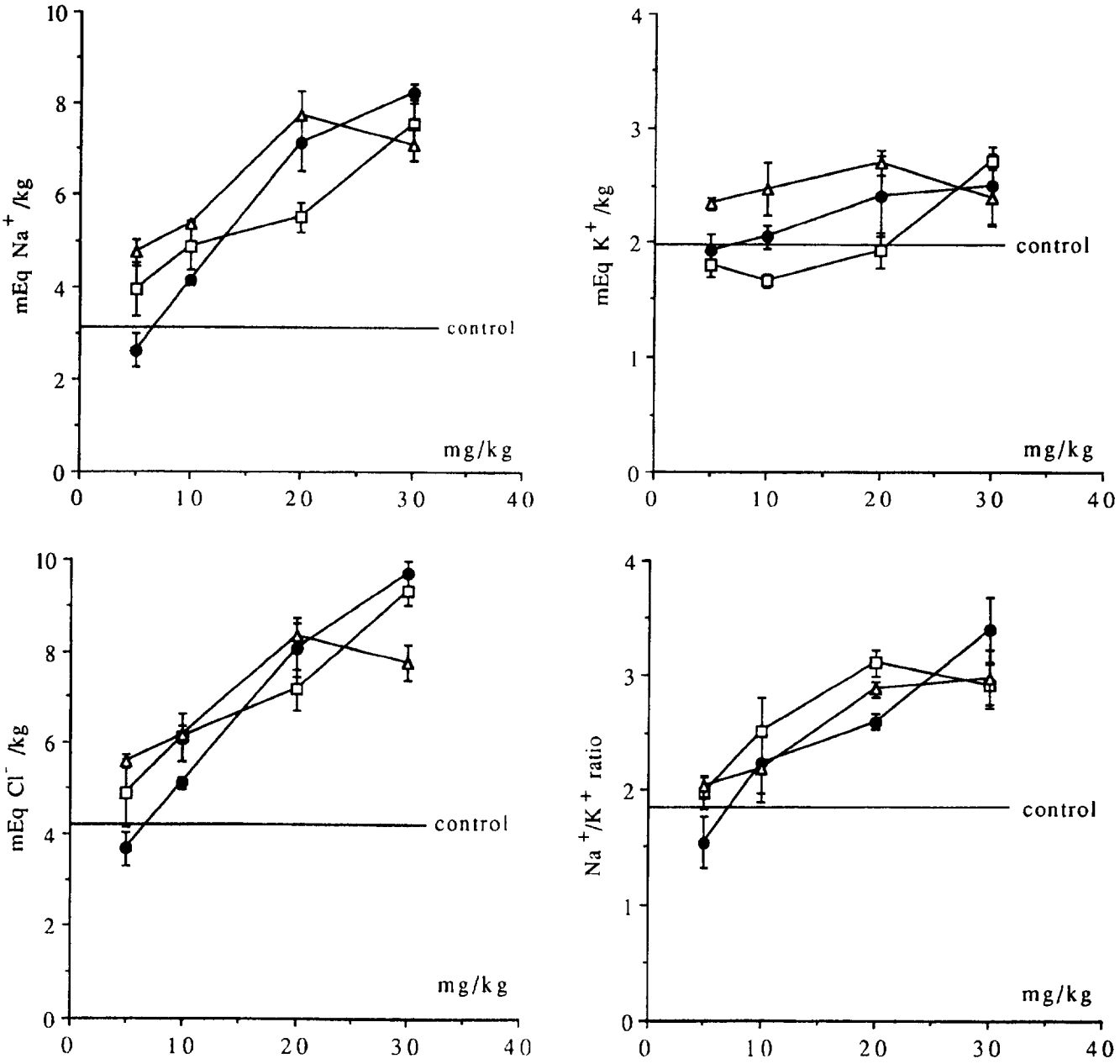


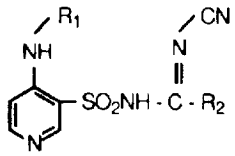
Fig 3. Effects of furosemide (●) and **21** (△) and **28** (□) on sodium, potassium and chloride excretion and Na^+/K^+ urinary ratio over 4 h after oral administration. Each point is mean ± SD of 9 rats.

