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# SYNTHESIS OF 2-SUBSTITUTED-1,3,4-THIADIAZOLE-5-SULPHONAMIDES AS NOVEL WATER-SOLUBLE INHIBITORS OF CARBONIC ANHYDRASE

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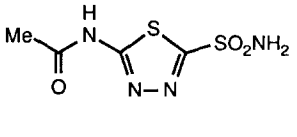
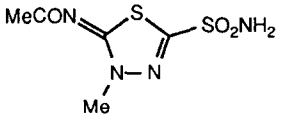
**Abstract**: The synthesis of novel 2-substituted-1,3,4-thiadiazole-5-sulphonamides which have high water-solubility and nanomolar activity as inhibitors of carbonic anhydrase *in vitro* are reported.

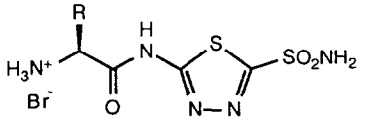
A wide variety of aromatic and heterocyclic sulphonamides are known to be good inhibitors for carbonic anhydrase, carbonate hydro-lyase (EC.4.2.1.1). Certain such inhibitors (CAIs), notably acetazolamide (**1**) and methazolamide (**2**), have been used for more than 37 years to decrease intra-ocular pressure (IOP) in patients with glaucoma.[1] However, their therapeutic value has been limited by serious side effects when administered systemically.[2-5] Consequently, considerable activity is being directed currently towards the development of a water-soluble CAI which can be formulated in aqueous solution for topical ocular application. This presents a particular case of the general problem of transforming an enzyme inhibitor, specified for binding at a hydrophobic site in an enzyme, into a water-soluble form without loss of activity.




**1** Acetazolamide




**2** Methazolamide

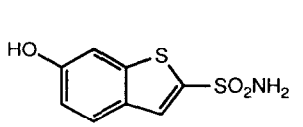



**3**

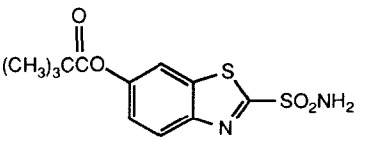
Recently, a number of CAIs[6,7] has been reported to lower IOP when applied directly into animal eyes. Disappointingly, many of these new topical agents, for example L-650,719,[8] 2-sulfamoylbenzothiazol-6-yl 2,2-dimethylpropionate,[9] and other ethoxzolamide derivatives[10] have shown only a modest effect in clinical trials although MK-927 seems to possess clinically relevant IOP-lowering activity.[11]

As part of an ongoing programme in the search for a water-soluble CAI,[12] we report the structural manipulation of acetazolamide (**1**) in order to improve those physical properties necessary for corneal penetration, whilst still retaining carbonic anhydrase inhibitory activity. We describe here the results of the introduction of an

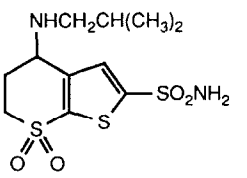
aminoacyl function derived from a natural amino-acid in place of the acetyl group in (**1**) as shown in formula (**3**).



L-650,719



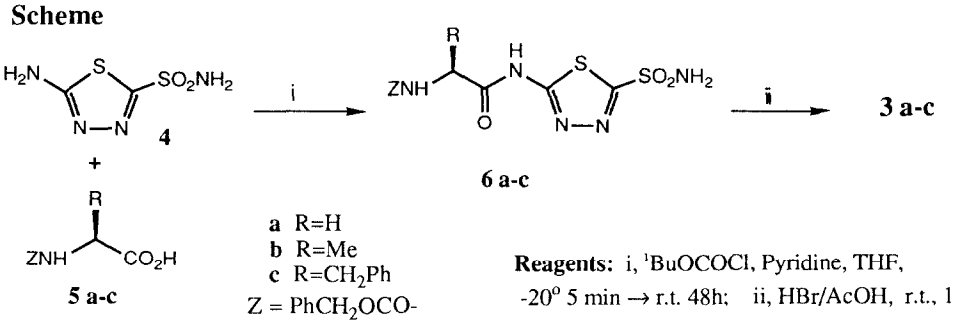
2-Sulfamoylbenzothiazol-6-yl 2,2-dimethylpropanoate



MK-927

The starting material, 2-amino-1,3,4-thiadiazole-5-sulphonamide[13] (**4**), was acylated at the 2-amino group by condensation with an appropriate *N*-protected amino acid using a suitable activating agent. Thus, reaction of (**4**) with *N*-carbobenzyloxy derivatives of glycine (**5a**), L-alanine (**5b**), or L-phenylalanine (**5c**) in the presence of isobutyl chloroformate and pyridine in tetrahydrofuran gave the 1,3,4-thiadiazoles, (**6a-c**) respectively, in good yields (Scheme).

