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Inactivation of GABA transaminase by 3-chloro-1-(4-hydroxyphenyl)propan-1-one

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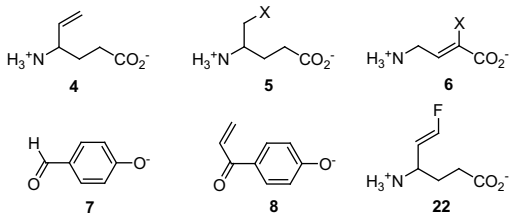
ABSTRACT

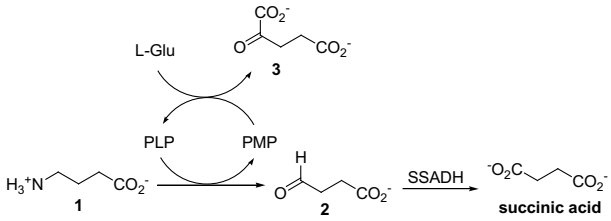
Previously it was found that 4-hydroxybenzaldehyde is a competitive inhibitor of GABA transaminase. Here 3-chloro-1-(4-hydroxyphenyl)propan-1-one (**9**), a 4-hydroxybenzaldehyde analogue, was found to inactivate potently the enzyme in a time-dependent manner. α-Ketoglutarate prevented the enzyme from inactivation, suggesting that the inactivation occurs in its active site. Several experiments indicated that the inactivation is irreversible. This study provides a novel strategy for the design of more effective inhibitors.

γ-Aminobutyric acid (GABA, **1**) is the major inhibitory neurotransmitter in the mammalian central nervous system.[1] The pathway for its degradation is via transamination with α-ketoglutarate (**3**) catalyzed by GABA transaminase (GABA-T) (Scheme 1).[2] GABA-T has been validated as a target for neuroactive drugs,[3,4] because its inhibition in brain tissues increases the concentration of GABA, and could have therapeutic applications in neurological disorders including epilepsy, Parkinson's disease, Huntington's chorea, and Alzheimer's disease.[5] Recently, it has been found that an increase in GABA also blocks the effects of drug addiction.[6] Vigabatrin,[7] a mechanism-based GABA-T inactivator, is already successfully utilized in the treatment of epilepsy.[8] So far most GABA-T inhibitors are analogues of GABA, including vigabatrin (**4**) analogues,[5,9,10] 4-amino-5-halopentanoic acid analogues (**5**),[11] and (Z)-4-amino-2-butenoic acid analogues (**6**).[12] Vigabatrin inactivates the enzyme by two pathways: a Michael addition mechanism (Scheme 2, pathway a) and an enamine mechanism (pathway b).[9]

Previously we found that 4-hydroxybenzaldehyde (**7**) inhibited GABA-T in a competitive manner with respect to α-ketoglutarate.[13] Consequently, we attempted to evaluate 4-hydroxybenzaldehyde analogues as GABA-T inhibitors, and then found that 4-acryloylphenol (**8**) inactivates GABA-T in a time-dependent manner.[14] 3-Chloro-1-(4-hydroxyphenyl)propan-1-one (**9**) was incidentally obtained in synthesis of **8** according to Scheme 2. Compound **9** was passingly examined for inhibition of GABA-T and was found to be a potent inhibitor (IC_{50} of **9** is 3.99 μM, but IC_{50} of **7** is 16.5 μM). As a part of an ongoing program, here we report inactivation of GABA-T by **9**.



Synthesis of **9** (Scheme 3) was achieved by a Fries rearrangement in 64% yield. GABA-T and succinic semialdehyde dehydroge-



Scheme 1. GABA shunt. PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; SSADH, succinic semialdehyde dehydrogenase; L-Glu, L-glutamic acid.

