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Perspective

Design of Potential Anticonvulsant Agents: Mechanistic Classification of GABA Aminotransferase Inactivators†

Shrenik M. Nanavati and Richard B. Silverman*

Department of Chemistry and Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60208-3113. *Received April 14, 1989*

Epilepsy is a disease that was described over 4000 years ago in early Babylonian and Hebrew writings.[1] If it is broadly defined as any central nervous system disease characterized by recurring convulsive seizures, then one-half to one percent of the world population has epilepsy.[2] It is categorized as *primary* or *idiopathic* when no cause for the seizure is known, and *secondary* or *symptomatic* when the etiology has been identified. Symptomatic epilepsy can result from specific physiological phenomena such as brain tumors, syphilis, cerebral arteriosclerosis, multiple sclerosis, Buerger's disease, Pick's disease, Alzheimer's disease, sunstroke or heat stroke, acute intoxication, lead poisoning, head trauma, vitamin B_6 deficiency, hypoglycemia, and labor.[3,4] The biochemical mechanism leading to central nervous system electrical discharges and epilepsy are unknown, but there may be multiple mechanisms involved. However, it has been shown that convulsions arise when there is an imbalance in two principal neurotransmitters in the brain, L-glutamic acid, an excitatory neurotransmitter, and γ-aminobutyric acid (GABA), an inhibitory neurotransmitter. The concentrations of these two amino acids are regulated by two PLP-dependent enzymes, L-glutamic acid decarboxylase (EC 4.1.1.15), which converts glutamate (1) to GABA (2) and GABA aminotransferase (EC 2.6.1.19), which degrades GABA to succinic semialdehyde (3) (Scheme I). Although succinic semialdehyde is toxic to cells,[5] there is no buildup of this metabolite, because it is efficiently oxidized to succinic acid by the enzyme succinic semialdehyde dehydrogenase (EC 1.2.1.24). GABA system dysfunction has beem implicated in the symptoms associated with Huntington's disease,[6] Parkinson's disease,[7] and tardive dyskinesia.[8] When the concentration of GABA diminishes below a threshold level in the brain, convulsions begin.[9] If a convulsion is induced in an animal, and GABA is injected directly into the brain, the convulsions cease.[10,11] It would seem, then, that an ideal anticonvulsant agent would be GABA; however, peripheral administration of GABA produces no anticonvulsant effect. This was shown to be the result of the failure of GABA, under normal circumstances, to cross the blood–brain barrier, a membrane that surrounds the capillaries of the circulatory system in the brain and protects it from passive diffusion of undesirable chemicals from the bloodstream. Another approach for increasing the brain

† Abbreviations and acronyms used herein include: GABA, γ-aminobutyric acid; GABA-T, γ-aminobutyric acid aminotransferase; GAD, L-glutamic acid decarboxylase; Glu, L-glutamic acid; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; SSADH, succinic semialdehyde dehydrogenase; α-KG, α-ketoglutaric acid; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate reduced form.

(1) Isaacson, E. I; Delgado, J. N. In *Burger's Medicinal Chemistry*, 4th ed.; Wolff, M. E., Ed.; Wiley: New York, 1981; Part III, p 829.
(2) Jasper, H. H.; Ward, A. A.; Poper, A. *Basic Mechanism of the Epilepsies*; Little, Brown and Co.: Boston, 1969.
(3) Wyke, B. *Principles of General Neurology*; Elsevier: London, 1969.
(4) Biemond, A. *Brain Diseases*; Elsevier: London, 1970.
(5) Hearl, W. G.; Churchich, J. E. *J. Biol. Chem.* **1984**, *259*, 11459.
(6) Perry, T. L.; Hansen, S.; Kloster, M. N. *N. Engl. J. Med.* **1973**, *288*, 337.
(7) Lloyd, K. G.; Shemen, L.; Hornykiewicz, O. *Brain Res.* **1977**, *127*, 269.
(8) Palfreyman, M. G.; Hout, S.; Lippert, B.; Schechter, P. J. *Eur. J. Pharmacol.* **1978**, *50*, 325.
(9) Karlsson, A.; Fonnum, F.; Malthe-Sorrensen, D.; Storm-Mathisen, J. *Biochem. Pharmacol.* **1974**, *23*, 3053.
(10) Purpura, D. P.; Girado, M.; Smith, T. A.; Callan, D. A.; Grundfest, H. *J. Neurochem.* **1959**, *3*, 238.
(11) Tower, D. B. In *GABA in Nervous System Function*; Roberts, E., Chase, T. N., Tower, D. B., Ed.; Raven Press: New York, 1976; pp 461–478.

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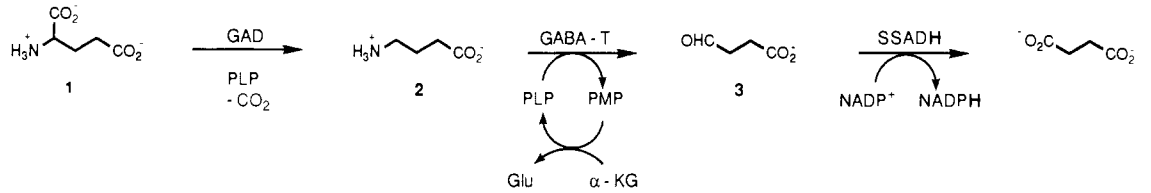
Scheme I. Metabolism of L-Glutamate



GABA concentration, however, would be to design a compound capable of permeating the blood–brain barrier that subsequently inactivated GABA aminotransferase, the enzyme that catalyzes the degradation of GABA. Provided that GAD also is not inhibited, GABA concentrations should rise. This, in fact, has been shown to be an effective approach to the design of anticonvulsant agents. Compounds that both cross the blood–brain barrier and inhibit GABA aminotransferase in vitro have been reported to increase whole brain GABA levels in vivo and possess anticonvulsant activity.[12,13] However, the anticonvulsant effect does not correlate with whole brain GABA levels, but, rather, with an increase in the GABA concentration at the nerve terminals of the substantia nigra.[14,15] γ-Vinyl GABA (7, Scheme III; vigabatrin), for example, shows a lag time prior to its anticonvulsant activity, even though whole brain GABA levels rise rapidly; the anticonvulsant activity, however, correlates with the increase in GABA levels at the substantia nigra.[14,15]

γ-Vinyl GABA is a mechanism-based enzyme inactivator, that is, an unreactive compound with a structure similar to that of the substrate or product for the target enzyme that converts it by its normal catalytic mechanism into an activated form that inactivates the target enzyme prior to its release from the active site.[16] The potential of mechanism-based enzyme inactivators in drug design has been reviewed recently.[17] Their advantage over competitive reversible inhibitors is that steady-state concentrations of mechanism-based inactivators do not need to be maintained in order to sustain the inhibitory effect, since they are generally irreversible inhibitors. Their advantage over affinity labeling irreversible inactivators, that is, reactive compounds that react covalently with enzymes, is that mechanism-based inactivators are unreactive so they potentially can be more specific for the target enzyme and, therefore, be less toxic. Despite their great potential as drugs, no rationally designed mechanism-based enzyme inactivator for a specific enzyme has yet appeared on the American drug market. There are, however, quite a few mechanism-based inactivators in current medical use. These drugs, however, were not *designed* as mechanism-based inactivators, but, rather, were determined to be so ex post facto.

