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β-LACTAMS: A NEW CLASS OF CONFORMATIONALLY-RIGID INHIBITORS OF γ-AMINOBUTYRIC ACID AMINOTRANSFERASE

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A structural similarity of several monobactams (**2–4**), 3-aminonocardicinic acid (**6**), 6-aminopenicillanic acid (**7**), 7-aminocephalosporanic acid (**8**), and 7-aminodesacetoxycephalosporanic acids (**9**, **10**) to γ-aminobutyric acid (GABA) and to known inhibitors and substrates of GABA aminotransferase is described. Because of this, the above-mentioned compounds were tested as competitive inhibitors and as inactivators of pig brain GABA aminotransferase. All of the compounds were competitive inhibitors of GABA aminotransferase. On the basis of the inhibitory potency of these conformationally-rigid GABA analogues it is hypothesized that GABA is bound at the active site with its amino and carboxylate groups in a *syn* orientation. None of the compounds inactivates GABA aminotransferase. These β-lactam analogues represent the first examples of a new class of inhibitors of GABA aminotransferase.

KEY WORDS: β-Lactams, γ-aminobutyric acid, γ-aminobutyric acid aminotransferase, monobactams, penicillins, cephalosporins.

INTRODUCTION

Many studies have been carried out on the relationship between the γ-aminobutyric acid (GABA) system and convulsions; for example, the perturbation of GABA metabolism,[1] the impairment of GABA-utilizing neurons,[2] and the blockage of postsynaptic GABA-receptors by drugs like bicuculine or picrotoxin[2] are believed to be involved in the origin of various types of experimental epilepsy. It is known that inhibition of GABA aminotransferase, the enzyme that catalyzes the degradation of the inhibitory neurotransmitter GABA, also can produce an anticonvulsant effect.[3] Currently, an irreversible inactivator of GABA aminotransferase, vigabatrin (γ-vinyl GABA), is on the drug market for use in the treatment of convulsions.[4] Because of the importance of inhibitors of GABA aminotransferase to the treatment of convulsive disorders, we have been interested in the design of new classes of compounds that might inhibit this enzyme.[5]

GABA and ethanolamine *O*-sulfate (**1**), a potent time-dependent inactivator of GABA aminotransferase,[6] can be drawn in a conformation that strongly resembles the structure of the monobactams **2–4**. Consequently, the monobactams are

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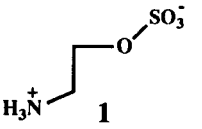

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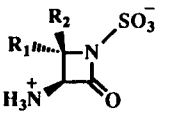
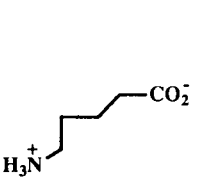


	R_1	R_2
2; SQ 30,384	H	H
3; SQ 27,129	H	CH_3
4; SQ 26,771	CH_3	H

conformationally-rigid GABA analogues. Likewise, 5-aminopentanoic acid (**5**),[7] a substrate for GABA aminotransferase, can be drawn in a conformation that resembles the structures of 3-aminonocardicinic acid (**6**), 7-aminopenicillanic acid (**7**), 6-aminocephalosporanic acid (**8**), and 7-aminodesacetoxycephalosporanic acids (**9** and **10**). These



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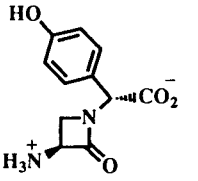

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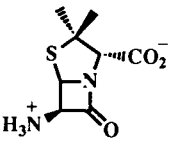

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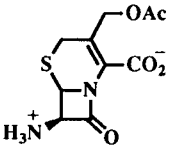

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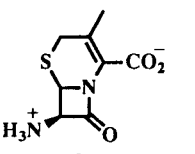

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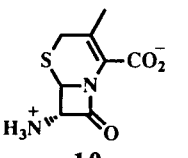

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β-lactams, then, are conformationally-rigid analogues of 5-aminopentanoic acid. Because of this potential structural similarity of the β-lactam antibiotic analogues to inhibitors and substrates of GABA aminotransferase, the present study was carried out to determine if any of these β-lactams have inhibitory effects on the activity of GABA aminotransferase.