of **11** (yield: 72%). Mp: 199–201°C; IR (KBr) 2170 cm^{-1} (C≡N st); ^{1}H-NMR (DMSO-d_6) δ 0.89 (3H, t, CH_3), 0.97 (6H, 1s, $(CH_3)_2CH$), 1.61 (2H, m, CH_2CH_3), 3.39 (2H, m, CH_2NH), 3.68 (1H, m, >CHNH), 6.23 (1H, br s, C=N(CN)NH), 7.06 (1H, d, 5H-pyridine), 8.16 (1H, d, 6H-pyridine), 8.21 (1H, m, NH-pyridine), 8.39 (1H, s, 2H-pyridine). Anal $C_{13}H_{20}N_6O_2S$ (C, H, N, S).

N-Cyano-N''-{[4-(cyclohexylamino)pyrid-3-yl]sulfonyl}-N'-isopropylguanidine ***16***

The title compound was obtained from 4-cyclohexylaminopyrid-3-ylsulfonamide **5** (1.01 g, 3.96 mmol) and *N*-cyano-*N'*-isopropyl-*S*-methylcarbamimidothioate **7b** (0.65 g, 4.13 mmol) following the experimental conditions described for **11**. The reaction gave 1.0 g of **16** (yield: 69%).

Table IV. Influence of ethanol on the pK_a value of the sulfonylcyanoguanidine function of **11**, **21** and **28**.



Compd	R_1	R_2	Ethanol % (v/v)				
			0	10	20	30	40
11	$CH_3(CH_2)_2$	$(CH_3)_2CHNH$	6.78 ± 0.01	6.76 ± 0.02	6.77 ± 0.02	6.76 ± 0.01	6.77 ± 0.02
21	c-C_7H_{13}	C_2H_5NH	-[a]	-[a]	6.84 ± 0.02	6.84 ± 0.01	6.85 ± 0.01
28	3-$CH_3C_6H_4$	$(CH_3)_2CHNH$	-[a]	-[a]	6.01 ± 0.01	5.99 ± 0.01	6.00 ± 0.01

Mp: 231–233°C; IR (KBr) 2168 cm^{-1} (C≡N st); ^{1}H-NMR (DMSO-d_6) δ 0.98 (6H, 1s, $(CH_3)_2CH$), 1.14–1.93 (10H, m, cyclohexyl), 3.76–3.85 (2H, 2m, >C*H*NH and $(CH_3)_2CH$), 6.12 (1H, br s, C=N(CN)NH), 7.07 (1H, d, 5H-pyridine), 7.91 (1H, d, NH-pyridine), 8.13 (1H, d, 6H-pyridine), 8.41 (1H, s, 2H-pyridine). Anal $C_{16}H_{24}N_6O_2S$ (C, H, N, S).

N-Cyano-N''-{[4-(cycloheptylamino)pyrid-3-yl]sulfonyl}-N'-ethylguanidine ***21***

The title compound was obtained from 4-cycloheptylamino-pyrid-3-ylsulfonamide **5** (1.00 g, 3.71 mmol) and *N*-cyano-*N'*-ethyl-*S*-methylcarbamimidothioate **7a** (0.55 g, 3.84 mmol) following the experimental conditions described for **11**. The reaction gave 0.96 g of **21** (yield: 70%). Mp: 227–229°C; IR (KBr) 2166 cm^{-1} (C≡N st); ^{1}H-NMR (DMSO-d_6) δ 0.94 (3H, t, CH_3), 1.22–2.16 (12H, m, cycloheptyl), 3.08 (2H, m, CH_2), 3.86 (1H, m, >C*H*NH), 6.48 (1H, br s, C=N(CN)NH), 7.01 (1H, d, 5H-pyridine), 7.92 (1H, d, NH-pyridine), 8.18 (1H, d, 6H-pyridine), 8.42 (1H, s, 2H-pyridine). Anal $C_{16}H_{24}N_6O_2S$ (C, H, N, S).