Many of the difficulties in getting these compounds to the drug market are the same as for any drug candidate, namely, unpredicted problems with pharmacokinetics, metabolism, and toxicity. However, mechanism-based enzyme inactivators have an additional complication with which to contend. They are designed on the basis of hypothesized chemistry by which the target enzyme is supposed to convert them to the form responsible for inactivation. What if the hypothesized chemistry is incorrect? Or what if structural modifications of the substrate to give the mechanism-based inactivator lead to a different chemistry than the chemistry of normal substrate turnover? What if the activated product generated can follow more than one reaction pathway, but only one route leads to inactivation? These questions may remain unanswered until detailed product analyses can allow more intelligent postulates to be made regarding the actual chemistry involved. At some point an investigator must stop and consider multiple inactivation pathways that are catalyzed by a target enzyme so that a better defined basis for the design of effective mechanism-based inactivators can be established. This is the perspective we present here with regard to the design of mechanism-based inactivators of GABA aminotransferase.

In order to discuss inactivation mechanisms catalyzed by GABA aminotransferase, its normal substrate catalytic mechanism must be presented first. GABA aminotransferase is a typical PLP-dependent aminotransferase characterized by a cycle of two half reactions (a ping-pong BiBi mechanism).[18] As shown in Scheme II, PLP is present in the active site as a Schiff base with the ε-amino group of a lysine residue.[19] Transimination with GABA generates a new PLP imine (4) which undergoes rate-determining[20] enzyme-catalyzed γ-deprotonation of the *pro-S* proton[21] to the PMP-aldimine 6. Hydrolysis of this intermediate gives the product succinic semialdehyde and the reduced form of the cofactor, PMP, which is converted back to PLP concomitant with conversion of α-ketoglutarate to L-glutamate via a mechanism that is the inverse of the first half reaction. It is interesting to note that the action of GABA aminotransferase destroys one molecule of inhibitory neurotransmitter (GABA) and produces one molecule of excitatory neurotransmitter (L-glutamate), and, therefore, in one cycle changes the GABA/glutamate imbalance by two molecules.

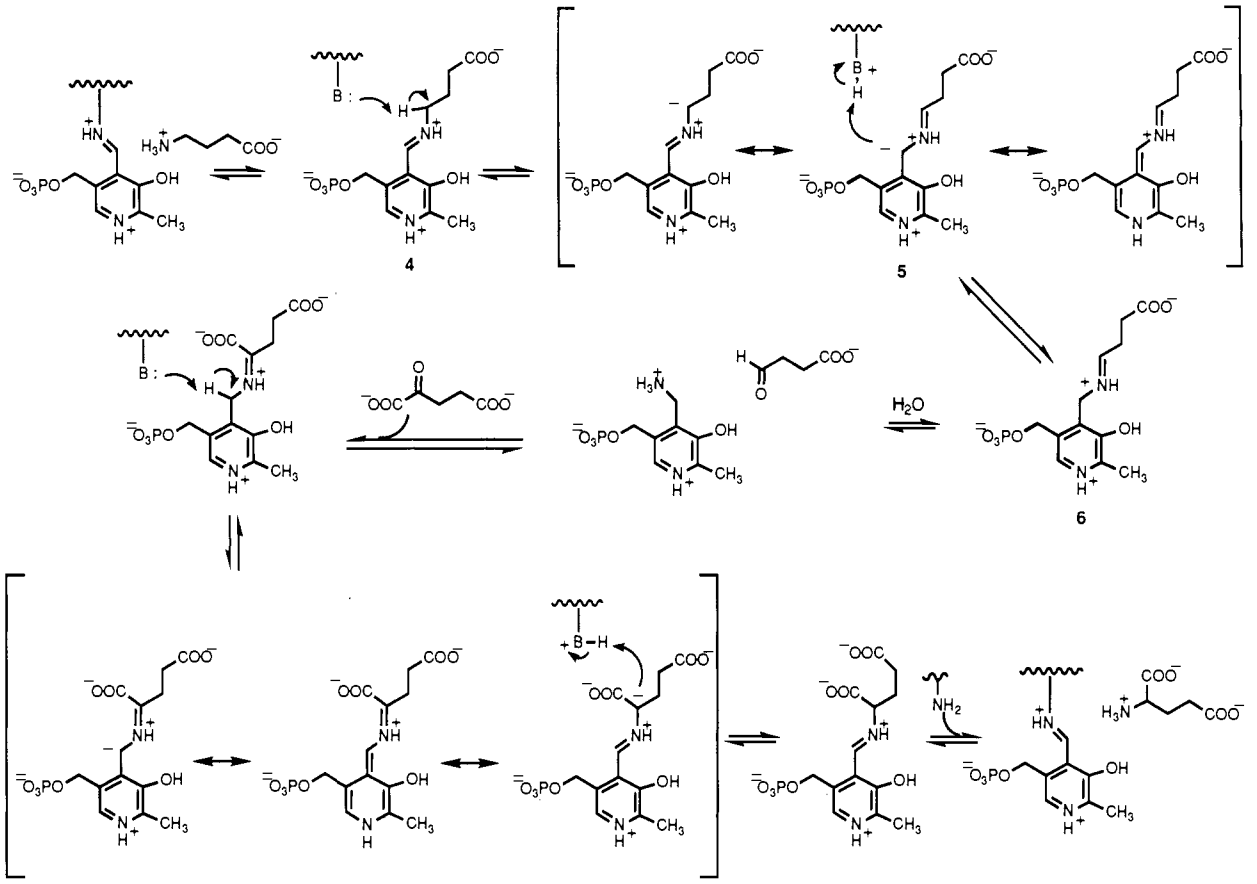
Mechanistic Classification of GABA Aminotransferase Inactivators

On the basis of results from our laboratory and numerous reports in the literature, we now classify mechanism-based inactivators of GABA aminotransferase into one of four generalized inactivation mechanisms.

