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A Sulphonylthiourea (BM 20) Related to Torasemide: a new Loop Diuretic with Relative Potassium-sparing Properties

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Abstract—A series of sulphonylthioureas related to torasemide, a high ceiling loop diuretic, were synthesized and found to inhibit the Na^+ $2Cl^-$ K^+ co-transporter of the thick ascending limb of the loop of Henlé. Their diuretic properties were studied (30 mg kg^{-1}) after oral administration to rats. Lipophilic derivatives, very active in-vitro, were found inactive orally and intraperitoneally in rats. The four most active compounds were examined for their dose-dependent diuresis. Three of them showed a potency, water and electrolyte excretion similar to torasemide. The fourth molecule, a sulphonylthiourea (BM 20), exhibited relative potassium-sparing properties and a minimal diuretic dose of 0·001 mg kg^{-1}, 200 times lower than torasemide.

Torasemide is the leader of a new class of loop diuretics (Delarge 1988; Friedel & Buckley 1991) more potent and more potassium-sparing than frusemide (Uchida et al 1990, 1991). Torasemide exhibits antihypertensive properties at non-diuretic doses (Spannbrucker et al 1988) probably due to vasodilatation caused by an increase of intracellular cAMP (Schoeffer et al 1987; Yamanaga et al 1992). Sulphonylthioureas related to torasemide have been synthesized and found to inhibit the renal torasemide target, the luminal Na^+ $2Cl^-$ K^+ co-transport of the thick ascending limb of the loop of Henlé (Masereel et al 1992).

According to the structure-activity relationships postulated (Delarge et al 1980, 1981; Wittner et al 1987; Masereel et al 1992) new sulphonylthioureas have been prepared (Fig. 1) and screened for their diuretic properties.

Materials and Methods

Chemistry (Fig. 1)

Sulphonylthioureas BM 35, BM 36 and BM 37 (Table 3) were prepared by an original route. Other compounds were synthesized from 4-cycloalkylamino pyrid-3-yl sulphonamide according to general synthetic pathways previously described (Delarge 1973; Delarge et al 1980; Masereel et al 1992). Elemental analyses of all molecules for C, H, N and S were within 0·4% of the theoretical values. NMR and IR spectra were in accordance with chemical structures. All reactions were routinely checked by thin layer chromatography.

Synthesis of 4-cycloalkylamino pyrid-3-yl sulphonylimino carbodithioate potassium salt. The appropriate sulphonamide (0·01 mol) was suspended in absolute ethanol (30 mL) and dissolved by adding powdered KOH (0·03 mol). Carbon disulphide (10 mL) was added and the solution stirred at room temperature (21°C) for 24 h. The resulting precipitate was collected, washed with absolute ethanol, then with ether, and dried. The yield was 30–40%.

Synthesis of 4-cycloalkylamino pyrid-3-yl sulphonyl methylmercapto thiocarbamate. The appropriate potassium carbodithioate (0·01 mol) was suspended in methanol (50 mL). Iodomethane (0·011 mol) was added and the mixture stirred at room temperature (21°C). At the end of the reaction, water (100 mL) was added to the solution. The mixture was adjusted to pH 7·0 with 2 M HCl and the formed precipitate was filtered, washed with water and dried. The yield was 52–77%.

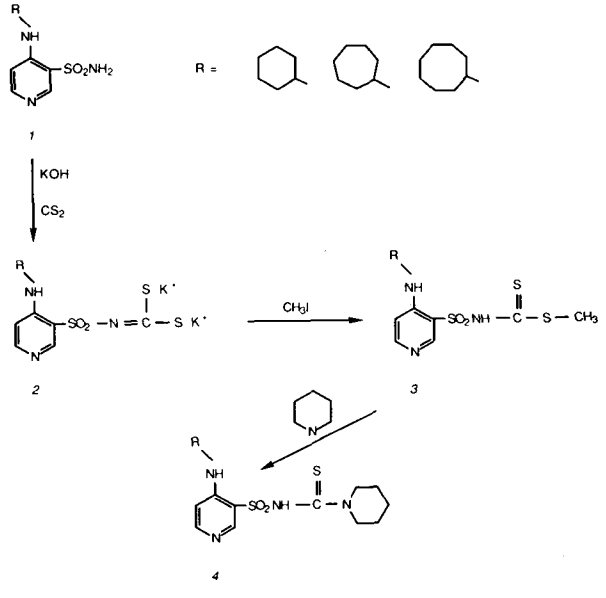

FIG. 1. Synthetic pathway of new sulphonylthioureas.

