J. Pharm. Pharmacol. 1992, 44: 589–593
Received November 13, 1991

Design, Synthesis and Biological Activity of a Series of Torasemide Derivatives, Potent Blockers of the Na^+ $2Cl^-$ K^+ Co-transporter: In-vitro Study

B. MASEREEL, E. LOHRMANN*, M. SCHYNTS, B. PIROTTE, R. GREGER* AND J. DELARGE

*Department of Medicinal Chemistry, University of Liege, rue Fusch 3, 4000 Liege, Belgium and *Physiologisches Institut der Albert-Ludwigs-Universität, Hermann Herder Strasse 7, 7800 Freiburg, Germany*

Abstract—Pharmacomodulation of the torasemide molecule, a loop diuretic inhibiting Na^+ $2Cl^-$ K^+ co-transport in the thick ascending limb of the loop of Henlé has been performed in order to obtain new long-acting diuretics. The aim of this study was to decrease the metabolism of the drug and to slow down its rate of excretion by increasing its hydrophobicity. The present study describes the synthesis and the inhibitory potency of new torasemide derivatives in the bioassay system of the cortical thick ascending limb of rabbit. A correlation between the lipophilicity (log P') of these substances and their activity as inhibitors of the Na^+ Cl^- K^+ co-transporter was observed. The present design led to compounds more active than torasemide. Structure-activity relationships permit us to propose an interaction model between torasemide derivatives and the Na^+ $2Cl^-$ K^+ co-transport system of the cortical thick ascending limb.

Torasemide and many of its derivatives are well known as loop diuretics. Their diuretic activity is mostly the result of the inhibition of the luminal Na^+ $2Cl^-$ K^+ co-transport system present in the thick ascending limb of the loop of Henlé (Delarge 1988). Their ability to block chloride channels in the basolateral membrane of this same nephron segment is probably not relevant to the diuretic effect (Witnner et al 1987).

On the basis of the structure-activity relationships previously described (Delarge et al 1980, 1981; Witnner et al 1987), new related compounds were synthesized with the intention of generating potentially long-acting diuretics as a result of either a reduction in their metabolism or by slowing down their excretion rate by enhancing their hydrophobicity.

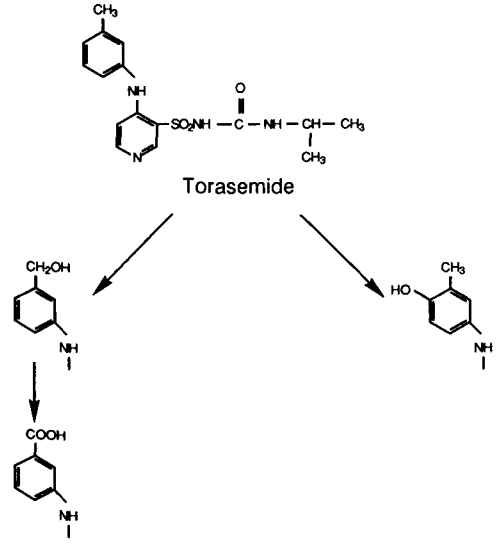


FIG. 1. Structure of torasemide and its metabolites.

Correspondence: B. Masereel, Department of Medicinal Chemistry, University of Liege, rue Fusch 3, 4000 Liege, Belgium.

The metabolism site on the torasemide molecule, the *m*-toluyl moiety (Fig. 1) (Karnes et al 1989), was replaced by a putative more resistant cycloalkyl residue (R_1) since most metabolizing reactions first occur at the most reactive centres such as hydroxyl or amino groups, allylic carbons and aromatic rings (Williams 1974). Hydrophobicity could be increased by the replacement of the aminoisopropyl side chain with a second cycloalkylamino moiety (R_2). For other compounds, the distal nitrogen of the sulphonylurea function was included in a saturated ring (R_2). Finally, and for the same reasons, sulphonylthioureas were synthesized.

The compounds prepared in this study (Table 1) were screened for their lipophilicity (octanol/phosphate buffer pH 7·4 partition, expressed as log P') and their inhibitory potency on the Na^+ $2Cl^-$ K^+ co-transporter in the rabbit cortical thick ascending limb.