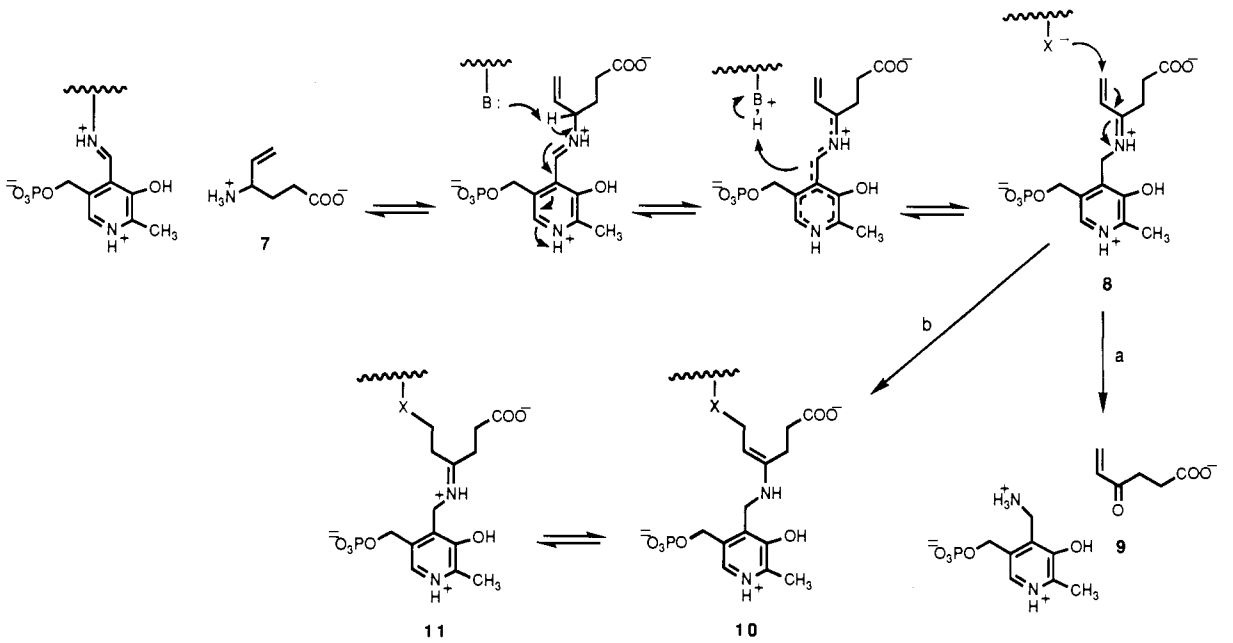
Class I GABA Aminotransferase Inactivators. With the mechanism for GABA aminotransferase shown in Scheme II in mind, Metcalf and co-workers[22] proposed

(12) Leach, M. J.; Walker, J. M. G. *Biochem. Pharmacol.* **1977**, *26*, 1569.
(13) Philips, N. I.; Fowler, L. J. *Biochem. Pharmacol.* **1982**, *31*, 2257.
(14) Gale, K.; Iadarola, M. J. *Science (Washington, D.C.)* **1980**, *208*, 288.
(15) Iadarola, M. J.; Gale, K. *Science (Washington, D.C.)* **1982**, *218*, 1237.
(16) Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*; CRC Press: Boca Raton, FL, 1988; Vols. I and II.
(17) Silverman, R. B. *J. Enz. Inhib.* **1988**, *2*, 73.
(18) White, H. L.; Sato, T. L. *J. Neurochem.* **1978**, *31*, 41.
(19) Kim, D. S.; Churchich, J. E.; Schlitz, E. In *Prog. Clin. Biol. Res. 144B (Chemical and Biological Aspects of Vitamin B_6 Catalysis Part B)*; Evangelopoulos, A. E., Ed.; Alan R. Liss, Inc.: New York, 1984; pp 161–167.
(20) Yu, P. H.; Durden, D. A.; Davis, B. A.; Boulton, A. A. *J. Neurochem.* **1987**, *48*, 440.
(21) Burnett, A.; Walsh, C. *J. Chem. Soc., Chem. Commun.* **1979**, 826.

Scheme II. Catalytic Mechanism for GABA Aminotransferase



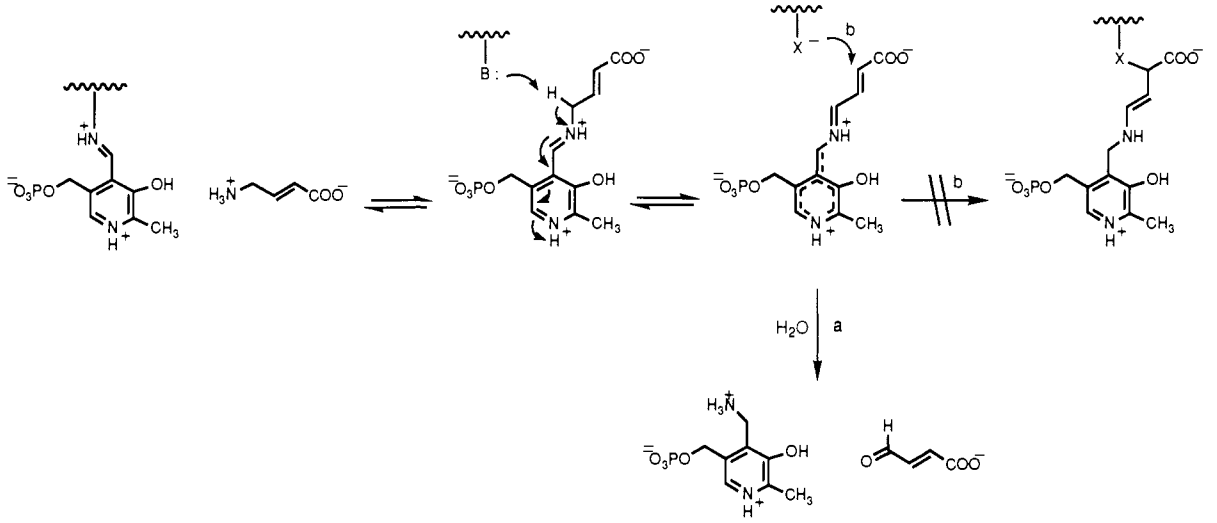
Scheme III. Proposed Inactivation Mechanism for GABA Aminotransferase by γ-Vinyl GABA



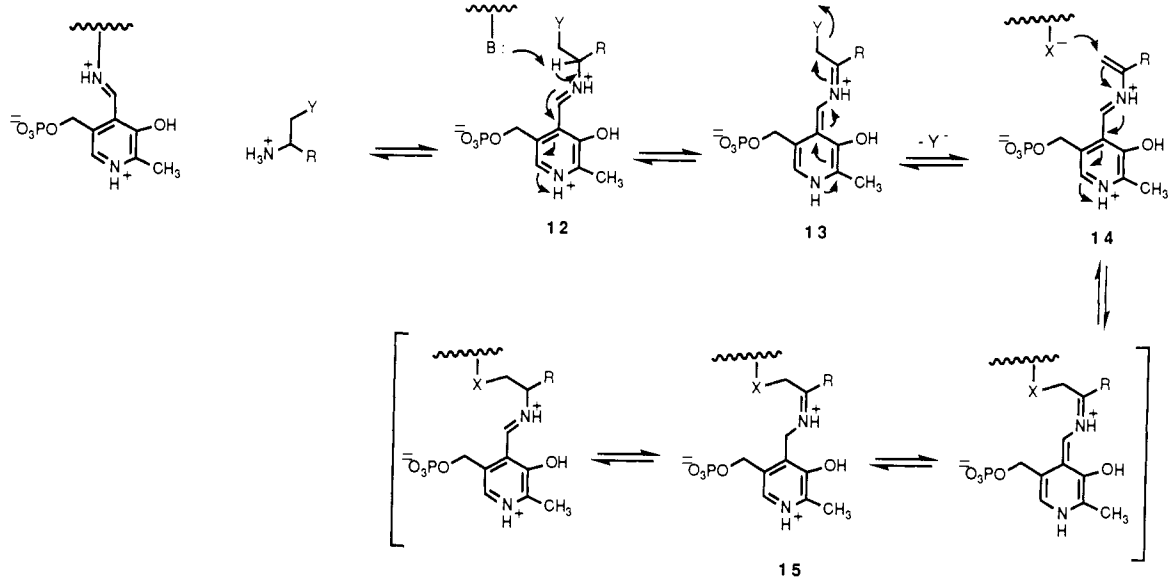
that 4-amino-5-hexenoic acid (γ-vinyl GABA; **7**) should inactivate the enzyme by the mechanism shown in Scheme III. By comparison of Schemes II and III it is apparent that identical mechanisms are proposed up to **6** (Scheme II) and **8** (Scheme III). In the case of GABA turnover, hydrolysis of **6** leads to the formation of succinic semialdehyde and PMP. The same hydrolysis reaction could occur on **8** (pathway a), which would produce the corre-