**Scheme**



**a** R=H
**b** R=Me
**c** R=$CH_2Ph$
Z = $PhCH_2OCO$-

**Reagents:** i, $^t$BuOCOCl, Pyridine, THF, -20° 5 min → r.t. 48h; ii, HBr/AcOH, r.t., 1h

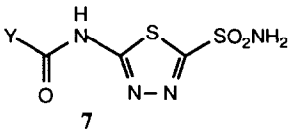
Attempts to remove the *N*-carbobenzyloxy group (Z in Scheme) by catalytic hydrogenolysis (10% Pd/C) proved ineffective. Deprotection was, however, efficiently achieved by the treatment of (**6a-c**) with hydrogen bromide in glacial acetic acid (32% w/w) to give the hydrobromide salts, (**3a-c**) respectively, in excellent yield.

All six compounds (**6a-c**) and (**3a-c**) were fully characterised and then tested for their *in vitro* inhibition of carbonic anhydrase (EC.4.2.1.1) using the method of Maren.[14] The inhibition data are listed in the Table. All of the compounds except (**3c**) proved to be more effective inhibitors of carbonic anhydrase than acetazolamide (**1**) on a molar basis.

The amine hydrobromides (**3a**) and (**3b**) described here exhibit high water solubility (0.896 and 0.701 M, respectively) with the phenylalanine product (**3c**) being typically some 5-6 times less soluble in water (as well as in organic solvents). In addition, all three compounds show excellent CAI activity, as do their carbobenzyloxy-protected precursors (**6a-c**) (Table). It follows that the *N*-carbobenzyloxy derivatives (**6a-c**) are also promising compounds for systemic administration in glaucoma therapy despite their low water solubility, as for instance through formulation in suitable gels.

Since the inception of our programme, another research group has independentantly sought water-solubility

for 1,3,4-thiadiazole-5-sulphonamides primarily through the use of ω-aminoacyl and ω-carboxyacyl substituents at the 2-position. The most potent inhibitors described in that work[15] are the compounds (**7a**) and (**7b**) [we have observed (**7b**) to be formed regularly as a by-product from the synthesis of compounds (**3**) as it results from the direct side-reaction between isobutyl chloroformate and the amine (**4**)]. We note that the most successful acetazolamide-related compounds described in that work (namely **7a-b**) show $IC_{50}$ CAI activities of 370 nM and 570 nM with 0.7 mM and 0.103 M solubility in water respectively, which makes them some 40-50 times less potent than compounds (**3a-b**) and very much less water-soluble (Table).




**7**

**a** Y = $NH_2(CH_2)_4$

**b** Y = $(CH_3)_2CHCH_2O$

**Table** Physical characteristics and CAI and water-solubility data for compounds **3**, **6**, and **7**.

| Compound | Mp / °C | Mass Spectrum [a] Parent ion (*%Base peak*) | $IC_{50}$ for Carbonic anhydrase[b] / nM | Limiting Water Solubility / M at 20°C |
|---|---|---|---|---|
| **1** | 248-249d | 222 (*14*) $(M)^{\cdot+}$ | 16 | < 0.0001 |
| **6 a** | 199-201 | 372 (*700*) $(M + 1)^+$ | 8.0 | < 0.0001 |
| **6 b** | 203-205 | 385 (*18*) $(M)^{\cdot+}$ | 7.0 | < 0.0001 |
| **6 c** | 192-194 | 353 (2) $(M - C_6H_5CH_2O)^+$ | 5.0 | < 0.0001 |
| **3 a** | 207-209 | 238 (*100*) $(M - Br)^+$ | 10 | 0.896 |
| **3 b** | 193-197 | 252 (*100*) $(M - Br)^+$ | 9.0 | 0.701 |
| **3 c** | 188-192 | 328 (*100*) $(M - Br)^+$ | 30 | 0.132 |
| **7 a**[15] | n.a. | n.a. | 440 | 0.0007 |
| **7 b**[15] | n.a. | n.a. | 550 | 0.103 |

[a] Kratos MS80 using electron impact ionisation.