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Synthesis of 4-cycloalkylamino pyrid-3-yl sulphonyl piperidinocarbamate. The required thiocarbamate (1 g) was dissolved in piperidine (10 mL) and refluxed for 24–36 h. At the end of the reaction, excess piperidine was removed under reduced pressure. The crude residue was dissolved in 1 M NaOH (100 mL) and washed three times with ether. The alkaline solution was adjusted to pH 7·0 with acetic acid and the resulting precipitate was crystallized from ethanol/ether. The yield was 70–75%.

Diuresis

Preliminary screening. An oral single dose study was conducted in male Wistar rats, 189–231 g. The rats were allowed free access to food and water until the beginning of the experiment. At 0900 h, the drug or the vehicle (NaCl 0·9% with sodium carmellose 0·1%) was administered by gastric lavage in a dose volume of 40 mL kg^{-1}. The rats were housed in plastic metabolism cages (three rats/cage) and urine collected for 4 h. Three rats received each drug at a dose of 30 mg kg^{-1}.

Oral dose-dependent diuresis. Torasemide, BM 15, BM 17, BM 20 and BM 24 were given orally (20, 10, 5, 2·5, 1·25, 0·5 and 0·1 mg kg^{-1}). Nine rats received each dose of drug or vehicle in a dose volume of 40 mL kg^{-1}. Urine was collected 1, 2, 4 and 8 h following gavage. The urinary concentrations of sodium and potassium were determined by flame photometry and chloride by argentimetry.

Intraperitoneal dose-response study. Torasemide, BM 6, BM 9 and BM 27 were injected at a dose of 20 and 5 mg kg^{-1}. Each rat received 30 mL kg^{-1} vehicle orally and 10 mL kg^{-1} drug intraperitoneally. The protocol was similar to the oral dose-dependent diuresis study.

Results and Discussion

Preliminary screening

Urine excretion was induced by reference compounds (Table 1) and torasemide derivatives (Tables 2, 3) at a single oral dose of 30 mg kg^{-1}. Torasemide-induced diuresis (88 mL kg^{-1}/4 h) is similar to that obtained by Delarge et al (1980) (93 mL kg^{-1}/4 h). Bumetanide, as potent as torasemide in-vitro (Schlatter et al 1983; Wittner et al 1987) is only slightly diuretic (38 mL kg^{-1}/4 h) in rats due to its extensive metabolism (Ostergaard et al 1972).

Of the torasemide derivatives, Tables 2 and 3, many compounds could be considered as weakly diuretic (< 40 mL kg^{-1}/4 h). BM 2, BM 3, BM 21, BM 23, BM 35 and BM 37 induced high urinary volume excretion (> 70 mL kg^{-1}/4 h) and were more potent than frusemide (49 mL kg^{-1}/4 h). Sulphonylureas, BM 15 and BM 17 (Table 2) and sulphonylthioureas, BM 20 and BM 24 (Table 3), were shown to be the most diuretic molecules. As expected, BM 1, BM 18, BM 19, and BM 28, all inactive in-vitro, did not exhibit diuretic properties probably due to the steric hindrance of the R1-cyclododecyl (BM 18, BM 19, BM 28) moiety of the R2-amantadine residue (BM 1) (Table 2). Surprisingly, high potent co-transport inhibitors in-vitro such as BM 6, BM 9, BM 10, BM 11, BM 12 and BM 27 produced only small urine excretion.

Table 1. Lipophilicity (log P′) and diuresis induced by oral administration of reference loop diuretics (30 mg kg^{-1}). Values represent the urine volume (mL kg^{-1}) collected for 4 h following gavage of three rats, and are uncorrected for the vehicle.