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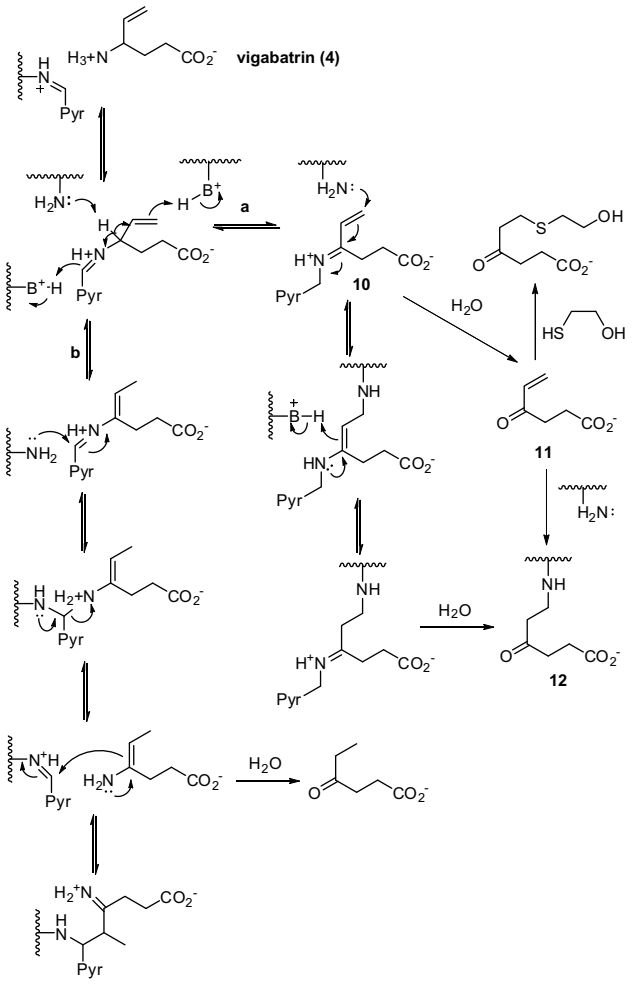
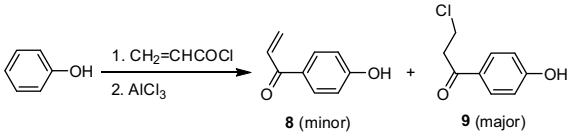


Scheme 2. Mechanism of inactivation of GABA-T by vigabatrin (**4**).



Scheme 3. Synthesis of **9**.

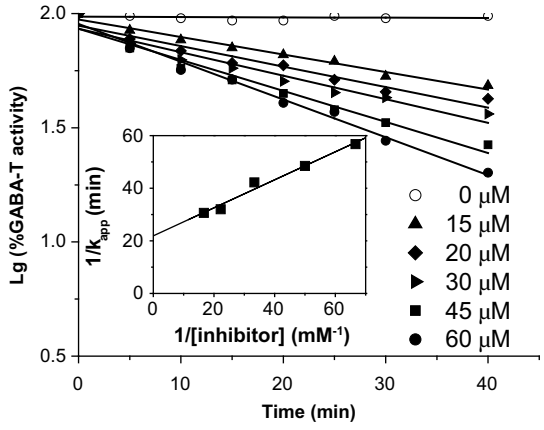


Figure 1. Time course of inactivation of GABA-T by **9** and its Kitz–Wilson plot.

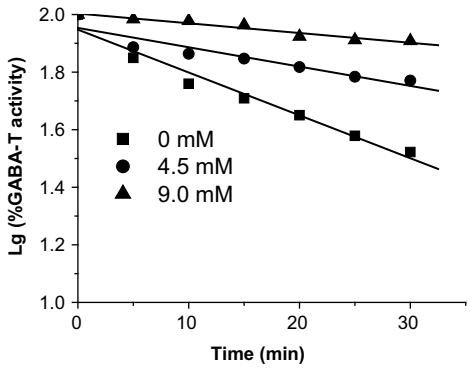


Figure 2. Effect of α-ketoglutarate on inactivation of GABA-T by **9**. The enzyme was simultaneously incubated with GABA, 45 μM **9** and varying concentrations of α-ketoglutarate for 40 min.

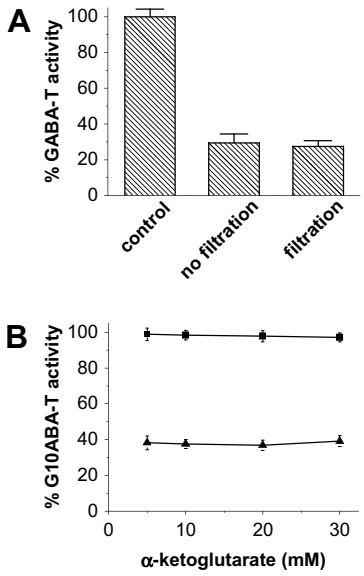
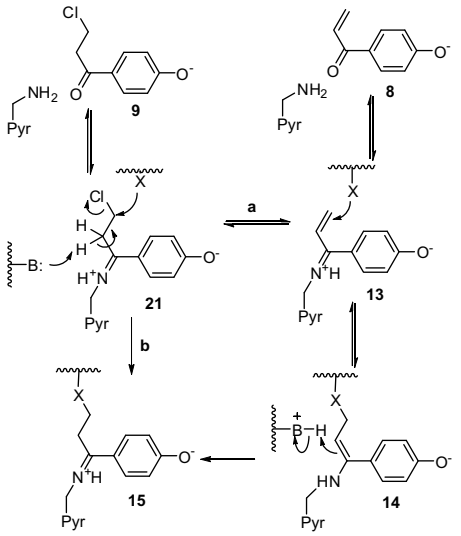


