ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 54 (1999) 838–841

IL FARMACO

Short Communication

# Anticonvulsant activity of analogues of acetazolamide

E.E. Chufán [a], J.C. Pedregosa [a], O.N. Baldini [b], L. Bruno-Blanch [b,*]

[a] *Química Inorgánica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina*

[b] *Química Medicinal, División Farmacia, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Casilla de Correo, 243-1900 La Plata, Argentina*

Received 30 March 1999; accepted 23 September 1999

**Abstract**

The anticonvulsant activity of 5-tertbutyloxycarbonylamido-1,3,4-thiadiazole-2-sulfonamide (B–$H_2$ats) and 5-amino-1,3,4-thiadiazole-2-sulfonamide (Hats) was compared in mice, to that of acetazolamide ($H_2$acm). These compounds exhibit potent anticonvulsant activity and low minimal motor impairment. © 1999 Elsevier Science S.A. All rights reserved.

*Keywords:* Anticonvulsant activity; Maximal electroshock test; Acetazolamide; Sulfonamides; Carbonic anhydrase inhibition

## 1. Introduction

Seizures are the prime manifestation of epilepsy, and the main aim of antiepileptic drugs (AEDs) is to suppress them without inducing undesirable side effects. Epilepsy has been found to have point prevalence rates in the range of 4–10/1000 in the general population [1]. The majority of these cases (60–70%) occur without a well-defined etiology. Despite this, anticonvulsant drugs are estimated to be useful in treating 90% of the epileptic patients. However, all the anticonvulsant currently approved, and already in use, have dose-related toxicity and idiosyncratic side effects [2], which are mainly manifested during metabolization by liver enzymes [3].

Due to the fact that non-pharmacologic therapies for the treatment of epilepsy do not become evident in the near future, the search for new antiepileptic drugs with lower toxicity and fewer side effects still continues [4].

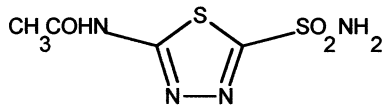
As a part of this research, several sulfonamides have been synthesized and tested for a broad spectrum of biological activity during the last year. Sulfonamides represent a minor class of antiepileptic agents [5,6]. Some of them, such as acetazolamide (N-[5-sulfamoyl-1,3,4-thiadiazole-2-yl]acetamide), methazolamide, ethoxazolamide, sulthiame and disamides, which are known to possess potent diuretic action, have been used as anticonvulsants for some time. Presently, topiramate and zonizamide (1,2-benzisoxazole-3-methanesulfonamide) are used; the last in conjunction with carbamazepine for the treatment of partial seizures. Zonisamide is a potent anticonvulsant, capable of suppressing maximal electroshock induced seizure (MES), but ineffective against the seizure induced by subcutaneous pentylenetetrazol (scMet). Zonisamide has been already approved as an antiepileptic drug in Japan [5]. The anticonvulsant mechanism associated with its activity does not seem to depend on carbonic anhydrase inhibition but is more likely to be similar to that of phenytoin, involving the interaction with $Na^+$ channels [7,8].

Among the unsubstituted sulfonamides that inhibit the enzyme carbonic anhydrase and have shown to have anticonvulsant properties in experimental animals and humans, the most extensively studied is acetazolamide ($H_2$acm, Fig. 1). It is presently used in the treatment of some kinds of epilepsies.

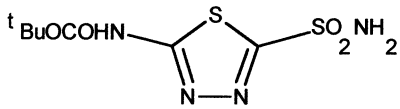
In the search for new antiepileptic agents, we have tested in mice the anticonvulsant activity of two sulfonamides derived from ($H_2$acm, see Fig. 1) and synthesized in our laboratory. We have also measured the

* Corresponding author. Fax: +54-221-422 3409.
*E-mail address:* lbb@nahuel.biol.unlp.edu.ar (L. Bruno-Blanch)

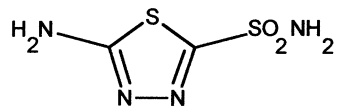
0014-827X/99/$ - see front matter © 1999 Elsevier Science S.A. All rights reserved.
PII: S0014-827X(99)00096-8




Acetazolamide




5-terbutyloxycarbonylamido-1,3,4-thiadiazole-2-sulfonamide
(B-$H_2$ats)




5-amino-1,3,4-thiadiazole-2-sulfonamide
(Hats)

Fig. 1. Structures of the compounds.

inhibition properties against carbonic anhydrase correlating these results with those derived from the pharmacology study.