Compound	log P′	Diuresis
Vehicle		20
Frusemide	−0·92	49
Bumetanide	−0·30	38
Piretanide	−0·60	86
Torasemide	+0·45	88
		93*

* Delarge et al (1980).

No correlation was found between the in-vitro inhibitory potency of the luminal Na^+ $2Cl^-$ K^+ co-transporter and the in-vivo diuretic properties throughout this series. However, a good correlation ($r = 0·84$; $P < 0·001$; $n = 26$) was established between lipophilicity and urine volume (Fig. 2). The lipophilicity (log P′) was expressed as the logarithm of the partition

Table 2. Lipophilicity (log P′) and diuresis induced by oral administration of sulphonylureas (30 mg kg^{-1}). Values represent the urine volume (mL kg^{-1}) collected for 4 h following gavage of three rats, and are uncorrected for the vehicle.

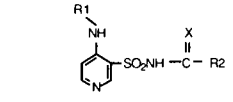
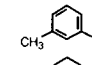
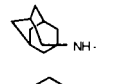
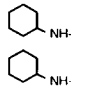
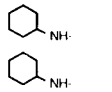
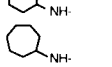
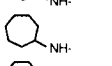
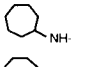
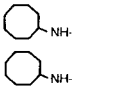
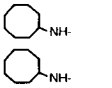
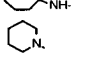
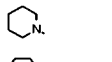
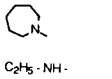
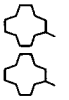
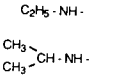
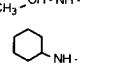
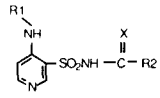
N°	Internal N°	R1	R2	X	log P′	Diuresis
						
5	BM 1			O	+2.35	28
6	BM 2			O	+1.33	73
7	BM 3			O	+1.67	72
8	BM 10			O	+2.06	40
9	BM 8			O	+1.72	42
10	BM 27			O	+2.06	47
11	BM 6			O	+2.45	40
12	BM 4			O	+2.07	30
13	BM 12			O	+2.44	35
14	BM 9			O	+2.70	27
15	**BM 15**			O	+1.24	**86**
16	**BM 17**			O	+1.65	**86**
17	BM 16			O	+1.53	36
18	BM 18		C_2H_5-NH-	O	+2.27	20
19	BM 19		CH_3 CH_3 >CH-NH-	O	+2.56	43
20	BM 28			O	+3.45	23

Table 3. Lipophilicity (log P') and diuresis induced by oral administration of sulphonylthioureas (30 mg kg^{-1}). Values represent the urine volume (mL kg^{-1}) collected for 4 h following gavage of three rats, and are uncorrected for the vehicle.

N°	Internal N°	R1	R2	X	log P'	Diuresis
						
21	BM 24		$C_2H_5 \cdot NH \cdot$	S	+0 20	9 6
22	BM 20		$C_2H_5 \cdot NH \cdot$	S	+0 96	105
23	BM 21		$(CH_3)_2CH \cdot NH \cdot$	S	+0 96	79
24	BM 23		$(CH_3)_2CH \cdot NH \cdot$	S	+1 30	76
25	BM 11			S	+1 81	44
26	BM 34			S	+2 14	29
27	BM 35			S	+0 85	76
28	BM 36			S	+1 25	67
29	BM 37			S	+1 60	82

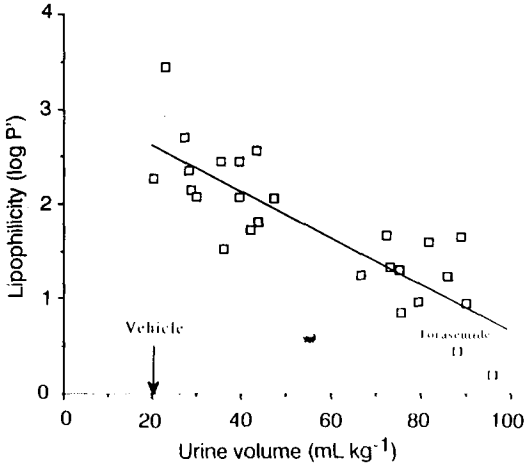


FIG. 2. Correlation between the urine volume (mL kg^{-1}) induced by torasemide and its derivatives (30 mg kg^{-1}, p.o.) and their lipophilicity (log P'). r = 0·84, $P < 0·001$, n = 26.