N-Cyano-N''-{[4-(cyclooctylamino)pyrid-3-yl]sulfonyl}piperidinoamidine ***25***

The title compound was obtained from 4-cyclooctylylamino-pyrid-3-ylsulfonamide **5** (1.00 g, 3.53 mmol) and *N*-cyano-methylthiopiperidinoimine **7c** (0.80 g, 4.36 mmol) following the experimental conditions described for **11**. The reaction gave 0.76 g of **25** (yield: 52%). Mp: 199–201°C; IR (KBr) 2161 cm^{-1} (C≡N st); ^{1}H-NMR (DMSO-d_6) δ 1.18–2.02 (20H, m, -CH_2- from cyclooctyl and piperidine), 3.76–3.84 (5H, 2m, >C*H*NH and C=N(CN)N<$(CH_2)_2$), 7.03 (1H, d, 5H-pyridine), 7.92 (1H, d, NH-pyridine), 8.20 (1H, d, 6H-pyridine), 8.39 (1H, s, 2H-pyridine). Anal $C_{20}H_{30}N_6O_2S$ (C, H, N, S).

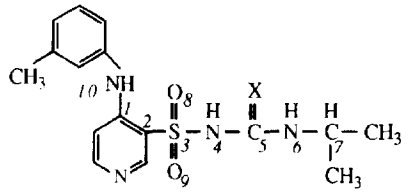
N-Cyano-N'-isopropyl-N''-{[4-(3'-methylphenylamino)pyrid-3-yl]sulfonyl}guanidine ***28***

The title compound was obtained from 4-(3'-methylphenyl-amino)pyrid-3-ylsulfonamide **5** (1.00 g, 3.80 mmol) and *N*-cyano-*N'*-isopropyl-*S*-methylcarbamimidothioate **7b** (0.63 g, 4.00 mmol) following the experimental conditions described for **11**. The reaction gave 0.52 g of **28** (yield: 37%). Mp: 187–189°C; IR (KBr) 2164 cm^{-1} (C≡N st); ^{1}H-NMR (DMSO-d_6) δ 0.96 (1s, 6H, $(CH_3)_2CH$), 2.28 (s, 3H, CH_3-phenyl), 3.76 (m, 1H, NHC*H*), 6.42 (br s, 1H, C=N(CN)NH), 7.04 (d, 1H, 5H-pyridine), 7.28–7.47 (m, 4H, phenyl), 8.20 (d, 1H, 6H-pyridine), 8.66 (s, 1H, 2H -pyridine), 9.66 (s, 1H, NH-pyridine). Anal $C_{17}H_{20}N_6O_2S$ (C, H, N, S).

Lipophilicity

The lipophilicity of the compounds listed in table III is expressed as the logarithm of the partition coefficient (log *P*) in *n*-octanol/phosphate buffer pH 7.40 by using the shake-flask technique [17]. A RP-HPLC system was also loaded to determine the log *P* of other drugs (tables I and II) [18]. A series of standards (table III) with a wide range of lipophilicity determined by the shake-flask method was run and a calibration curve was established for each session. KNO_3 was injected to determine the void volume and log *k'* ($\log(t_r - t_0)/t_0$) was measured for each sample, where t_r is the drug retention time and t_0 the NO_3^- retention time. A correlation curve was calculated from log *P* and log *k'* of standards: log *P* = (3.903 x log *k'*) + 0.709; *n* = 5; *r* = 0.997. Log *P* values of other compounds (tables I and II) were obtained by interpolation of the standard curve.

Table V. Comparative evaluation of the conformers of torasemide **2** and **28**



$o_1 = C_1 - C_2 - S_3 - N_4$ $o_2 = C_2 - S_3 - N_4 - C_5$

$o_3 = S_3 - N_4 - C_5 - N_6$ $o_4 = N_4 - C_5 - N_6 - C_7$

Compound	Conformer	ϕ_1 (°)	ϕ_2 (°)	ϕ_3 (°)	ϕ_4 (°)	E (kcals/mol)
torasemide (2) (X = O)	α	101.2 (-90)	+69.5 (+90)	+173.4 (180)	-177.1 (180)	99.72
	β	+81.2 (+90)	+57.5 (+90)	-170.2 (180)	-7.3 (0)	124.35
	γ	+65.8 (+90)	+71 (+90)	+171.3 (180)	+179.9 (180)	102.13
28 (X = N-CN)	δ	+65.9 (+90)	+117.3 (+90)	+27.8 (0)	-179.9 (180)	218.78
	β	+78.0	+50.7	+164.5	+8.4	212.61
	γ	+75.1	+54.4	+173.7	-174.9	215.65

The energy and the torsional angle values were calculated from crystallographic structural data optimized by Mopac. The typical angle values are in brackets.