(22) Lippert, B.; Metcalf, B. W.; Jung, M. J.; Casara, P. *Eur. J. Biochem.* **1977**, *74*, 441.
(23) Jung, M. J.; Metcalf, B. W. *Biochem. Biophys. Res. Commun.* **1975**, *67*, 301.
(24) Kolb, M.; Barth, J.; Heydt, J.-G.; Jung, M. J. *J. Med. Chem.* **1987**, *30*, 267.
(25) Jung, M. J.; Heydt, J.-G.; Casara, P. *Biochem. Pharmacol.* **1984**, *33*, 3717.
(26) Bey, P.; Gerhart, F.; Jung, M. *J. Org. Chem.* **1986**, *51*, 2835.
(27) Bey, P.; Jung, M. J.; Gerhart, F.; Schirlin, D.; Van Dorsselaer, V.; Casara, P. *J. Neurochem.* **1981**, *37*, 1341.
(28) Silverman, R. B.; George, C. *Biochemistry* **1988**, *27*, 3285.

Scheme IV. Turnover of 4-Amino-2-butenoic Acid



Scheme V. Proposed Michael Addition Pathway for Inactivation of GABA Aminotransferase by Compounds Containing a Leaving Group β to the γ-Position



sponding transamination products, 4-oxo-5-hexenoic acid (**9**) and PMP. However, **8** is an electrophile, a Michael acceptor, which can undergo conjugate addition by an active-site nucleophile and produce inactivated enzyme (pathway b; **10** or **11**). Compounds that inactivate GABA aminotransferase by conjugate addition of an active-site nucleophile to an enzyme-generated Michael acceptor (Table I) and thus become attached to an active-site amino acid residue constitute Class I inactivators. Similar mechanisms can be drawn for the other compounds in Table I.

Note that the hydrolysis pathway to **9** is simply product formation. The ratio of molecules converted to a product per inactivation event has been termed the partition ratio.[29] With regard to drug design the ideal case is that of no product formation (partition ratio of zero) because turnover may release a toxic metabolite. Compound **9** is an example of just such a potentially toxic product because it is a Michael acceptor.

It is interesting to note that a mechanism related to that shown in Scheme III could be drawn for 4-amino-2-butenoic acid (Scheme IV), but this compound is only a substrate for GABA aminotransferase.[30] Hydrolysis to 4-oxo-2-butenoic acid and PMP (pathway a), presumably, is the only viable pathway in this case. However, when a strong electron withdrawing group, such as fluorine, is attached to the 2-position (4-amino-2-fluoro-2-butenoic acid, Table I), then it, apparently, activates the Michael acceptor sufficiently to induce active-site nucleophilic attack once in 750 turnovers.[28]

Table I. Class I GABA Aminotransferase Inactivators[a]

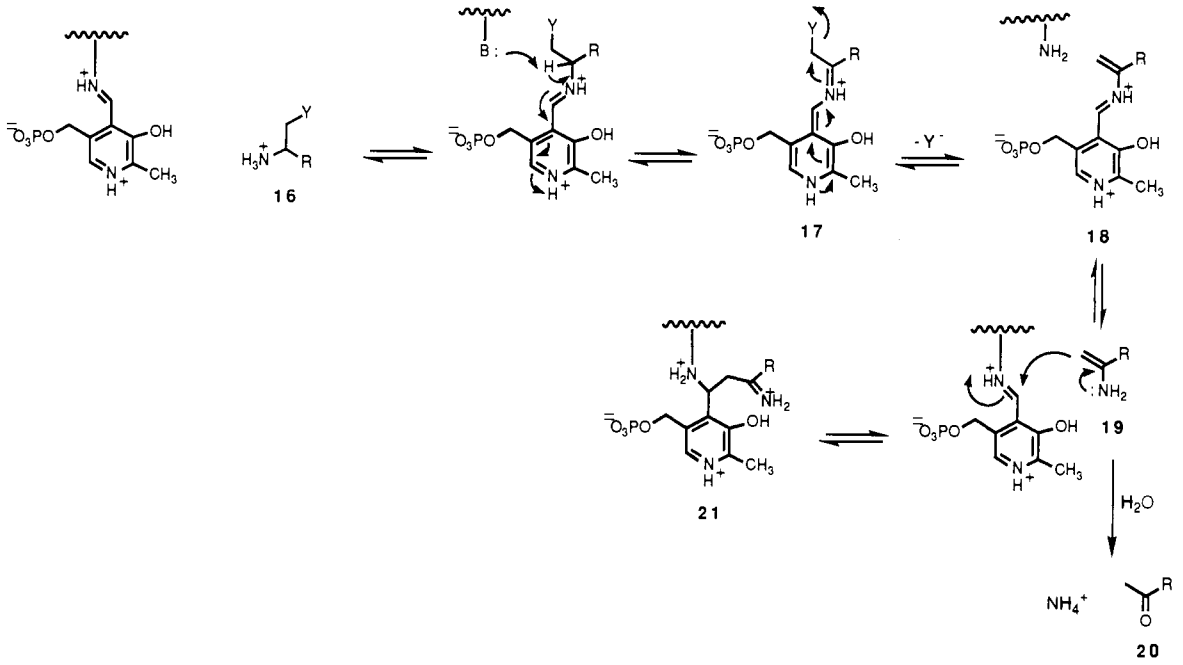
compound	ref
4-amino-5-hexenoic acid	22
4-amino-5-hexynoic acid	23
(*Z*)-4-amino-6-fluoro-5-hexenoic acid	24
4-amino-6,6-difluoro-5-hexenoic acid	24
4-amino-5,6-heptadienoic acid	25
(*E*)-4-amino-2,5-hexadienoic acid	26
3-amino-4-pentynoic acid	27
(*Z*)-4-amino-2-fluoro-2-butenoic acid	28
3-amino-4-pentenoic acid	27

[a] See Scheme III for example.

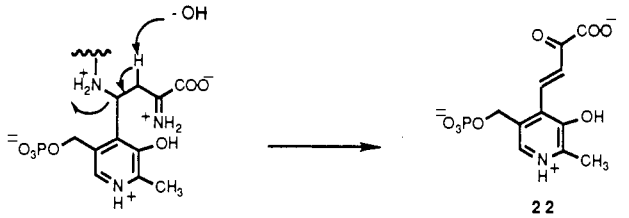
(29) Walsh, C.; Cromartie, T.; Marcotte, P.; Spencer, R. *Methods Enzymol.* **1978**, *53D*, 437.