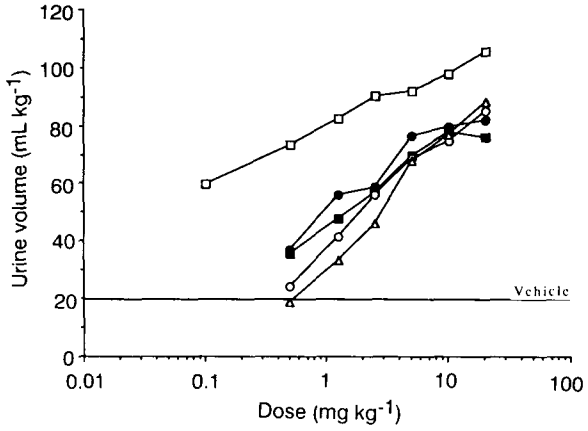


FIG. 3. Effects of torasemide (■), BM 15 (○), BM 17 (△), BM 20 (□) and BM 24 (●) on urine volume (mL kg^{-1}) for a 4 h period following oral administration. Data represent mean of nine rats.

coefficient in *n*-octanol/phosphate buffer (pH 7·4) system (Cloux et al 1988). This correlation showed that the diuretic activity significantly decreased for molecules with log P' > 1·7. The lack of biological response of such hydrophobic compounds could be caused by a poor bioavailability, a different tissue distribution or a modification of their excretion route.

To test the hypothesis of poor oral absorption, intravenous testing would be appropriate. However, the hydrophobic sulphonylureas (BM 6, BM 9 and BM 27), which were very active in-vitro (Masereel et al 1992) were so poorly soluble, even as sodium salts or with a co-solvent, that the intraperitoneal route was preferred (Table 4). The mode of administration did not modify the urine excretion induced by torasemide. At 5 mg kg^{-1}, the three lipophilic compounds did not exhibit any diuretic properties whatever the route used. As shown in the oral screening (30 mg kg^{-1}), BM 9 orally or intraperitoneally and BM 6 orally had no diuretic activity at 20 mg kg^{-1}. BM 27 intraperitoneally or orally could be considered as a very weak diuretic agent.

Dose-dependent diuresis

The most active compounds, two sulphonylureas, BM 15 and BM 17 (Table 2), and two sulphonylthioureas, BM 20 and BM 24 (Table 3), were selected to study their dose-dependent diuretic properties at oral doses ranging from 0·1 to 20 mg kg^{-1}. Torasemide was chosen as a reference compound. Dose-related increases in urine flow were

Table 4. Urine volume (mL kg^{-1}) excreted for a 4 h period after oral or intraperitoneal administration of 20 or 5 mg kg^{-1} torasemide, BM 6, BM 9 and BM 27.

		20 mg kg^{-1}		5 mg kg^{-1}	
N°	Compound	Oral	Intraperitoneal	Oral	Intraperitoneal
	Vehicle	19·8 ± 1·6	10·4 ± 1·4	19·8 ± 1·6	10·4 ± 1·4
	Torasemide	73·9 ± 4·2***	82·1 ± 1·4***	69·7 ± 2·7***	60·1 ± 3·3***
11	BM 6	18·6 ± 2·0	28·1 ± 1·8***	12·5 ± 1·4	6·0 ± 1·1
14	BM 9	23·0 ± 1·1	17·5 ± 3·0	20·9 ± 1·0	5·6 ± 1·3
10	BM 27	28·3 ± 0·7**	32·9 ± 1·9***	22·3 ± 4·7	12·3 ± 3·6

Data are expressed as mean ± s.e.m. of three cages. *** $P < 0·001$, ** $P < 0·01$.