Ionization constants

The pK_a of compounds **11**, **21** and **28** were determined by dynamic titration. Each compound was dissolved at a concentration of 2 mM in a mixture (50 ml) containing 0.010685 N NaOH (15 ml), water and ethanol ranging from 0 to 20 ml. This solution (10 ml) was titrated with increments (80 µl) of $HClO_4$ (0.010758 N) using a dosimat Metrohm 665 and a titroprocessor Metrohm 670 combined with a glass electrode Metrohm 6.0204.100. The pK_a values obtained correspond to the pH of half-neutralization and were corrected according to the equation described by Albert *et al* [20]. Corrected pK_a values represent the mean of three independent determinations performed at 25°C.

Diuresis

Each drug or vehicle (NaCl 0.9% with methocel 0.1%) was orally administered in rats (male Wistar 189–231 g) in a dose volume of 40 ml/kg. For the preliminary screening, 3 rats received each drug at a dose of 30 mg/kg. The animals were housed in metabolism cages and urine collected over 4 h. The diuresis is expressed in ml/kg over 4 h. In dose-dependent experiments, each dose of **1–3**, **21** or **28** was given orally to nine rats. Diuresis (ml/kg over 4 h) and the urinary concentrations of sodium, potassium and chloride were determined.

Molecular modeling

The molecular and structure design, search process and energy calculations were performed using Sybil 6.03 software package [21] on a Silicon Graphics Personal Iris Indigo Elan workstation. The starting coordinates of **2** [23, 24] and **28** [32] were taken from crystal structure analysis. The conformational spaces of both compounds were explored using Sybil automatic search facility. Torsion angles were defined around the

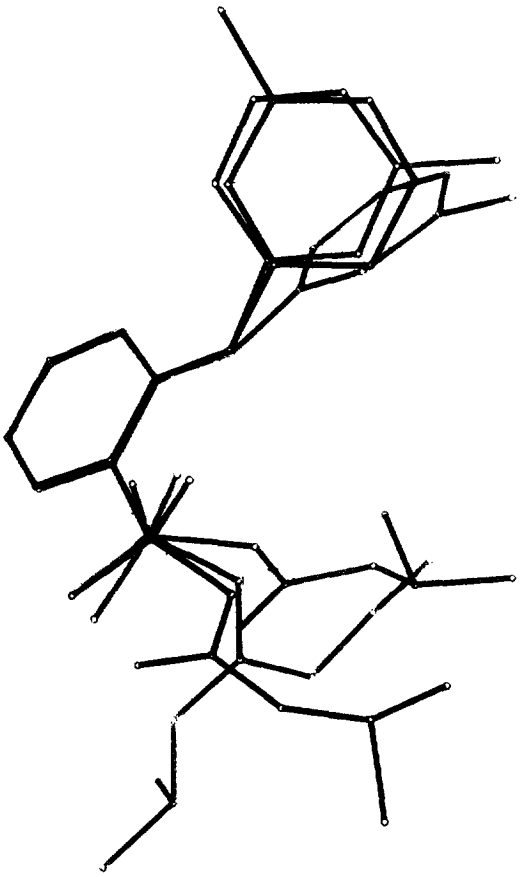


Fig 4. Superimposition of the δ (green) conformer of **28** with the β (black) and γ (red) conformers of torasemide (**2**).

single bonds S_1-N_3, N_3-C_6 and C_6-N_5 of **2** and **28**. The bonds were allowed to rotate with a 360° revolution by 30 and 10° increments. The lowest-energy conformers thus obtained were submitted to AM1 calculations (Mopac 5.0) [22] to optimize their geometry and determine atomic charge distributions.