(30) Johnston, G. A. R.; Curtis, D. R.; Beart, P. M.; Game, C. J. A.; McCulloch, R. M.; Twitchin, B. *J. Neurochem.* **1975**, *24*, 157.

Scheme VI. Enamine Mechanism for Inactivation of PLP Enzymes by Amino Acids Containing Leaving Groups Capable of β-Elimination



Scheme VII. Evidence for the Structure of Ternary Complex 14



Class II GABA Aminotransferase Inactivators. Another approach to generate an enzyme-bound Michael acceptor would be by β-elimination of a leaving group adjacent to the incipient carbanion that is generated by enzyme-catalyzed proton removal. In this mechanism, generalized in Scheme V, Schiff base formation with the PLP (12) and proton removal (13) are comparable to the first two steps of GABA turnover. Elimination of a leaving group Y^- to generate Michael acceptor 14 competes with normal reprotonation (5 in Scheme II). Theoretically, 14 is capable of undergoing conjugate addition with an active-site amino acid residue to give inactivated enzyme (15). In fact, there are numerous examples of this proposed inactivation mechanism in the literature for a wide variety of PLP-dependent enzymes. However, Metzler and coworkers carefully reinvestigated this general inactivation mechanism for the enzymes glutamic acid decarboxylase[31] and aspartate aminotransferase[32] using serine *O*-sulfate (Scheme VI; 16, R = COO^-, Y = OSO_3^-) as their inactivator. They concluded that the Michael addition mechanism was incorrect, and proposed a mechanism involving an enamine intermediate (Scheme VI). An active-site lysine (presumably the same lysine to which the PLP is bound in the native enzyme) transiminates with 18 to give enzyme-bound PLP and enamine 19. The enamine then undergoes nucleophilic attack on the electrophilic immonium carbon of the bound PLP to give a ternary adduct of the enzyme, the PLP, and the inactivator (21). The structure of the ternary adduct was deduced by raising the pH to 11, then isolating and characterizing 22, presumably derived by the mechanism shown in Scheme VII. The results of these studies changed the way enzymologists think about these inactivators. This enamine inactivation mechanism was shown later to be relevant to the inactivation of a broad specificity amino acid racemase[33] and a narrow specificity alanine racemase[34] by β-fluoroalanine and to the inactivation of GABA aminotransferase by 4-amino-5-fluoropentanoic acid.[35] Compounds that inactivate GABA aminotransferase by the enamine mechanism (Scheme VI) and form a ternary adduct constitute Class II inactivators; these are listed in Table II. Note that upon transimination a change in polarization of the inactivator occurs from that of an electrophile (18, Scheme VI) to that of a nucleophile (19). Since the only reactive electrophile in the active site of GABA aminotransferase is the protonated imine of lysine-bound PLP, this inactivation mechanism must, by necessity, lead to adduct

Table II. Class II GABA Aminotransferase Inactivators[a]

compound	ref
(*S*)-4-amino-5-fluoropentanoic acid	35
(*S*,*E*)-4-amino-5-fluoro-2-pentenoic acid	36
3-amino-4-fluorobutanoic acid	27
3-amino-4,4-difluorobutanoic acid	27
4-amino-5,5-difluoropentanoic acid	27
5-amino-6-fluorohexanoic acid	27
5-amino-6,6-difluorohexanoic acid	27
ethanolamine-*O*-sulfate	37
3-amino-2,4-difluorobutanoic acid	38
3-amino-4-chloro-4-fluorobutanoic acid	38

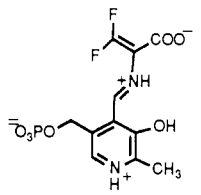
[a] See Scheme VI for example.

(31) Likos, J. J.; Ueno, H.; Feldhaus, R. W.; Metzler, D. E. *Biochemistry* **1982**, *21*, 4377.
(32) Ueno, H.; Likos, J. J.; Metzler, D. E. *Biochemistry* **1982**, *21*, 4387.
(33) Roise, D.; Soda, K.; Yagi, T.; Walsh, C. T. *Biochemistry* **1984**, *23*, 5195.
(34) Badet, B.; Roise, D.; Walsh, C. T. *Biochemistry* **1984**, *23*, 5188.
(35) Silverman, R. B.; Invergo, B. J. *Biochemistry* **1986**, *25*, 6817.
(36) Silverman, R. B.; George, C. *Biochem. Biophys. Res. Commun.* **1988**, *150*, 942.
(37) Fowler, L. J.; John, R. A. *Biochem. J.* **1972**, *130*, 569.
(38) Schirlin, D.; Baltzer, S.; Heydt, J.-G.; Jung, M. J. *J. Enz. Inhib.* **1987**, *1*, 243.

formation with the cofactor. Because the cofactor is bound to the protein, a ternary adduct forms (21). The revelation of the enamine mechanism by Metzler and co-workers[31,32] indicated that the most obvious mechanism is not necessarily the correct one.

When large amounts of enzyme are readily available, as in the case of GAD[31] and aspartate aminotransferase,[32] differentiation of Class I versus Class II mechanisms involves enzyme inactivation, dialysis, or gel filtration, then an increase of the pH to 11 or 12 followed by denaturation and HPLC chromatography. Class I inactivators would release PLP and/or PMP; class II inactivators would release **22** or a related analogue. When much less enzyme is available, as in the case of GABA aminotransferase, apoenzyme can be reconstituted with [^{3}H]PLP and the cofactor metabolites can be isolated with unlabeled carrier standards.[35] A second experiment can be carried out to confirm the results of the first, i.e., inactivation with a radioactively labeled inactivator. Class I inactivators remain bound to the protein after the above treatment. It is unwise to carry out only the second experiment because base treatment and/or denaturation of a Class I inactivation may result in no radioactivity bound to the protein if the adduct is labile to those conditions.