Materials and Methods

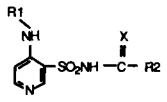
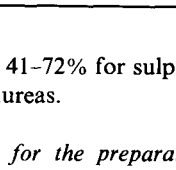
Chemistry (Fig. 2)

Elemental analyses of all synthesized compounds for C, H, N and S were within ±0·4% of the theoretical values. All reactions were routinely checked by thin layer chromatography. The general procedures for the preparation of 4-cycloazkylamino sulphonamides have been described previously (Delarge 1973; Delarge et al 1980).

General procedure for the preparation of sulphonylthioureas and sulphonylureas. Routes 1 & 2

The appropriate sulphonamide (0·07 mol) was dissolved in 20 mL of a mixture of acetone/water (50 : 50). Triethylamine (0·07 mol) and the appropriate isothiocyanate or isocyanate (0·1 mol) were added. The reaction mixture was stirred and refluxed. At the end of the reaction, the solvent was removed under reduced pressure. The residue was dissolved in 1 M NaOH (100 mL) and washed with ether. The aqueous solution was adjusted to pH 7·0 with acetic acid and the resulting precipitate was filtered and crystallized from etha-

Table 1. Torasemide and its synthesized derivatives. Lipophilicity (log P′) and biological activity expressed as the concentration (IC50 μM) to inhibit 50% of the short-circuit current in the rabbit cortical thick ascending limb.

N°	Internal N°	R1	R2	X	log P′	IC50
						
7	TORA			O	+0.45	0.30
8	JDL 888			O	+0.68	0.70
9	BM 2			O	+1.33	19
10	BM 3			O	+1.67	3.5
11	BM 10			O	+2.06	0.47
12	BM 8			O	+1.72	9.6
13	BM 27			O	+2.06	2.8
14	BM 6			O	+2.45	2.0
15	BM 4			O	+2.07	14
16	BM 12			O	+2.44	1.7
17	BM 9			O	+2.70	0.56
18	BM 28			O	+3.45	>100
19	BM 21			S	+0.96	1.3
20	BM 11			S	+1.79	0.79
21	C 2947			O	+0.62	0.82
22	BM 15			O	+1.24	0.25
23	BM 17			O	+1.65	0.15
24	BM 16			O	+1.53	0.80
25	FURO				-0.92	3.0

nol. The yield was 41–72% for sulphonylthioureas and 55–77% for sulphonylureas.

General procedure for the preparation of sulphonylcarbamates. Route 3

The appropriate sulphonamide (0·01 mol) was dissolved in pyridine (25 mL) and stirred at room temperature (21°C). Excess ethylchloroformate (20 mL) was added dropwise. The mixture was heated for 20 min. Pyridine and ethylchloroformate were evaporated to dryness in-vacuo. The crude residue was dissolved in 1 M NaOH (50 mL) and the solution was adjusted to pH 7·0 with acetic acid. The resulting precipitate was collected, washed with water and dried. The yield was 54–77%.

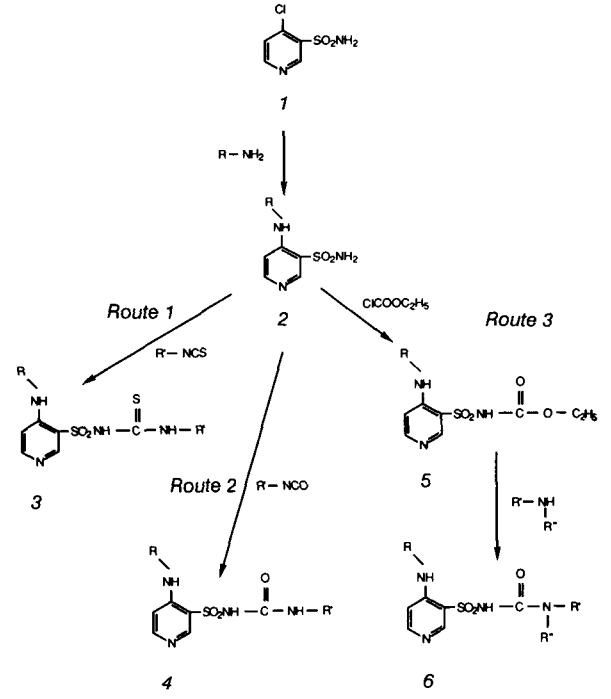


FIG. 2. Synthetic pathways of torasemide derivatives. For further explanation see text.