Figure 3. Compound **9** inhibits GABA-T in an irreversible manner. (A) GABA-T was incubated with 60 μM **9** at 25 °C. Aliquots were removed at 30 min and assayed for enzyme activity. Residual incubation mixture was applied to Sephadex G50 column to remove small molecules. An aliquot was removed and assayed for enzyme activity. An identical sample containing no inactivator served as the control. Gel filtration did not result in recovery of enzyme activity. The results ($n = 6$) are the mean ± SEM of assays performed. (B) GABA-T was incubated with **9** (30 μM; filled triangle) for 40 min followed by addition of increasing concentrations of α-ketoglutarate. Control (no inactivator) is denoted by the filled square. The results ($n = 6$) are the mean ± SEM of assays performed.

nase (SSADH) were isolated from rat brains by a modified procedure,[15,16] respectively. Time-dependent inactivation of GABA-T by **9** was carried out using the previous procedure.[14] The logarithm of remaining activity was plotted against time for each inactivator concentration; then the inverse slopes from these plots were plotted against the inverse of the concentration. K_I and k_{inact} values were determined using a Kitz and Wilson plot.

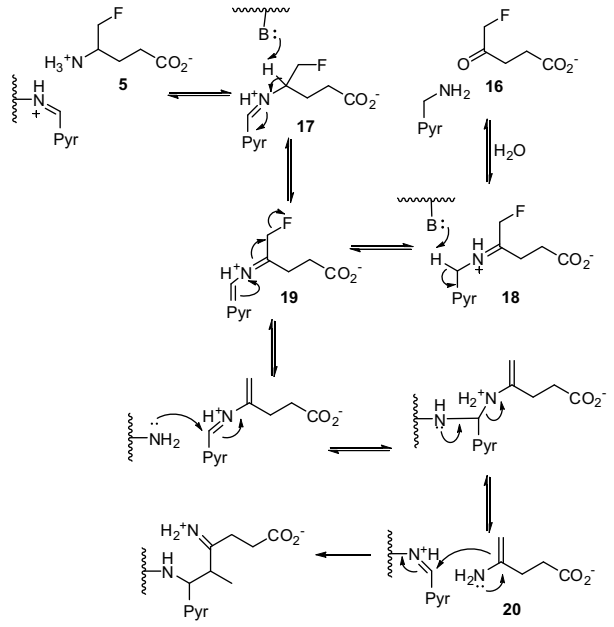
Compound **9** inactivated GABA-T in both a time- and concentration-dependent manner (Fig. 1). The inactivation follows a pseudo-first kinetic behavior. A reciprocal plot of the pseudo-first-order constants, taken from the slopes of the lines in Figure 1, gives a straight line ($K_I = 24.3 \pm 3.5$ μM, $k_{inact} = 0.0457 \pm 0.0052$ min^{-1}, and $k_{inact}/K_I = 1.88 \pm 0.23$ mM^{-1} min^{-1}). As with **8**, the K_I value is very low, indicating that the phenol moiety has even a stronger affinity for the active site of the enzyme than the carboxylic acid of its substrate.



Scheme 4. Potential mechanism of inactivation of GABA-T by **8** and **9**.

Inactivation by **9** was prevented by addition of α-ketoglutarate to the incubation (Fig. 2), indicating that the inactivation occurs at the active site and **9** is bound to the pyridoxamine 5′-phosphate (PMP) form of the coenzyme to produce a Schiff base. Most of published GABA-T inactivators are GABA analogues and thus are bound to the pyridoxal 5′-phosphate (PLP) form of the coenzyme. Like **7** and **8**, however, **9** is a α-ketoglutarate analogue.

Because of the surprising inhibition of GABA-T by **9**, the inhibition type was studied further via three methods. As shown in Figure 3A, incubation of GABA-T with **9** for 30 min followed by removal of excess inactivator using gel filtration did not result in recovery of enzyme activity. No activity was also restored when enzyme that had been inactivated by **9** was dialyzed at 4 °C (data not shown). Moreover, GABA-T was exposed to **9** for 40 min followed by addition of increasing concentrations of α-ketoglutarate. Even at 30 mM α-ketoglutarate, no increase in activity was observed (Fig. 3B). These observations indicated that the adduct formed between GABA-T and **9** is stable and the inactivation is an irreversible process.