It should be noted that Class II inactivators may be restricted to compounds containing only one leaving group. It has been shown that β,β,β-trifluoroalanine is a mechanism-based inactivator of γ-cystathionase[39] and of the alanine racemase from *Salmonella typhimurium* and *Bacillus stearothermophilus*,[40] and that this inactivator becomes attached to an active-site lysine residue,[40,41a] suggesting a Class I mechanism. The product of β-elimination of one of the fluoride ions would be a Michael acceptor that is doubly activated with two electron-withdrawing groups (**23**), and this may be sufficiently more



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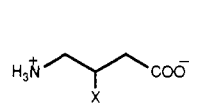
electrophilic to alter the pathway of attack of the active-site lysine residue. This would result in the labeling of the protein (Class I inactivator) rather than the cofactor. An alternative rationalization for the change in inactivation mechanism could be that active-site nucleophilic addition to the terminal alkene of the activated intermediate **18** (Scheme VI) occurs, but is reversible, whereas addition to the halogenated intermediate **23** is irreversible because that addition ultimately leads to ejection of a second fluoride ion. If this is the case, then all of the multi gem-halogenated compounds in Table II may belong, instead, in Table I, the Class I inactivators. A preliminary experiment to test this possibility was carried out recently.[41b] GABA aminotransferase, reconstituted with [^{3}H]PLP, was inactivated with 4-amino-5,5-difluoropentanoic acid (gift of Dr. Philippe Bey, Merrell Dow Pharmaceuticals). After the usual workup[35] *both* [^{3}H]PLP and a new tritiated metabolite, which eluted by HPLC with a t_R similar to that of the metabolite formed by 4-amino-5-fluoropentanoic acid inactivation, were isolated. This preliminary result suggests that *both* Class I and Class II mechanisms may be involved in this inactivation. Unfortunately, neither 4-amino-5,5,5-trifluoropentanoic acid nor 3-amino-4,4,4-trifluorobutanoic acid inactivates GABA aminotransferase;[27] consequently, it cannot be determined if trihalo-substituted compounds inactivate GABA aminotransferase exclusively by a Class I mechanism, which appears to be the case with other PLP-dependent enzymes.[39-41a]

The alternative to ternary adduct formation (**21**) is release of the enamine (**19**) from the enzyme. Hydrolysis of the enamine would give the ketone (**20**, Scheme VI) and ammonium ion. Unlike the transamination products from Class I inactivator turnover, the hydrolyzed Class II products are not potent Michael acceptors; nonetheless, they would be undesirable metabolites from a drug design standpoint.

The key to lowering the partition ratio for the Class II inactivators is the geometry of the enamine that is generated. If its formation occurs so that it cannot readily react with the cofactor, then release will be favored, and the partition ratio will be high. 4-Amino-5-fluoropentanoic acid was shown to inactivate GABA aminotranserase as a Class II inactivator[35] having a partition ratio of zero;[42] 4-amino-5-fluoro-2-pentenoic acid also is a Class II inactivator,[36] but with a partition ratio of 4.[43] Related compounds such as 4-amino-3-halobutanoic acids (**24**)[44] and 4-amino-2-(halomethyl)-2-butenoic acids (**25**),[45] however, were designed as Class I inactivators of GABA aminotransferase, but were found to be only substrates, not inactivators, for the enzyme, presumably because they proceed by a Class II mechanism. The enamines generated, however (**26** and **27**, respectively), are poorly juxtaposed for attachment to the cofactor, and, therefore, are released and hydrolyzed exclusively.



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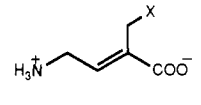

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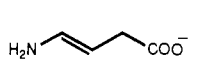

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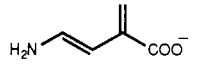

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These results suggest that if a Class II mechanism is desired, a leaving group capable of β-elimination should be incorporated into the design of the inactivator. However, it is possible to draw a Class II type mechanism for compounds such as γ-vinyl and γ-ethynyl GABA; Scheme VIII depicts an enamine mechanism for the former. Instead of protonation of the cofactor in **28** (Scheme VIII) followed by Michael addition as shown in Scheme II, protonation of the terminal alkene could occur to give **29**. Transimination with the active-site lysine would give enamine **30**, which could inactivate the enzyme by the Class II mechanism. However, our preliminary results[46] indicate that both γ-vinyl GABA and γ-ethynyl GABA inactivate

(39) Silverman, R. B.; Abeles, R. H. *Biochemistry* **1977**, *16*, 5515.
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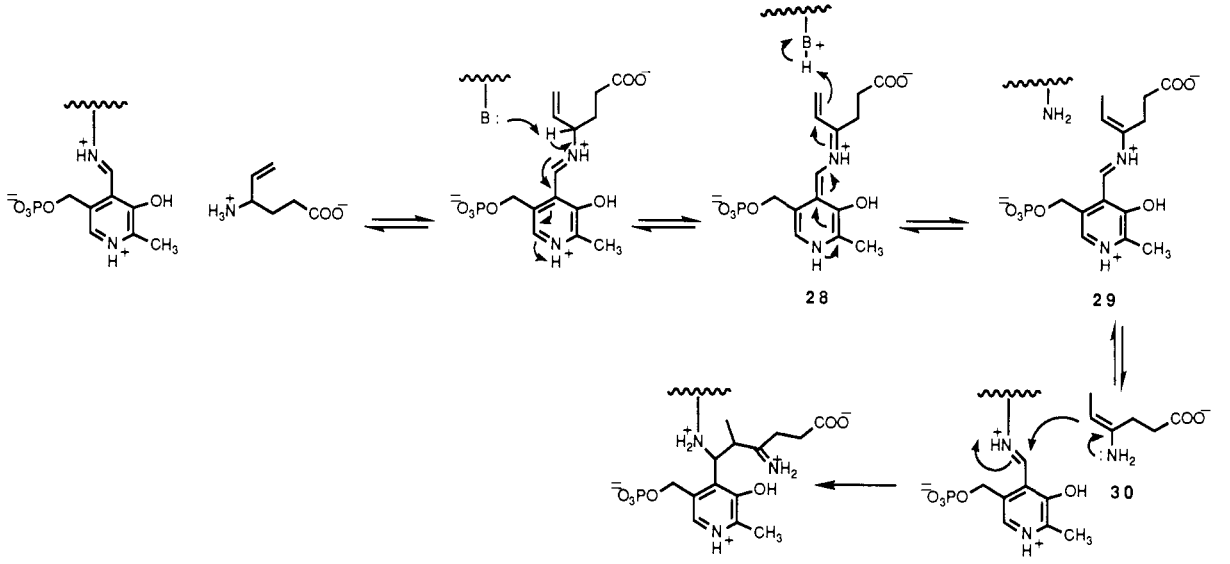
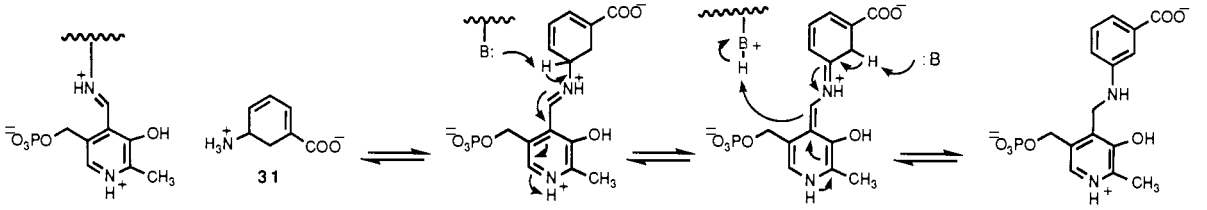
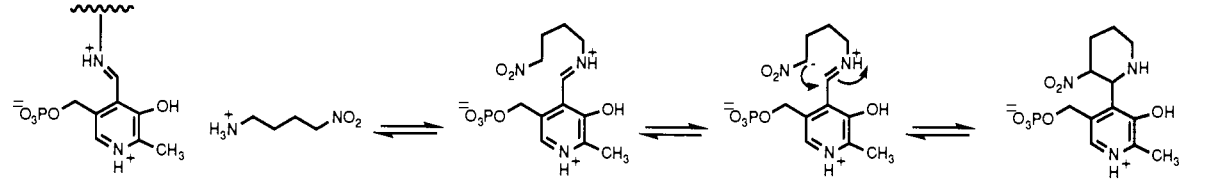
Scheme VIII. Hypothetical Enamine Inactivation Mechanism for γ-Vinyl GABA



Scheme IX. Inactivation of GABA Aminotransferase by Gabaculine



Scheme X. Inactivation of GABA Aminotransferase by 4-Nitro-1-butanamine



GABA aminotransferase by a Class I mechanism, further supporting the requirement for a leaving group to favor a Class II inactivation mechanism.