## 2. Experimental

### 2.1. Chemistry

Acetazolamide was supplied by John Wyeth Laboratories; B–$H_2$ats was synthesized by the method described by Pedregosa et al. [9] and Hats was synthesized by acidic hydrolysis of acetazolamide [10].

### 2.2. Pharmacology

#### 2.2.1. Enzymatic inhibition studies

Acetazolamide, B–$H_2$ats and Hats were assayed by Maren's micromethod [11] at 0°C, under the conditions of the E–I (enzyme–inhibitor) technique, in the concentration range of $10^{-4}$–$10^{-9}$ M. Inhibitors and enzyme (a concentration of $5 \times 10^{-9}$ M of pure carbonic anhydrase isoenzyme II was used) were preincubated for 10 min prior to assay, in order to allow the formation of the enzyme–inhibitor adduct.

In a special $CO_2$ bubbler cell, 0.3 ml distilled water was added, followed by 0.4 ml of phenol red indicator solution and 0.1 ml enzyme solution and 0.1 ml inhibitor solution (the last two solutions preincubated as mentioned before). The hydration reaction was initiated by the addition of 0.1 ml of buffer solution (pH 7.3), and the time taken for the color to change, was recorded with a stopwatch. Enzyme specific activity (with and without inhibitors) was calculated using Maren's formula [12]. For the enzymatic inhibition studies of the sulfonamides, solutions ($10^{-2}$ M) in dimethylsulfoxide were prepared and dilutions up to $10^{-9}$ M were subsequently performed with distilled–deionized water. In this concentration range, DMSO is not a carbonic anhydrase inhibitor. The $IC_{50}$ values (molarity of inhibitor producing a 50% decrease of enzyme specific activity) were obtained from semilogarithmic plots of specific activity versus inhibitor concentration (Table 1).

#### 2.2.2. Anticonvulsant activity

The pharmacological evaluation of the compounds tested were performed according to the procedures

Table 1
Inhibition with acetazolamide, B–$H_2$ats and Hats, against carbonic anhydrase isoenzyme II

| Comp. | $IC_{50}$ [a] $\times 10^{-9}$ (M) |
|---|---|
| Acetazolamide | 12 |
| B–$H_2$ats | 3.3 |
| Hats | 60 |

[a] Molarity of inhibitor producing a 50% decrease of enzyme specific activity.

Table 2
Anticonvulsant activity and rotorod toxicity of acetazolamine; B–$H_2$ats and Hats at different times and doses

| Comp. (dose μmol/k) | Test | Time (h) | | | |
|---|---|---|---|---|---|
| | | 0.5 | 1 | 2 | 4 |
| Acetazolamide (315) | MES [a] | 6/6 | 3/5 | 2/5 | 2/4 |
| | TOX [b] | 1/6 | 0/5 | 0/5 | 0/4 |
| B–$H_2$ats (315) | MES | 3/4 | 4/4 | 4/4 | 3/5 |
| | TOX | 0/4 | 0/4 | 0/4 | 2/5 |
| B–$H_2$ats (180) | MES [a] | 2/4 | 4/5 | 3/4 | 4/5 |
| | TOX [b] | 1/4 | 0/5 | 1/4 | 0/5 |
| Hats (315) | MES [a] | 4/4 | 2/4 | 2/4 | 2/4 |
| | TOX [b] | 0/4 | 0/4 | 0/4 | 0/4 |

[a] Maximal electroshock test — MES (number of animals protected/number of animals tested).