Scheme 5. Mechanism of inactivation of GABA-T by **5** and **16**.

Two published mechanism including a Michael addition and an enamine mechanism cannot elucidate the present inactivation. We previously speculated that inactivation of GABA-T by **8** accords with a Michael addition mechanism (Scheme 4).[14] Compound **9** is not a Michael acceptor, so the inactivation cannot be described by a Michael addition mechanism. On the other hand, 5-fluoro-4-oxopentanoic acid (**16**)[17] inactivates only the PMP form of GABA-T by the mechanism shown in Scheme 5. Compound **16** initially was described as inactivating GABA-T by Michael addition mechanism; however, this work was published prior to the report on the enamine mechanism. In fact, this mechanism starts as Schiff base formation with PMP until it reaches the same intermediate **19** produced by the reaction of 4-amino-5-fluoropentanoic acid (**5**) with the PLP form of GABA-T. Consequently, **16** undoubtedly inactivates the enzyme by an enamine mechanism rather than a Michael addition mechanism.[18] These findings suggest that the inactivation via an enamine mechanism requires introduction of a halogen atom to the α-position of the carbonyl group or the β-position of the amino group; in other words, the β-position of the amino group for **5** is equal to the α-position of the carbonyl group for **16**. By careful comparison between **9** and **16**, it can be found that the halogen atom is introduced to the β-position of carbonyl group for **9**, differing from α-position for **16**. Therefore, an enamine mechanism cannot account for the inactivation by **9**.

Two mechanisms were proposed to rationalize the inactivation by **9** (Scheme 4). An active-site nucleophile may react directly with **9** via an SN2 mechanism, accounting for the inactivation. When the electropositive carbon atom adequately approaches to an active-site nucleophile of GABA-T, an SN2 reaction occurs (Scheme 4). Silverman et al. have reported that an SN2 pathway is one of the inactivation mechanisms of **22**,[19] suggesting that an SN2 reaction of an active-site nucleophile for GABA-T with halide may take place. Alternatively, proton abstraction from the α-carbon and subsequent chloride elimination may result in transient formation of a Michael acceptor (**13**) which then irreversibly reacts with an active-site nucleophile. While participation of a Michael acceptor in the inactivation process has not been demonstrated, there is some evidence of other enzymes to support a β-elimination-Michael addition mechanism, such as inactivation of HMG-CoA synthase by β-chloropropionyl CoA.[20] Thus, the above two hypotheses are realistic possibilities. The distinction between an SN2 mechanism and β-elimination-Michael addition mechanism must await the results of future investigation. In contrast to **9**, **7** is a reversible inhibitor and **8** inactivates via a Michael addition pathway,[14] suggesting that modifications in the inactivator structures can lead to major difference in inhibitory mechanisms.

Regardless of the identity of the actual inactivating agent, there is no doubt that our previous and present results may also give a clue to the design of a novel class of GABA-T inhibitors inactivating only the PMP form of the enzyme. GABA analogues could potentially inhibit PLP-dependent glutamate decarboxylase (GAD) and thus inhibit synthesis of GABA. Gabaculine, for example, an irreversible inactivator of GABA-T,[21] inactivates GAD potently.[22] Therefore, analogues of succinic semialdehyde (**2**) are useful as selective inactivators of GABA-T. So far, however, only two analogues of succinic semialdehyde, 3,5-dioxocyclohexane-carboxylic acid[23] and **16**,[17] were found to inactivate its PMP form.

Introduction of phenol group may increase lipophilicity of drug candidates, which is extremely important to central nervous system drugs. Phenol moiety is very useful to design GABA-T inhibitors that could be more effective at crossing the blood–brain barrier. Moreover, design of conformationally rigid analogues of vigabatrin has received significant attention in recent years, because vigabatrin has been found to lead to irreversible visual

field defects[24] and is catalyzed by GABA-T to produce a potent electrophile.[9] The presence of the phenol moiety would undoubtedly increase the conformational rigidity of its inhibitors.

In summary, **9** inactivated potently GABA-T in a time-dependent and irreversible manner. The inactivation was protected by α-ketoglutarate, indicating that it is active site-directed. This study provides a novel strategy for design of more effective GABA-T inhibitors.

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