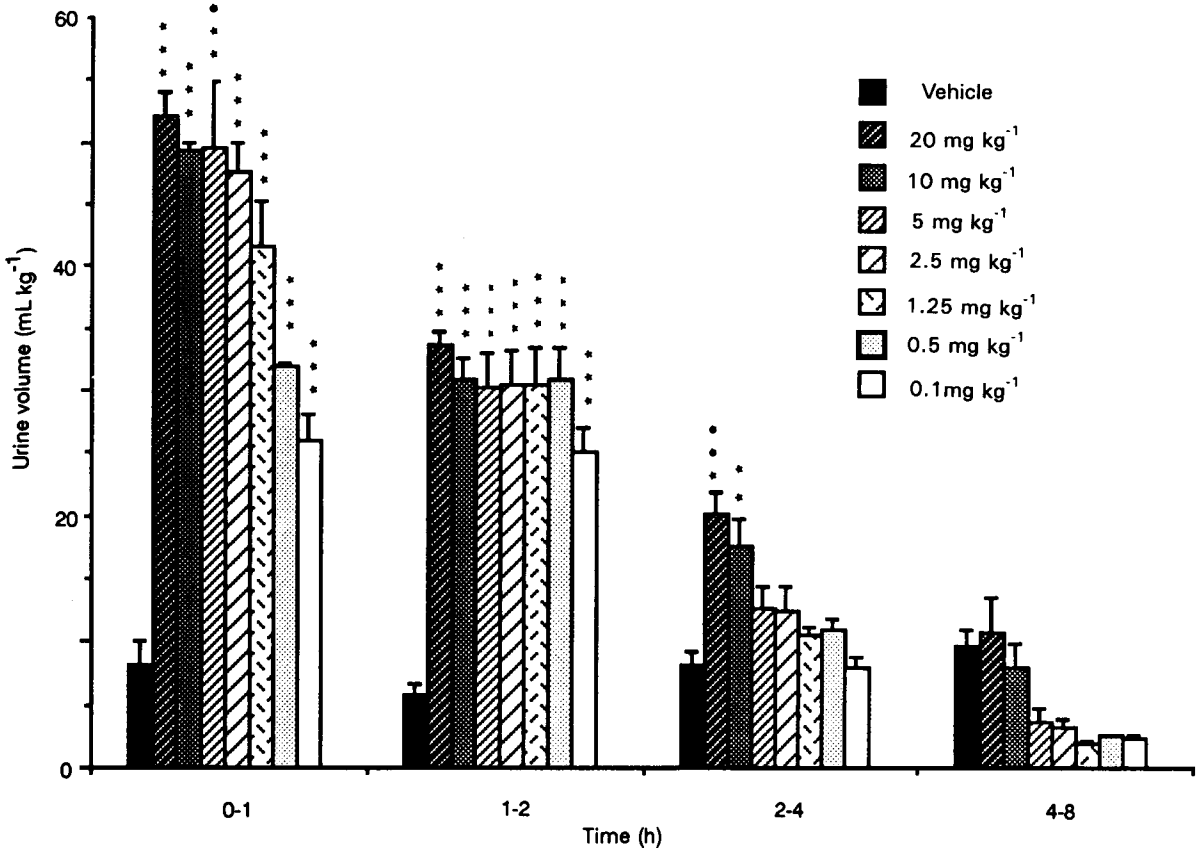


FIG. 4. Time-course of urine excretion (mL kg^{-1}) after oral administration of BM 20. Drug was administered at doses ranging from 0·1 to 20 mg kg^{-1} and urine collected at 1, 2, 4 and 8 h. Data represent mean ± s.e.m. of nine rats. *** $P<0·001$, ** $P<0·01$.