MATERIALS AND METHODS

Reagents

6-Aminopenicillanic acid (**7**), 7-aminocephalosporanic acid (**8**), 7-aminodesacetoxycephalosporanic acid (**9**), β-mercaptoethanol, β-aminobutyric acid, and α-ketoglutarate were purchased from Sigma Chemical Co. 3-Aminonocardicinic acid (**6**) was a generous gift of Professor Craig Townsend of Johns Hopkins University. SQ, 26,771 (**4**), SQ 27,129 (**3**), and SQ 30,384 (**2**) were gifts of the Bristol-Meyers Squibb Company, and A-44177 (**10**) was a gift of Abbott Laboratories.

TABLE I
K_i values for compounds **2–4** and **6–10** with GABA aminotransferase. See Experimental Section for details

Compound	K_i (mM)
2	21.8
3	34.4
4	3.9
6	2.9
7	12.4
8	12.4
9	10.1
10	167

Enzymes and Assays

GABA aminotransferase was purified to homogeneity from pig brains by the method of Churchich and Moses.[8] The enzyme showed one band on $NaDodSO_4$-PAGE at pH 7.0 and had a specific activity of 4.23 units/mg of protein. One unit is defined as the amount of enzyme that catalyzes the transamination of 1 μmol of GABA/min at 25°C. Enzyme activity was measured on a Perkin-Elmer Lambda 1 UV/vis spectrophotometer as previously described.[9] Enzyme activity was recorded on a Beckman DU-40 UV/vis spectrophotometer. Succinic semialdehyde dehydrogenase was prepared from GABAse (Sigma Chemical Co. and Boehringer-Mannheim Biochemicals) as described by Jeffery *et al.*[10]

*Inactivation of GABA aminotransferase by β-lactams **2–4** and **6–10***

Testing for time-dependent inhibition of GABA aminotransferase by the compounds listed in Table I was carried out as previously described.[11]

*Inhibition of GABA aminotransferase by **2–4** and **6–10***

The competitive inhibition of GABA aminotransferase by the compounds listed in Table I was carried out and analyzed as previously described.[12]

RESULTS AND DISCUSSION

All of the β-lactams tested were competitive inhibitors of GABA aminotransferase; the K_i values are summarized in Table I. The fact that these conformationally-rigid compounds are competitive inhibitors of GABA aminotransferase is consistent with their structural similarity to the natural substrate, GABA, and to other known competitive inhibitors and substrates of the enzyme (such as **1** and **5**).[6,7] This suggests that the conformation of bound substrate is not too different from the rigid geometry of the β-lactams. The fact that **10** is a very weak inhibitor of GABA aminotransferase, but that **9**, the enantiomer of **10**, is a much stronger inhibitor, indicates the importance of the absolute configuration of the amino substituent. Since these compounds are GABA analogues, this orientation also should be relevant to the geometry at the active site of bound GABA. Likewise, the fact that **2** and **3** are poor competitive

inhibitors of GABA aminotransferase but that **4**, the diastereomer of **3**, is a much better competitive inhibitor, suggest that there are binding interactions with **4** that are not available for **2** and **3**. Although no X-ray structural data are available for these monobactams at this time, the method of synthesis of these compounds should produce the thermodynamically more stable isomers. Therefore, the sulfo group should occupy a geometry *trans* to the adjacent methyl group. If that is the case, then in **3**, the sulfo group would be *trans* to the amino group, but in **4** the sulfo group would be *cis* to the amino group. Since the sulfo group, apparently, mimics the carboxylate of GABA, the geometry of the monobactam **4** more likely resembles the geometry of bound GABA than does that of **3**. This suggests that GABA is oriented in the active site with the amino and carboxylate groups *syn*, a geometry that also is consistent with the above-mentioned increased inhibitory activity of **9** over **10**.