General procedure for the preparation of sulphonylureas. Route 3

The appropriate sulphonylcarbamate (0·01 mol) was suspended in anhydrous toluene. The appropriate cycloalkylamine (0·015 mol) was added and the mixture refluxed. At the end of the reaction, toluene was removed under reduced pressure. The residue was dissolved in 1 M NaOH (50 mL) and washed three times with ether. The aqueous solution was adjusted to pH 7·0 with acetic acid and the resulting precipitate was crystallized from ethanol/water. The yield was 64–85%.

Lipophilicity

Shake-flask method. Lipophilicity (log P′) is expressed as the logarithm of the partition coefficient in n-octanol/phosphate buffer (pH 7.4). The shake-flask method used was as previously described (Cloux et al 1988).

Reversed phase high performance liquid chromatography. A reversed phase, high performance liquid chromatography (RP- HPLC) system was also used for the determination of the log P′ of all drugs. A reversed phase column (RP-18) was equilibrated with n-propanol/phosphate buffer pH 7·4 (30:70). Compounds were dissolved and eluted with the same solution. A series of standards with a wide range of lipophilicity, as 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 $k' = \log (t_r - t_o)/t_o$ was determined for each sample, where t_r = drug retention time and $t_0 = NO_3^-$ retention time. Calibration curves were calculated using log P′ and log k′ values

of standards (n=6; r=0·997). Log P′ values of other compounds were obtained by interpolation of the standard curves.

Bioassay. For this study, the cortical thick ascending limbs of the loop of Henlé of female white New Zealand rabbits, 500–800 g, were used. The tubules were dissected and perfused according to the technique described by Burg et al (1966), with modifications as previously reported (Greger 1981; Greger & Hampel 1981).

The rate of lumen perfusion in all experiments was kept high (10–20 nL min^{-1}) to prevent changes in the ionic composition of the perfusate along the perfused tubule (Rocha & Kokko 1973). Bath flow rate was 15–20 mL min^{-1}. As control perfusate, a solution containing (mM): NaCl 145, K_2HPO_4 1·6, KH_2PO_4 0·4, $MgCl_2$ 1, Ca gluconate 1·3 and glucose 5, was used on both sides of the epithelium. pH was adjusted to 7·4.

The transepithelial potential (V_{te}) was recorded continuously on both ends of the tubule (Burg et al 1966). The specific transepithelial resistance (R_{te}) was calculated from the input resistance obtained by the injection of short-current pulses (25–30 nA, 800 ms) into the tubule lumen (Burg et al 1966). The ratio of both values (V_{te}/R_{te}) gives the equivalent short circuit current I_{sc}.

There is a direct relationship between the decrease of I_{sc} and the inhibition of the Na^+ $2Cl^-K^+$ co-transporter (Greger & Schlatter 1983). For each drug under study, dose-response curves were established by adding compounds at various concentrations to the luminal perfusate with at least three determinations for each concentration. These response curves (Fig. 3) were used to calculate the concentration necessary to block 50% of the short circuit current (IC50).

Results and Discussion

In-vitro studies

Synthesized compounds, torasemide (7) and frusemide (25) were screened as Na^+ $2Cl^-$ K^+ co-transport blockers on rabbit cortical thick ascending limb, where they cause a concentration-dependent inhibition of equivalent I_{sc} when added to the lumen perfusate of in-vitro perfused tubule segments (Witnner et al 1987).