[b] Neurotoxicity as measured by the rotorod test — TOX (number of animals exhibiting toxicity/number of animals tested in the rotorod test).

Table 3
Anticonvulsant activity and neurotoxicity, in mice, of B–$H_2$ats and Hats in comparison with acetazolamide

| Dose (μmol/kg) | | 20 | 40 | 60 | 100 | 140 | 180 | 260 | 280 | 315 | $DE_{50}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Acetazolamide | MES [a] | | | | 0/8 | 1/6 | 4/9 | 4/8 | 3/5 | 6/6 | 239 (174–327) |
| | TOX [b] | | | | 0/8 | 0/6 | 1/9 | 1/8 | 1/5 | 1/6 | |
| B–$H_2$ats | MES [a] | | 3/10 | 3/8 | 5/8 | | 4/5 | | | 4/4 | 74 (44–125) |
| | TOX [b] | | 0/10 | 0/8 | 0/8 | | 0/5 | | | 0/4 | |
| Hats | MES [a] | 1/9 | 3/8 | | 4/9 | 4/9 | | 9/10 | | 4/4 | 90 (50–159) |
| | TOX [b] | 0/9 | 0/8 | | 0/9 | 0/9 | | 0/10 | | 0/4 | |

[a] Maximal electroshock test (MES test) (number of animals protected/number of animals tested).
[b] Rotorod toxicity (number the animals exhibiting toxicity/number of animals tested in the rotorod test).

described by the Antiepileptic Drug Development (ADD) (Program of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS)) [13,14]. Tests were conducted with BALB/cN mice. Maximal electroshock induced seizures (MES) and subcutaneous pentylentetrazole seizure (scMet), tests were employed to determine the anticonvulsant activity. Minimal motor impairment test (rotorod test) was used to determine the neurological toxicity. The antiepileptic activity (AE) was expressed as $DE_{50}$ (the dose that is effective in 50% of the animals tested), and estimated, with their 95% confidence limits, by computer linear regression analysis (in a log dose–probit calculation, see Table 2).

## 3. Results and discussion

From the comparison of the $IC_{50}$ from the enzymatic inhibition study (Table 1), it becomes noticeable that B–$H_2$ats is three times more active than acetazolamide; on the other hand, Hats is less effective. It has been found that the inhibitory action on carbonic anhydrase is particularly increased in derivatives bearing lipophilic moieties [15]. This observation may explain the higher inhibitor potency of B–$H_2$ats, compared to acetazolamide and also the lower inhibition capability of Hats.

The anticonvulsant activity and rotorod toxicity of the sulfonamides investigated in this work are presented in Table 2. Data show that acetazolamide and Hats were maximally effective against the MES seizure at 0.5 h. On the other hand, B–$H_2$ats is maximally effective between 1 and 2 h. Hats, as acetazolamide, can be considered rapid in onset. The three sulfonamides show a long duration of activity. These sulfonamides, on the other hand, are not effective against PTZ-induced seizures.

B–$H_2$ats showed more potent activity in the MES test than acetazolamide (see Table 3) and these data can be correlated with those of carbonic anhydrase inhibition.

Hats exhibited potent activity in the MES test. As it has been found for topiramate [16], no apparent correlation exists between $IC_{50}$ and MES activity. Other mechanisms of action appear to be responsible for the anticonvulsant activity of these compounds.

## Acknowledgements

The authors are indebted to Dr Claudiu Supuran for the obtainment of the enzymatic inhibition data. This work was supported in part through grants from Universidad Nacional de La Plata, Laboratorios Bagó, and Colegio de Farmacéuticos de la Provincia de Buenos Aires, Argentina, CONICET(PID-CONICET 4929/96), Secretaría de Ciencia y Técnica UNSL.

