Letters

Design, Synthesis, and Biological Activity of a Difluoro-Substituted, Conformationally Rigid Vigabatrin Analogue as a Potent γ-Aminobutyric Acid Aminotransferase Inhibitor

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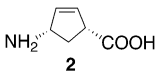
Abstract: Previously it was found that a conformationally rigid analogue (**2**) of the epilepsy drug vigabatrin (**1**) did not inactivate γ-aminobutyric acid aminotransferase (GABA-AT). A cyclic compound with an exocyclic double bond (**6**) was synthesized and was found to inactivate GABA-AT, but only in the absence of 2-mercaptoethanol. The corresponding difluoro-substituted analogue (**14**) was synthesized and was shown to be a very potent time-dependent inhibitor, even in the presence of 2-mercaptoethanol.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system.[1] When the level of GABA in the brain falls below a threshold level, convulsions occur.[2] Compounds that inhibit γ-aminobutyric acid aminotransferase (GABA-AT), the enzyme that degrades GABA, exhibit anticonvulsant activity. One of the most effective inactivators of GABA-AT is the antiepilepsy drug vigabatrin (**1**),[3] which inactivates the enzyme by two pathways: a Michael addition mechanism (Scheme 1, pathway a) and an enamine mechanism (pathway b).[4]

Previously, **2**, a conformationally rigid vigabatrin analogue, was synthesized.[5] Surprisingly, **2** was not a GABA-AT inactivator but was a very good substrate



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with a specificity constant almost five times greater than that of GABA. It was later determined by computer modeling[6] that the endocyclic double bond is not in the right orientation for Michael addition (pathway a, Scheme 1), nor is it an effective enamine for enzyme inactivation. Therefore **6**, which has an exocyclic double bond, was designed. The synthesis (Scheme 2) started from **3**.[7] An addition reaction with (trimethylsilyl)-methylmagnesium chloride followed by elimination furnished **5**. Deprotection of the benzyl group and hydrolysis of the lactam gave the amino acid **6**.

Interestingly, inactivation of GABA-AT was observed with **6**, but when 2-mercaptoethanol was added to the incubation mixture, no inactivation occurred (data not shown). A possible mechanism accounting for this phenomenon is shown in Scheme 3. It is likely that **6** is only a substrate for GABA-AT. After formation of **7**, the double bond is not reactive enough, so this intermediate is not trapped by the enzyme, but rather is released from the active site in the form of an α,β-unsaturated ketone (**8**). In the presence of 2-mercaptoethanol, a reactive nucleophile, **8** is trapped to form **9**, giving no inactivation of the enzyme. In the absence of 2-mercaptoethanol, however, **8** may return to the active site of the enzyme and become covalently attached to the enzyme (**10**), leading to the enzyme's inactivation. According to the definition of mechanism-based enzyme inactivators,[8] **6** is not a mechanism-based inactivator because inactivation does not occur prior to the release of the active species from the active site.

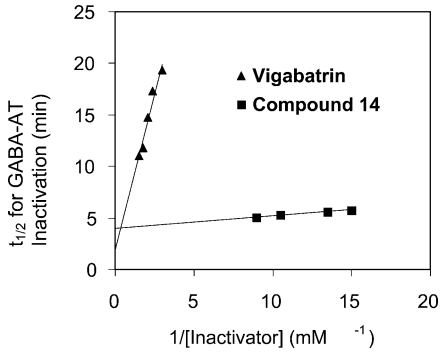

Figure 1. Comparison of the activities of vigabatrin and **14** with GABA-AT at pH 6.5, 25 °C.

Because **6** did not inactivate GABA-AT, we designed the corresponding, more reactive, difluoro-substituted analogue **14**. As a result of fluorine's similar size to hydrogen and its high electronegativity, a much more reactive intermediate than **7** is expected. Prior to its release, this species may be sufficiently reactive to become covalently attached to GABA-AT, leading to its inactivation.

The synthesis of **14** (Scheme 4) started from **11**.[7,9] A Horner−Wadsworth−Emmons reaction with diethyl (difluoromethyl)phosphonate inserted the 1,1-difluoromethylene moiety in **12**, which then underwent deprotection with ceric ammonium nitrate to give the lactam **13**. Hydrolysis of **13** gave **14**.