Class III GABA Aminotransferase Inactivators. Gabaculine (5-amino-1,3-cyclohexadiene-1-carboxylic acid, **31**, Scheme IX), is a natural product isolated from *Streptomyces toyacaensis* shown to inactivate GABA aminotransferase.[47] Originally, it was suggested that it may act as a Class I inactivator similar to the inactivation mechanism presented in Scheme III. However, gabaculine was later shown[48] to involve an aromatization mechanism that leads to modification of the cofactor without attachment of an active-site residue (Scheme IX). Compounds that inactivate GABA aminotransferase by a mechanism that alters the PLP and are not attached to an active-site residue are Class III inactivators. Since some inactivators in this class proceed by an aromatization mechanism and others do not, this class is subdivided; those that involve aromatization are Class IIIA and those that do not are Class IIIB. Examples of this class are listed in Table III. Several of the Class IIIB inactivators are not, strictly speaking, mechanism-based inactivators because their inactivation mechanisms are not initiated by the normal catalytic mechanism (i.e., γ-proton removal). These compounds include 5-nitro-L-norvaline,[52] 3-nitro-1-propanamine,[53] and 4-nitro-1-butanamine.[53] Scheme X shows the inactivation mechanism for 4-nitro-1-butanamine; deprotonation may not be an enzymatic process.

Table III. Class III GABA Aminotransferase Inactivators

compound	ref
class IIIA[a]	
5-amino-1,3-cyclohexadiene-1-carboxylic acid	47
3-amino-1,5-cyclohexadiene-1-carboxylic acid	49
3-amino-4,5-dihydrofuran-2-carboxylic acid	50
4-amino-4,5-dihydrothiophene-2-carboxylic acid	51
class IIIB[b]	
5-nitro-L-norvaline	52
3-nitro-1-propanamine	53
4-nitro-1-butanamine	53

[a] See Scheme IX for example. [b] See Scheme X for example.

Class IV GABA Aminotransferase Inactivators. The catalytic mechanism for GABA aminotransferase shown in Scheme II is fully reversible. According to the principle of microscopic reversibility, for a reversible re-

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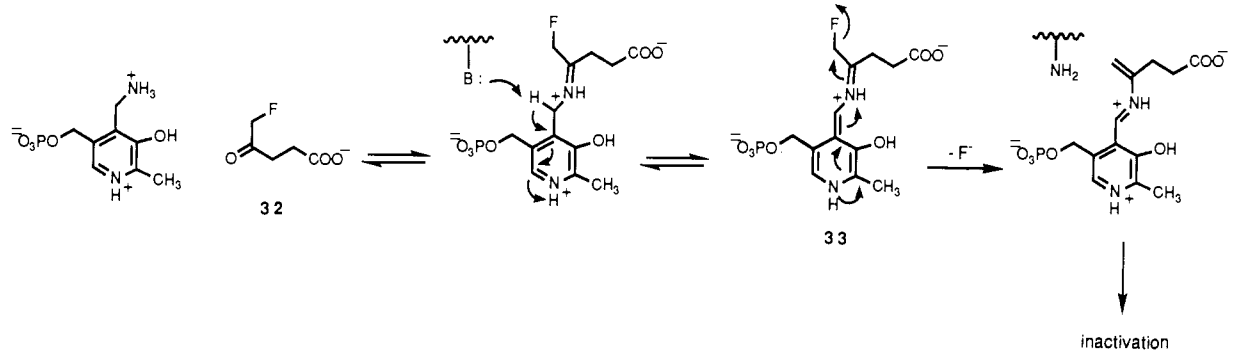
Scheme XI. Inactivation of the PMP Form of GABA Aminotransferase



Scheme XII. Inactivation of GABA Aminotransferase by 3,5-Dioxocyclohexanecarboxylic Acids

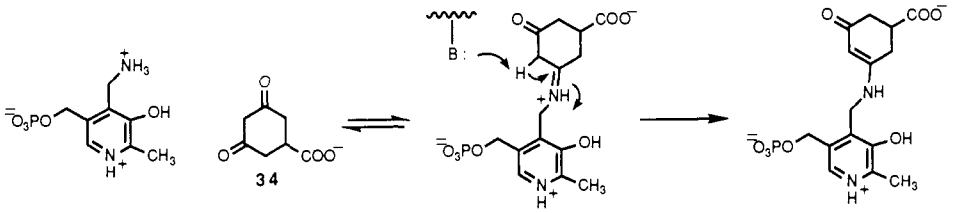


Table IV. Class IV GABA Aminotransferase Inactivators[a]

compound	ref
5-fluoro-4-oxopentanoic acid	54
3,5-dioxocyclohexanecarboxylic acid	55

[a] See Scheme XI for example.

action the mechanisms in the forward and reverse directions must be identical but opposite, and proceed through the same transition states. Thus, if GABA aminotransferase is converted to its PMP form in the absence of α-ketoglutarate, then succinic semialdehyde should be converted back to GABA under some set of conditions, and the mechanism must be that shown in the back direction of Scheme II. That being the case, then the keto form of a mechanism-based inactivator of GABA aminotransferase should be capable of inactivating the PMP form of the enzyme. Class IV inactivators are compounds that inactivate only the PMP form of the enzyme (Table IV). 5-Fluoro-4-oxopentanoic acid (**32**) inactivates GABA aminotransferase by the mechanism shown in Scheme XI.[54] Note that this mechanism starts as the reverse of the mechanism in Scheme II until it reaches the same intermediate produced by the reaction of 4-amino-5-fluoropentanoic acid (**16**, Y = F, R = $CH_2CH_2COO^-$, Scheme VI) with the PLP form of the enzyme (compare **17** in Scheme VI with **33** in Scheme XI). It should be noted that in the literature[54] **32** is described as inactivating GABA aminotransferase by what we refer to as a Class I mechanism (Michael addition); however, this work was published prior to the work of Metzler and co-workers[31,32] on the Class II mechanism. Consequently, **32** undoubtedly inactivates GABA aminotransferase by an enamine mechanism rather than by a Michael addition mechanism.

To date, the only other inactivator of the PMP form of GABA aminotransferase that has been reported is 3,5-dioxocyclohexanecarboxylic acid (**34**).[55] However, this is not, strictly speaking, a mechanism-based inactivator of GABA aminotransferase because it does not involve the normal catalytic mechanism (Scheme XII); the deprotonation step could be nonenzymatic.