## References

[1] (a) C.W. Bazil, T.A. Pedley, Advances in the medical treatment of epilepsy, Annu. Rev. Med. 49 (1998) 135–162. (b) D.M. Woodbury, J.W. Kemp, Other antiepileptic drugs: sulfonamides and derivatives: acetazolamide, in: R. Levy, R. Mattson, B. Meldrun, J.K. Penry, F.E. Dreifuss (Eds.), Antiepileptic Drugs, third ed., Raven Press, New York, 1989 (Chapter 61). (c) S.D. Shorvon, Epidemiology, classification, natural history, and genetics of epilepsy, Lancet 336 (1990) 93–96.
[2] M.J. Brodi, Established anticonvulsants and treatment of refractory epilepsy, Lancet 336 (1990) 350–354.
[3] D. Lindhout, J.G.C. Omtzigt, Teratogenic effects of antiepileptic drugs: implications for management of epilepsy in women of child bearing age, Epilepsia 35 (1994) S19–S28.
[4] P. Jallon, The problem of intractability: the continuing need for new medical therapies in epilepsy, Epilepsia 38 (1997) S37–S42.
[5] I.E. Leppik, Antiepileptic drugs in development: prospects for the near future, Epilepsia 35 (1994) S29–S40.
[6] M. Bialer, S.I. Johannessen, H.J. Kupferberg, R.H. Levy, P. Loiseau, E. Perucca, Progress report on new antiepileptic drugs: a summary of the third Ellat conference, Epilepsica Res. 52 (1997) 589–593.
[7] I.O. Edafigho, K.R. Scott, Anticonvulsant, in: Manfred E. Wolff (Ed.), Burger's Medicinal Chemistry and Drug Discovery, vol. 3, fifth ed., Wiley, New York, 1996, pp. 175–260 (Chapter 39).
[8] D.S. Ragsdale, M. Avoli, Sodium channels as molecular targets for antiepileptic drugs, Brain Res. Rev. 26 (1998) 16–28.
[9] J.C. Pedregosa, J. Casanova, G. Alzuet, J. Borrás, S. García-Granda, M.R. Díaz, A. Gutierrez-Rodriguez, Metal complexes of 5-tertbutyloxycarbonylamido-1,3,4-thiadiazole-2-sulfonamide (B-$H_2$ats), a carbonic anhydrase inhibitor. Synthesis and characterization of the copper(II) complex. Crystal structures of B-$H_2$ats and the $[Cu(B\text{-ats})(NH_3)_2]_2$ dimer complex, Inorg. Chim. Acta 232 (1995) 117–224.

[10] C. Supuran, A.T. Balaban, M.D. Gheorghiu, A. Schiketanz, A. Dinculescu, I. Puscas, Carbonic anhydrase inhibitors. Part 2. Membrane-impermeable derivatives of 1,3,4-thiadiazole-2-sulfonamide, Rev. Roum. Chim. 35 (1990) 399–403.
[11] T.H. Maren, Carbonic anhydrase: chemistry, physiology and inhibition Physiol. Rev. 47 (1967) 595–604.
[12] T.H. Maren, A simplified method for the determination of carbonic anhydrase and its inhibitors, J. Pharmacol. Exp. Ther. 130 (1960) 25–32.
[13] G.D. Gladding, H.J. Kupferberg, E.A. Swinyard, Antiepileptic drugs, in: H. Frey, D. Janz (Eds.), Handbook of Experimental Pharmacology 74, vol. 342, Springer, Berlin, 1985.
[14] H.S. White, J.H. Woodhead, M.R. Franklin, E.A. Swinyard, H.H. Wolf, Experimental selection, quantification and evaluation of anticonvulsants, in: R.H. Levy, R.H. Mattson, B.S. Meldrum (Eds.), Antiepileptic Drugs, fourth ed., Raven Press, New York, 1995, pp. 99–109.
[15] C.T. Supuran, in: I. Puscas (Ed.), Carbonic Anhydrase and Modulation of Physiologic and 1222 Pathologic Processes in the Organism, Helicon, Timisoara, Romania, 1994, pp. 29–113.
[16] I.O. Edafiogho, K.R. Scott, Anticonvulsants, in: M.E. Wolff (Ed.), Burger's Medicinal Chemistry and Drug Discovery, vol. 3, 1996, pp. 175–260 (Chapter 39).