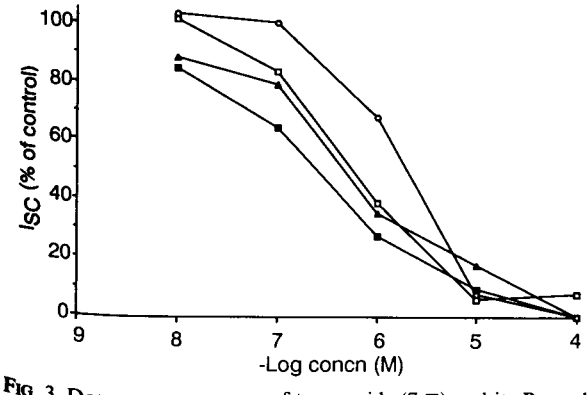


FIG. 3. Dose-response curves of torasemide (7 ■) and its R_1-cyclo-octylamino derivatives (11 ▲, 4 ○ and 17 □) for their inhibitory potency of the equivalent short circuit current (I_{sc}).

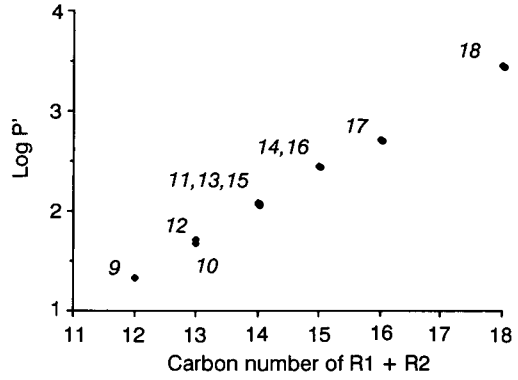


FIG. 4. Correlation of log P′ with the C-atom number of R_1+R_2 for cycloalkyl disubstituted sulphonylureas. r=0·99; significance level $P<0·001$.

The trend observed throughout this series (Table 1) is that an increase of the number of methylenes of the R_1 cycloalkyl group increases the inhibitory potency (9–11, 12–14, 15–17, 21–23) whatever the size of R_2. Optimum activity was observed with an 8-membered ring in the R_1 position. Indeed, a cyclododecyl moiety (18) in this position results in an inactive compound, probably as a consequence of steric hindrance. No optimum size could be shown for the R_2 aminocycloalkyl (Table 1). The incorporation of the distal nitrogen of the sulphonylurea moiety into a saturated ring leads to very active molecules (21–24). A piperidine ring in the R_2 position (22) increases inhibition potency compared with perhydroazepine (24).

It is also apparent that the newly synthesized sulphonyl-thioureas (19, 20) are as potent as their sulphonylurea counterparts (8, 10).

Comparison of log P′ with the C-number of R_1+R_2 for each cycloalkyl disubstituted compound (Fig. 4) indicates that an increase of one methylene results in an enhanced lipophilicity of some 0·35 (Δ log P′ $-CH_2-$). Moreover, there is a correlation between log P′ and inhibition potency (Fig. 5).

Structure-activity relationships

The structure-activity relationships of the torasemide derivatives, including those synthesized previously (Delarge et al

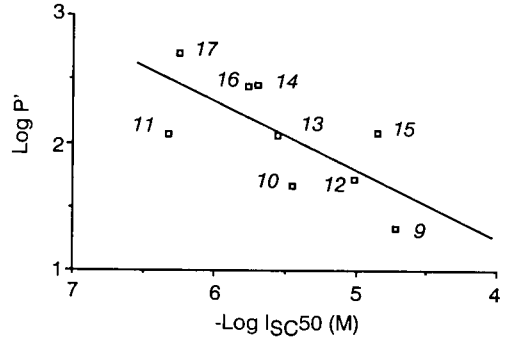


FIG. 5. Correlation between the inhibitory effect of cycloalkyl disubstituted sulphonylureas and their lipophilicity. r=0·71; $P=0.038$.

1980, 1981; Witnner et al 1987) can be summarized as follows. For R_1, the best moiety is a cyclo-octyl or a m-substituted phenyl ring where the substituent of choice is $-CH_3$, $-CF_3$ or $-Cl$. Alkyl groups (branched or not), cycloalkyl rings with a C-number <6 or heterocycles (2-furfuryl-, *N*-piperidyl-, *N*-methyl *N'*-piperazinyl-) strongly reduce the diuretic activity.