[b] $IC_{50}$: *In vitro* determinations of the concentration of inhibitor required to reduce enzyme activity by 50% (at 0-4°C)

While we have not yet engaged in detailed molecular modelling of the binding of these sulphonamides to CA, an inspection of the position of acetazolamide bound at the active site, as defined in the X-ray structure of human erythrocyte carbonic anhydrase C with acetazolamide[16] and other sulphonamides,[11] indicates that the cationic amino group of the compounds (**3**) may be capable of forming a salt bridge to an aspartate residue. This is located near to the edge of the conical binding pocket, of which the apex is the catalytic zinc ion that co-ordinates to the sulphonamide nitrogen as its fourth ligand.[17] If that proves to be the case, it could provide the binding energy needed to compensate for desolvation of the cationic amino-function of the 2-aminoacylamino substituent and facilitate the observed tight binding for compounds (**3**).

Preliminary results from *in vivo* studies using compounds (**3a-c**) to lower intraocular pressure are very

encouraging and will be published elsewhere in due course.

**References and Notes**


1 Becker, B. *Am. J. Ophthalmol.* **1954**, *31*, 13.

2 Foss, R. H. *Am. J. Ophthalmol.* **1955**, *36*, 336.

3 Green, H.; Leopold, I. H. *Am. J. Ophthalmol.* **1955**, *40(suppl)*, 137

4 Gloster, J.; Perkins, E. S. *Br. J. Opthhalmol.* **1955**, *39*, 647.

5 Stein, A. J.; Pinke, R.; Krupin, T.; Glabb, E.P.; Podor, S.M.; Serle, J.; Maren, T.H. *Am J. Ophthalmol.* **1983**, *95*, 222.

6 Maren, T.H.; Jankowska, L.; Sanyal, G.; Edelhauser, H.F. *Exp. Eye Res.* **1983**, *36*, 457.

7 Putnam, M.L.; Schoenwald, R.D.; Duffel, M.W.; Barfknecht, C.F.; Segarra, T.M.; Campbell, D.A. *Invest. Ophthalmol. Vis. Sci.* **1987**, *28*, 1373.

8 Bar-Ilan, A.; Pessah, N.I.; Maren, T.H. *J. Ocular Pharmacology* **1989**, *5(2)*, 99.

9 Sugrue, M.F.; Gautheron, P.D.; Schmitt, C.J.; Viader, M-P.; Conquet, P.; Smith, R.L.; Share, N.N.; Stone, C.A. *J.Pharmacol. Exp. Ther.* **1985**, *232*, 534.

10 Lewis, R.A.; Schoenwald, R.D.; Eller, M.G.; Barfknecht, C.F.; Phelps, C.D. *Arch. Ophthalmol.* **1984**, *102*, 1821.

11 Baldwin, J.J.; Ponticello, G.S.; Anderson, P.S.; Christy, M.E.; Murcko, M.A.; Randall, W.C.; Schwam, H.; Sugrue, M.F.; Springer, J.P.; Gautheron, P.; Grove, J.; Mallorga, P.; Viader, M-P.; McKeever, B.M.; Navia, M.A. *J. Med. Chem.* **1989**, *32*, 2510.

12 Trager, S.F.; Blackburn, G.M. U.S. Pat. 4,975,446, Dec. 4th 1990; *Chem. Abs.* **1991**, *114*, 164242d.

13 Robbin, R. O.; Clapp, J. W. *J. Am. Chem. Soc.* **1950**, *72*, 4890.

14 Maren, T. H. *J. Pharmacol. Exp. Ther.* **1960**, *130*, 26. The bovine erythrocyte carbonic anhydrase used (carbonate hydro-lyase, EC.4.2.1.1) was obtained from Sigma Chemical Co., Lot No 93F 9320.

15 Antonaroli, S.; Bianco, A.; Brufani, M.; Lo Baido, G.; Rende, G.; Poiter, E. Eur.Pat.Appl. EP 354,881, 14th Feb.1990; *Chem.Abs.*, **1990**, *113*, 152426h.

16. Kannan, K.K.; Vaara, I.; Notstrand, B.; Lovgren, S.; Borell, A.; Fridborg, K.; Petef, M. *Drug Action Mol.Level [Rep.Symp.]* Ed. Roberts, G.C.K., Univ.Park Press, Baltimore,Md., **1976**,73-91; *Chem.Abs.*, **1978**, *88*, 18229h; see also reference 11.

17. Blackburn, G.M.; Mann, B.E.; Taylor, B.F.; and Worrall, A.F. *Eur.J Biochem.* **1985**, *153*, 553; and references therein.