There also was the possibility that these β-lactams may act as affinity-labeling agents or as mechanism-based inactivators.[13] Penicillin derivatives are known to inactivate β-lactamase by initial active site nucleophilic attack at the β-lactam carbonyl followed by various isomerizations to give stable adducts.[14] By analogy to the β-lactamase inactivators, the β-lactams **2–4** and **6–10**, following Schiff base formation with PLP, may acylate an active site nucleophile, possibly the lysine residue that holds the PLP into position. If this is the only covalent reaction, then these compounds would be defined as affinity labeling agents. However, subsequent to acylation they could undergo enzyme-catalyzed elimination to an α,β-unsaturated ester, which could react with another active site nucleophile. Unfortunately, compounds **2–4** and **6–10** produced no time-dependent inactivation of GABA aminotransferase, even at concentrations of 100 mM for 24 h. This indicates that irreversible inactivation is not occurring. In addition, there was no evidence for any reaction with the PLP, as evidenced by the lack of any change in the UV spectrum of the enzyme monitored between 300–500 nm during its incubation with **9**.

In conclusion, compounds **2–4** and **6–10** were all determined to be competitive inhibitors of GABA aminotransferase. On the basis of the K_i values for the various compounds it appears that in bound GABA the amino and the carboxylate groups are in a *syn* orientation. Although all of these compounds have the potential to inactivate GABA aminotransferase, none does. One explanation could be that when the β-lactams bind to the active site, there is no nucleophile properly juxtaposed for acylation or the rigidity of these compounds interferes with appropriate binding modes for inactivation. Nonetheless, these β-lactam analogues represent the first examples of a new class of inhibitors of GABA aminotransferase.

Acknowledgements

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References

1. Baxter, C. (1970) Handbook of Neurochemistry, Lajtha, A. ed.) Vol. 3, pp. 289–353. New York: Plenum Press.
2. Meldrum, B. (1975) *Int. Rev. Neurobiol.* , **17**, 1–36.

3. (a) Leach, M.J. and Walker, J.M.G. (1977) *Biochem. Pharmacol.*, **26,** 1569–1572. (b) Phillips, N.I. and Fowler, L.J. (1982) *Biochem. Pharmacol.*, **31,** 2257–2261.
4. (a) Tassinari, C.A., Michelucci, R., Ambrosetto, G. and Salvi, F. (1987) *Arch. Neurol.*, **44,** 907–910. (b) Browne, T.R., Mattson, R.J., Penry, J.K., Smith, D.B., Treiman, D.M., Wilder, B.J., Ben-Menachem, E., Miketta, R.M., Sherry, K.M. and Szabo, G.K. (1989) *Br. J. Clin. Pharmacol.*, **27,** 95S–100S. (c) Sivenius, J.M.R., Ylinen, A., Murros, K., Matilainen, R. and Riekkinen, P. (1987) *Epilepsia*, **28,** 688–692.
5. Nanavati, S.M. and Silverman, R.B. (1989) *J. Med. Chem.*, **32,** 2413–2421.
6. Fowler, L.J. and John, R.A. (1981) *Biochem. J.*, **197,** 149–154.
7. Beart, P.M. and Johnston, G.A.R. (1973) *Brain Research*, **49,** 459–462.
8. Churchich, J.E. and Moses, U. (1981) *J. Biol. Chem.*, **256,** 1101–1104.
9. Silverman, R.B. and Levy, M.A. (1981) *Biochemistry*, **20,** 1197–1203.
10. Jeffery, D., Weitzman, P.D.J. and Lunt, G.G. (1988) *Insect Biochem.*, **18,** 347–349.
11. Silverman, R.B., Invergo, B.J. and Mathew, J. (1986) *J. Med. Chem.*, **29,** 1840–1846.
12. Andruszkiewicz, R. and Silverman, R.B. (1990) *J. Biol. Chem.*, **265,** 22288–22291.
13. Silverman, R.B. (1988) *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology;* vols. I and II. CRC Press: Boca Raton.
14. Silverman, R.B. (1988) *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*; vol. I, Chapter 5. CRC Press: Boca Raton.