The replacement of the secondary amine linked to R_1 by an ether, thioether, sulphone bond or by tertiary amines results in inactive compounds.

The optimum R_2 residue is isopropylamino, short alkylamino (C_1 to C_4) or cycloalkylamino (C_6 to C_8). Heterocyclic-amino residues and aromatic amines (with or without substitution) destroy the biological activity. The presence of a piperidine ring in the R_2 position leads to molecules (22,23) more active than torasemide.

All modifications of the sulphonylurea side chain (i.e. sulphonylguanidine) cause a loss in diuretic activity, except for the above sulphonylthioureas.

The replacement of the acidic proton (—SO_2—NH—CO—) with a methyl group inactivates the drug; previous data suggest that all loop diuretics act in their anionic form (Schlatter et al 1983) at a chloride binding site (Forbush & Palfrey 1983; Haas & McManus 1983). To examine this interpretation, BM 9 (17, $pK_a = 7{\cdot}7$) and frusemide were screened at two different pH values (pH 7.0 and 8·2) in in-vitro perfused rabbit thick ascending limb segments. 17 showed an increase in inhibitory potency from 1·2 μM at pH 7.0 to 0·25 μM at pH 8·2 as a consequence of its deprotonation. Under the same conditions, frusemide whose carboxylic acid function ($pK_a = 3{\cdot}8$), but not sulphonamide ($pK_a = 7{\cdot}5$, Orita et al 1976), was fully ionized at pH 7.0 and did not show any change in its biological activity at pH 8·2 in spite of an increase of the anionic level of the sulphonamide moeity. These experiments indicate that deprotonation of the sulphonylurea enhances biological activity and that ionization of the frusemide sulphonamide moeity is not required for activity. By using crystallographic data of frusemide (Lamotte et al 1978) and torasemide (Dupont et al 1978), Dupont & Dive (personal communication) suggested that the best molecular fits of these drugs could be the superimposition of the aromatic rings (benzene and pyridine) and the fitting of —SO_2NH_2 and C—COOH of frusemide onto SO_2NH— and —N= (pyridine) of torasemide, respectively. If this view was right, the transformation of the frusemide sulphonamide moiety into an isopropyl-sulphonylurea side-chain, as in torasemide, would probably lead to an active compound which is not the case. Therefore, we can suggest that frusemide, torasemide and its derivatives bind to their receptor by an ionic bond. The structure-activity study suggests three main sites of interaction between the Na^+ $2Cl^-$ K^+ co-transport system and torasemide derivatives (Fig. 6): R_1 residue with a hydrophobic receptor site; R_2 residue with a second hydrophobic receptor site and an ionic bond (—SO_2—CO—NH—). The nitrogen lone pair of pyridine could also H-bond to the receptor.

In conclusion, a series of Na^+ $2Cl^-$ K^+ co-transport blockers, based on torasemide has been prepared, leading to lipophilic molecules. A correlation between the log P' values (at pH 7·4) of these new compounds and their inhibitory potency of the Na^+ $2Cl^-$ K^+ co-transport system has been shown. Structure activity studies lead to a model for the interaction of the inhibitors related to torasemide with the Na^+ $2Cl^-$ K^+ co-transport receptor site.

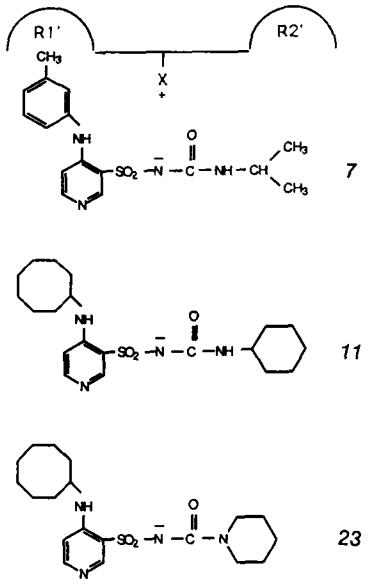


FIG. 6. Interaction model between torasemide and the Na^+ $2Cl^-$ K^+ co-transport system. For further explanation see text.