Acknowledgments

We thank P Neven and ML Pirard for technical assistance.

References

1 Schlatter E, Greger R, Weidtke C (1983) *Pflügers Arch* 396, 210–217
2 Wittner M, Di Stefano A, Wangemann P, Delarge J, Liégeois JF, Greger R (1987) *Pflügers Arch* 408, 54–62
3 Friedel HA, Buckley MMT (1991) *Drugs* 41, 81–103
4 Delarge J, Lapière CL, de Ridder R, Ghys A (1981) *Eur J Med Chem* 16, 65–68
5 Delarge J, Lapière CL, de Rldder R, Ghys A (1980) *Eur J Med Chem* 15, 299–304
6 Masereel B, Lohrmann E, Schynts M, Pirotte B, Greger R, Delarge J (1992) *J Pharm Pharmacol* 44, 589–593
7 Masereel B, Schynts M, Krzesinski JM, Pirotte B, Rorive G, Delarge J (1993) *J Pharm Pharmacol* 45, 720–724
8 Masereel B (1993) *Cardiovasc Drugs Rev* 11, 359–369
9 Petersen HJ (1974) *J Med Chem* 17, 101–104
10 Durant GJ, Emmett JC, Ganellin CR *et al* (1977) *J Med Chem* 20, 901–906
11 Takemoto T, Eda M, Okada T *et al* (1994) *J Med Chem* 37, 18–25
12 Manley PW, Quast U (1992) *J Med Chem* 35, 2327–2340
13 Lohrmann E, Nitschke RB, Nitschke R *et al* (1991) *In: Proc XIth Int Congr Nephrol* (Hatano M, ed) Springer-Verlag, Tokyo, Japan, 1072–1081
14 Haas M, McManus TJ (1983) *Am J Physiol* 245, C235–C240
15 Delarge J (1973) *Ann Pharm Fr* 31, 467–474
16 Hantzsch A, Wolvekamp M (1904) *Justus Liebigs Ann Chem* 331, 265–297
17 Cloux JL, Crommen J, Delarge J, Pirard ML, Thunus L (1988) *J Pharm Belg* 43, 141–151
18 Masereel B, Renard P, Schynts M, Pirotte B, de Tullio P, Delarge J (1994) *Eur J Med Chem* 29, 527–537
19 Masereel B, Ferrari P, Ferrandi M *et al* (1992) *Eur J Pharmacol* 219, 385–394
20 Albert A, Serjeant EP (1971) *In: The Determination of Ionization constants* (Albert A, Serjeant EP, eds) Chapman & Hall, London, UK, 23–43
21 Tripos Associates Inc, 1699, S Harley Rd, Suite 303, Saint Louis, MO, 63144, USA
22 Stewart JJP (1990) *J Comput-Aided Mol Des* 4, 1–105
23 Dupont L, Lamotte J, Campsteyn H, Vermeire M (1978) *Acta Cryst* B34, 1304–1310
24 Dupont L, Campsteyn H, Lamotte J, Vermeire M (1978) *Acta Cryst* B34, 2659–2662
25 Dupont L, Dideberg O, Vermeire M (1979) *Acta Cryst* B35, 1501–1504
26 Dupont L, Dideberg O, Lamotte J (1979) *Acta Cryst* B35, 2817–2820
27 Dupont L, Dideberg O, Toussaint J, Delarge J (1980) *Acta Cryst* B36, 2170–2173
28 Dupont L, Dideberg O, Delarge J (1980) *Cryst Struct Comm* 9, 1105–1110
29 Dupont L, Lewinski K, Stadnicka K, Delarge J (1981) *Cryst Struct Comm* 10, 925–930
30 Dupont L, Dideberg O, Delarge J, Dive G, Thunus L (1982) *Acta Cryst* B38, 1495–1500
31 Dupont L, Dideberg O, Masereel B, Delarge J, Schynts M, Pirotte B (1991) *Acta Cryst* C47, 2152–2156
32 Dupont L, Masereel B, de Tullio P, Pirotte B, Delarge J (1995) *Acta Cryst* C50 (in press)