Compound **14** was found to be a very potent GABA-AT inactivator, even in the presence of 2 mM 2-mercaptoethanol (Figure 1). Because of its high potency, k_{inact} and K_I values could not be determined accurately under optimal conditions (pH 8.5, 25 °C), where the enzyme exhibits maximum activity. When the concentration of **14** was comparable to that of the enzyme, the k_{inact}/K_I value for **14** was determined to be 186 times greater than that for (*S*)-vigabatrin! To obtain more accurate data, **14** and vigabatrin were compared under nonoptimal conditions. Even at 0 °C in pH 8.5 buffer no pseudo first-order kinetics were observed for **14**. At

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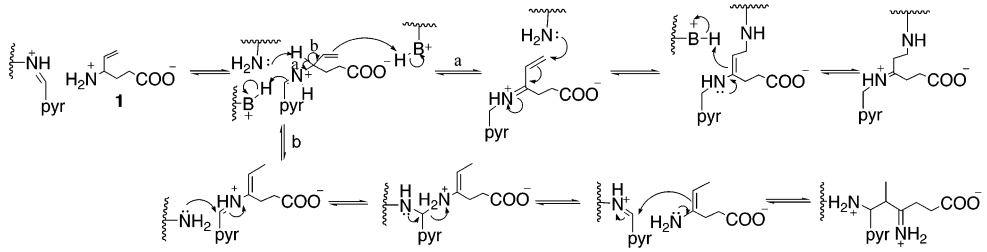
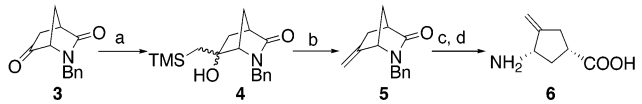
Scheme 1

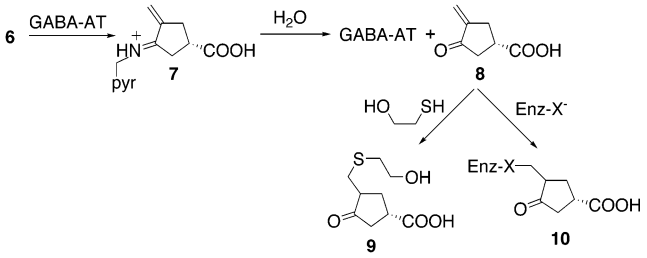


Scheme 2[a]

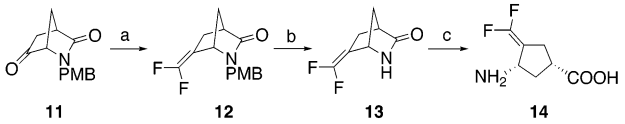


[a] Reagents and conditions: (a) $TMSCH_2MgCl$, −30 °C to RT, 38%; (b) $(CF_3CO)_2O$, DMAP, then TBABr, KF, 86%; (c) Na/NH_3/^{t}BuOH; (d) 4N HCl, 90%, two steps.

Scheme 3



Scheme 4[a]



[a] Reagents and conditions: (a) $CF_2HPO(OEt)_2$, ^{t}BuLi, 68%; (b) CAN, 68%; (c) 4 N HCl, 72%.

pH 6.5 (25 °C) **14** (K_I 31 ± 3 μM, k_{inact} 0.18 ± 0.02 min^{-1}, k_{inact}/K_I 5.7 ± 0.9 $mM^{-1}min^{-1}$) is 52 times more potent than (*S*)-vigabatrin (K_I 3.2 ± 1.0 mM, k_{inact} 0.37 ± 0.11 min^{-1}, k_{inact}/K_I 0.11 ± 0.03 $mM^{-1}min^{-1}$). The rigid conformation of **14**, which minimizes the entropic penalty on binding, probably contributes to its potency. The two fluorine atoms also make the possible intermediate highly reactive toward trapping by the enzyme. The partition ratio for **14** was determined to be 1.21 using α-keto[^{14}C]glutarate, which indicates that it takes 2.21 equiv of **14** to inactivate 1 equiv of the enzyme. The reason may be because the orientation of the double bond is the same as in **6** and therefore not optimal for trapping by the enzyme. Currently the inactivation mechanism is under investigation, which should lead to further optimization of **14**.

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Supporting Information Available: Experimental details and spectroscopic data for compounds **4**−**6** and **12**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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