Summary

Because of the importance of the inactivation of GABA aminotransferase to the design of anticonvulsant agents, a seemingly wide variety of inactivators has been investigated; all of the compounds, however, are analogues of GABA, β-alanine, or δ-aminovaleric acid, which are substrates for the enzyme. Relatively minor modifications in the inactivator structures result in major differences in inactivation mechanisms and enzyme adduct structures. Compounds that inactivate GABA aminotransferase by a Michael addition mechanism, leading to modification of an active-site residue are Class I inactivators. Those that proceed by an enamine mechanism and give ternary adducts are Class II inactivators. Class III inactivators modify only the PLP cofactor; if the inactivation involves aromatization of the inactivator, it is a Class IIIA inactivation, and if no aromatization is involved, then it is a Class IIIB inactivation. The last class of inactivators (Class IV) are not classified on the basis of the mechanism, but, rather, that they require the enzyme to be in the PMP form.

There appears to be no trend in partition ratio values when comparing Class I with Class II inactivators. Class III inactivations alter only the cofactor, so it may be possible for these adducts to diffuse slowly out of the active site; reactivation of the apoenzyme would require additional PLP. These inactivators also inactivate a variety of other PLP-dependent enzymes.

At this point there does not seem to be a therapeutic advantage of one class of inactivators over another, although the only current example of these inactivators to be useful clinically is γ-vinyl GABA (vigabatrin), a Class I inactivator recently approved for the drug market in France and the U.K. There *is* a mechanistic significance, however, for one class over another. If labeling of an active-site amino acid residue is desired, then Class I inactivators should be selected; desire for attachment of the inactivator to both the protein and the cofactor or just to the cofactor would determine whether Class II or Class III inactivators would be chosen.

The classification presented here should allow us to think about inactivator structures in terms of their mechanistic potential and, as a result of this, should afford

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us the opportunity to be able to make predictions regarding inactivation mechanisms for hypothetical new structural classes of inactivators. Since the different mechanistic pathways lead to different types of enzyme adducts, inactivator design may be driven by the class of adduct that is desired. This mechanistic approach should be useful to the rational design of GABA aminotransferase inactivators and, therefore, of potential anticonvulsant drugs.

Acknowledgment. The authors are grateful to the National Institutes of Health (NS 15703) for support of the work described here.

Articles

Synthesis and Antitubercular Activity of *N*-(2-Naphthyl)glycine Hydrazide Analogues

B. Ramamurthy* and M. V. Bhatt

Department of Organic Chemistry and Microbiology & Cell Biology Laboratory, Indian Institute of Science, Bangalore 560012, India. *Received April 6, 1988*

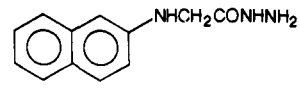
N-(2-Naphthyl)glycine hydrazide analogues were synthesized and tested for possible in vitro antitubercular activity. *N*-(2-Naphthyl)alanine hydrazide (**3**), *N*-methyl-*N*-(2-naphthyl)glycine hydrazide (**5**), *N*-(6-methoxy-2-naphthyl)glycine hydrazide (**7**), and 3-(2-naphthylamino)butyric acid hydrazide (**23**) showed potent inhibitory action against *Mycobacterium tuberculosis* $H_{37}R_v$ in Youman's medium at concentrations ranging from 0.5 to 10.0 μg/mL. These compounds showed significant inhibitory action against isonicotinic acid hydrazide and streptomycin-resistant strains of *M. tuberculosis*. *N*-(6-Quinolyl)glycine hydrazide (**18**) and 3-(2-quinolylamino)butyric acid hydrazide (**24**), which are bioisosteres of compounds **1** and **23**, showed loss of antitubercular activity at low concentrations.

Tuberculosis is generally regarded as the most important chronic communicable disease in the world. This disease continues to be one of the major health problems in India and other South Asian countries. The main reason for this has been the emerging resistance of *Mycobacterium tuberculosis*, the causative organism of this communicable disease, to currently available antitubercular drugs. The major problems associated with chemotherapy of tuberculosis are emergence of resistant strains, the variation of causative species, the failure of current antitubercular drugs to eradicate the mycobacterial infections quickly, and severe toxicity of certain available antitubercular drugs. Hence, many studies have been attempted in the past to develop new antitubercular compounds in order to resolve some of these problems.[1-10] Earlier, we found *N*-(2-naphthyl)glycine hydrazide (**1**) and its dihydrochloride to be potent in vitro inhibitors of *M. tuberculosis* $H_{37}R_v$ and the toxicity of compound **1** dihydrochloride was lower in animals compared to that of the primary antitubercular drug isonicotinic acid hydrazide.[7] Compound **1** dihydrochloride had certain favorable in vivo antitubercular activity.[11,12] Some of the favorable biological activities observed with compound **1** in our earlier studies[7,13] gave the impetus to synthesize new analogues of compound **1** and study their possible in vitro antitubercular activity. This paper describes the synthesis of *N*-(2-naphthyl)-glycine hydrazide analogues and their in vitro antitubercular activity.

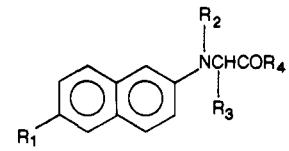
Chemistry

Hydrazides shown in Chart I were obtained by condensation of arylamines with ethyl haloacetate in aqueous medium followed by hydrazinolysis in ethanol as described in the Experimental Section.

Chart I

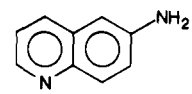


1



2: R_1 = H; R_2 = H; R_3 = CH_3; R_4 = OC_2H_5
3: R_1 = H; R_2 = H; R_3 = CH_3; R_4 = $NHNH_2$
4: R_1 = H; R_2 = CH_3; R_3 = H; R_4 = OC_2H_5
5: R_1 = H; R_2 = CH_3; R_3 = H; R_4 = $NHNH_2$
6: R_1 = OCH_3; R_2 = H; R_3 = H; R_4 = OC_2H_5
7: R_1 = OCH_3; R_2 = H; R_3 = H; R_4 = $NHNH_2$
8: R_1 = OCH_3; R_2 = H; R_3 = CH_3; R_4 = OC_2H_5
9: R_1 = OCH_3; R_2 = H; R_3 = CH_3; R_4 = $NHNH_2$
10: R_1 = H; R_2 = H; R_3 = H; R_4 = OH

Chart II



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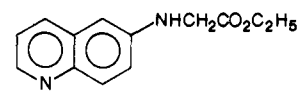

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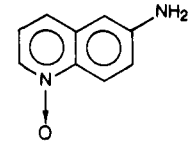

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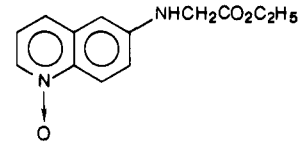

14

Reactions of various aromatic amines with ethyl haloacetate produced the corresponding condensed product

* Address correspondence to Dr. B. Ramamurthy, Bio-Organic Chemistry Laboratory, SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025.

(1) Sensi, P.; Maggi, N.; Furesz, S.; Maffii, G. *Antimicrob. Agents Chemother.* **1966**, 699.

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