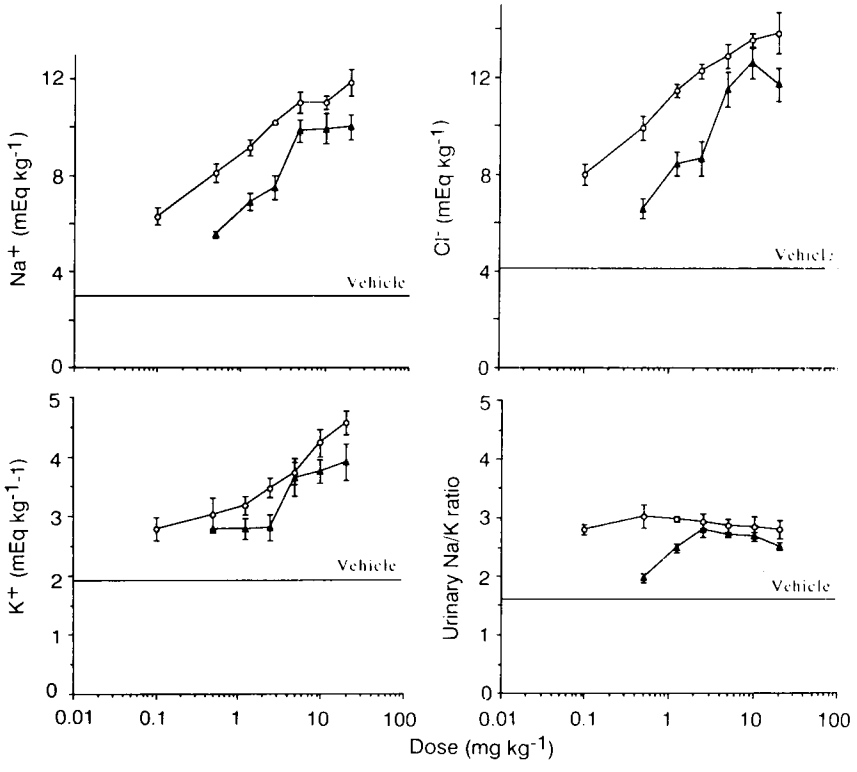


FIG. 5. Effects of torasemide (▲) and BM 20 (○) on sodium, potassium and chloride excretion and urinary Na/K ratio for 4 h after oral administration. Data represent mean ± s.e.m. of nine rats.

Table 5. Minimal diuretic dose (mg kg^{-1}) calculated from dose-response curve equations.

N°	Compound	Minimal diuretic dose
	Torasemide	0·2***
15	BM 15	0·3***
16	BM 17	0·6***
22	BM 20	0·001***
21	BM 24	0·2**

*** $P<0·001$, ** $P<0·01$.

observed with both sulphonylureas and sulphonylthioureas (Fig. 3) after oral administration to rats. For each compound, regression analysis of the total urinary volume excreted and the administered dose led to the establishment of dose-response curves from which the minimal diuretic efficient dose (mg kg^{-1}) was drawn (Table 5). This value is the higher oral dose which produces a urinary volume equal to that obtained with the vehicle for 4 h. BM 24 and both sulphonylureas (BM 15 and BM 17) showed a similar diuretic efficacy to that induced by torasemide, whose minimal diuretic dose (0·2 mg kg^{-1}) corresponds to that obtained by Ghys et al (1985) (0·1 mg kg^{-1}). The sulphonylthiourea BM 20 showed a minimal diuretic dose (0·001 mg kg^{-1}), 0·5% that of torasemide.

BM 20 induced a rapid onset of action in rats occurring the first two hours after oral administration (Fig. 4), indicating that this diuretic is rapidly absorbed after oral gavage. This effect was similar to what was observed with torasemide (data not shown) and typical of high-ceiling diuretics. Torasemide and BM 20 also produced dose-related increases in electrolyte excretion after oral gavage in rats (Fig. 5). The same quantities of sodium, potassium and chloride are excreted when a substantially lower dose of BM 20 is administered. At 0·5 and 1·25 mg kg^{-1}, the urinary Na/K ratio is significantly higher ($P<0·01$) after BM 20 gavage than after torasemide (Fig. 5) which was demonstrated to be more potassium-sparing than frusemide (Uchida et al 1991).

In conclusion, this study demonstrated that BM 20, a sulphonylthiourea related to torasemide, induced changes similar to torasemide in the pattern of urine, sodium, potassium and chloride excretion. Indeed, BM 20 has the profile of a new potent high-ceiling diuretic exhibiting a minimal diuretic dose 200-times lower than torasemide. Moreover the high potency and the relative potassium-sparing properties of BM 20 could be a therapeutic advantage compared with currently used loop diuretics.

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