Acknowledgement

This work was supported by grants from the Fonds National de la Recherche Scientifique (FNRS). B. Pirotte is a senior research assistant of the FNRS.

References

Burg, M. B., Grantham, J., Abramow, M., Orloff, J. (1966) Preparation and study of fragments of single rabbit nephrons. Am. J. Physiol. 210: 1293–1298

Cloux, J. L., Crommen, J., Delarge, J., Pirard, M. L., Thunus, L. (1988) Tentative de détermination de la constante fragmentaire (f) du groupement sulfonylurée. J. Pharm. Belg. 43: 141–151

Delarge, J. (1973) Nouveaux anti-inflammatoires dérivés de la pyridine. Ann. Pharm. Franç. 31: 467–474

Delarge, J. (1988) Chemistry and pharmacological properties of the pyridine-3-sulfonylurea derivative torasemide. Arzneim. Forsch. 38: 144–150

Delarge, J., Lapière, C. L., de Ridder, R., Ghys, A. (1980) Synthèse et propriétés diurétiques de quelques sulfonylurées apparentées au torasémide. Eur. J. Med. Chem. 15: 299–304

Delarge, J., Lapière, C. L., de Ridder, R., Ghys, A. (1981) Nouveaux dérivés du torasémide à propriétés diurétiques. Ibid. 16: 65–68

Dupont, L., Lamotte, J., Campsteyn, H., Vermeire, M. (1978) Structure cristalline et moléculaire d'un diurétique dérivé de l'alkyl-1[(phénylamino-4 pyridyl-3) sulfonyl]-3 urée la torasémide ($C_{15}H_{20}N_4SO_3$). Acta Cryst. B34: 1304–1310

Forbush, B., Palfrey, H. C. (1983) [^{3}H]Bumetanide binding to membranes isolated from dog kidney outer medulla. J. Biol. Chem. 258: 11787–11792

Greger, R. (1981) Cation selectivity of the isolated perfused cortical thick ascending limb of Henlé's loop of rabbit kidney. Pflüger's Arch. 390: 30–37

Greger, R., Hampel, W. (1981) A modified system for in vitro perfusion of isolated renal tubules. Ibid. 389: 175–176

Greger, R., Schlatter, E. (1983) Cellular mechanism of the action of loop diuretics on the thick ascending limb of Henle's loop. Klin. Wochenschr. 61: 1019–1027

Haas, M., McManus, T. J. (1983) Bumetanide inhibits (Na^+ K^+2Cl) cotransport at a chloride site. Am. J. Physiol. 245: C235–240

Karnes, H. T., Farthin, G. D., Besenfelder, E. (1989) Solid phase extraction with automated elution and HPLC of torasemide and metabolites from plasma. J. Liquid. Chromatogr. 12: 1809–1818

Lamotte, J., Campsteyn, H., Dupont, L., Vermeire, M. (1978) Structure cristalline et moléculaire de l'acide furfurylamino-2 chloro-4 sulfamoyl-5 benzoïque, la furosémide ($C_{12}H_{11}ClN_2O_5S$). Acta Cryst. B34: 1657–1661

Orita, Y., Ando, A., Urakabe, S., Abe, H. (1976) A metal complexing property of furosemide and bumetanide. Arzneim.-Forsch. 26: 11–13

Rocha, A. S., Kokko, J. P. (1973) Sodium chloride and water transport in the medullary thick ascending limb of Henle. J. Clin. Invest. 52: 612–623

Schlatter, E., Greger, R., Weidtke, C. (1983) Effect of 'high ceiling' diuretics on active salt transport in the cortical thick ascending limb of Henle's loop of rabbit kidney. Pflügers Arch. 396: 210–217

Williams, D. A. (1974) Drug metabolism: the pathways of drug metabolism. In: Foye, W. O. (ed.) Principles of Medicinal Chemistry. Lea & Febiger, Philadelphia, pp 104–131

Witnner, M., Di Stefano, A., Wangemann, P., Delarge, J., Liégeois, J. F., Greger, R. (1987) Analogues of torasemide: structure function relationships—experiments in the thick ascending limb of the loop of Henle of rabbit nephron. Pflügers Arch. 408: 54–62