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Structural modifications of (1*S*,3*S*)-3-amino-4-difluoromethylene-cyclopentanecarboxylic acid, a potent irreversible inhibitor of GABA aminotransferase

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Abstract—Low brain levels of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) lead to convulsions. Inhibition of GABA aminotransferase increases the concentration of GABA and can terminate the convulsions. Earlier we reported the synthesis of (1*S*,3*S*)-3-amino-4-difluoromethylenecyclopentanecarboxylic acid (**2**), which is 186 times more potent an inactivator of GABA aminotransferase than the epilepsy drug *S*-vigabatrin. The corresponding dichloromethylene analogue of **2** (compound **3**) has been made, but it shows only weak reversible inhibition of GABA aminotransferase. However, the tetrazole isostere of **2** (compound **4**) has been found to be a time-dependent inactivator of GABA aminotransferase. Although it is 20 times less potent than carboxylic acid **2**, it is 2.5 times more potent than *S*-vigabatrin. A calculation of the Clog *P* values indicates that **4** is the most lipophilic of the three, being 69 times more lipophilic than **2** and 55 times more lipophilic than *S*-vigabatrin, indicating potential for improved bioavailability.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). Low GABA levels are related to a series of neurological disorders including Parkinson's disease,[1] Huntington's chorea,[2,3] Alzheimer's disease,[4] and epilepsy.[5–7] γ-Aminobutyric acid aminotransferase (GABA-AT, E.C. 2.6.1.19), a pyridoxal 5'-phosphate (PLP)-dependent enzyme that degrades GABA, is a target for antiepilepsy drugs. Inhibition of GABA-AT results in an increase in the concentration of brain GABA and has an anticonvulsant effect. Inhibitors of GABA-AT have been developed as potential anticonvulsant agents. One of these, 4-amino-5-hexenoic acid (vigabatrin or Sabril®, **1**, Fig. 1), is a potent inactivator of GABA-AT[8] that has been marketed as an anticonvulsant drug,[9] although it may cause certain vision-related side effects after long-term use.[10–13]

Analogues of vigabatrin have been developed as drug candidates.[14] One approach to optimize the antiepilepsy drug vigabatrin is to rigidify its structure by formation of a five- or six-membered ring.[15–18] Recently, we synthesized (1*S*,3*S*)-3-amino-4-difluoromethylenecyclopentanecarboxylic acid (**2**, Fig. 1), a difluoro-substituted, conformationally rigid vigabatrin analogue,[18] and it was found to be one of the most potent inactivators of GABA-AT; it is approximately 186 times more potent than *S*-vigabatrin in vitro. The simple structure and superior potency of **2** make it a desirable lead compound for further modification.

The rigid conformation of **2** minimizes the entropic penalty on binding and, therefore, could contribute to its potency; however, the same structure with hydrogen in place of the fluorines binds much less well.[18] To obtain more structural information, we designed an analogue of **2** with a dichloromethylene moiety (**3**, Fig. 1), which can be used to explore the importance of the size of the fluorine atoms in the interaction between the enzyme and the inactivator as well as the importance of the lipophilicity of those groups.

Despite its high in vitro activity, the relatively low lipophilicity of **2** caused by its carboxylic acid and ammonium groups may limit its permeability to the blood-brain

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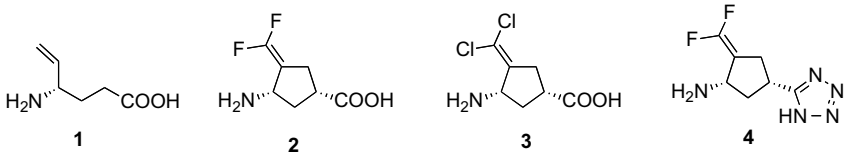


Figure 1. Modifications of **2**.

barrier (BBB), and therefore, may result in lower in vivo potency.[19,20] The BBB permeability of a compound, affected by its lipophilicity, hydrogen-bond desolvation potential, pK_a/charge, and molecular size,[21–26] is an important concern in drug delivery. Generally, uncharged, lipophilic compounds with low molecular weights are expected to cross the BBB and provide higher bioavailabilities.[27] To increase the lipophilicity of the molecule and to achieve better in vivo activity, **4**, a tetrazole analogue of **2**, also was designed. The tetrazole group is a well-known bioisostere of a carboxylic acid with higher lipophilicity.[28] In addition, our previous studies showed that the tetrazole analogue of vigabatrin is a potent inactivator of GABA-AT, which indicates that tetrazole may be a good replacement for carboxylic acid group in GABA analogues.[29] In this note we report the synthesis and the results of enzymatic studies of **3** and **4**.

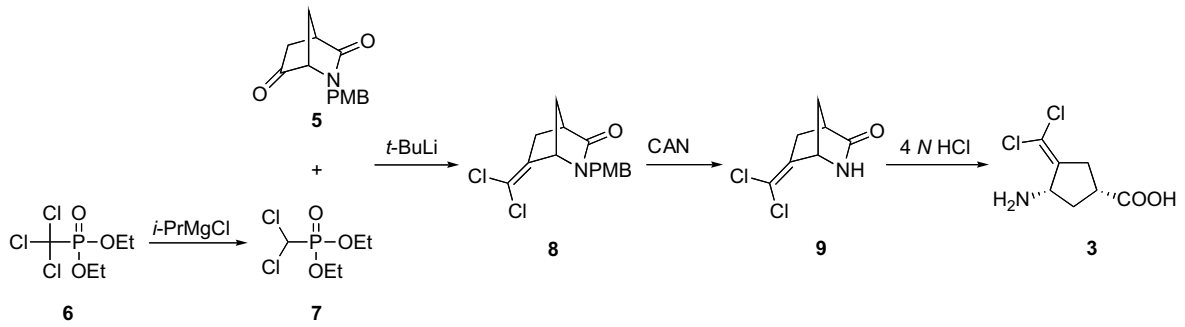
Compound **3** was synthesized from **5** (Scheme 1). The previously synthesized intermediate **5**[16,30] was treated with *tert*-butyl lithium and **7**, which was synthesized from **6**,[31] to give **8**. Deprotection of the PMB group with ceric ammonium nitrate afforded lactam **9**; hydrolysis of **9** with hydrochloric acid gave **3** in the form of a hydrochloride salt.

Compound **4** was synthesized from **2** as shown in Scheme 2. The amino group in **2** was protected with phthalic anhydride to give **10**. The carboxylic acid group in **10** was converted to amide **11**, which underwent dehydration to give nitrile **12**. The method of Amantini and coworkers[32] was used to obtain tetrazole **13**. Treatment of **13** with 6 N hydrochloric acid deprotected the phthalic group and provided **4**.

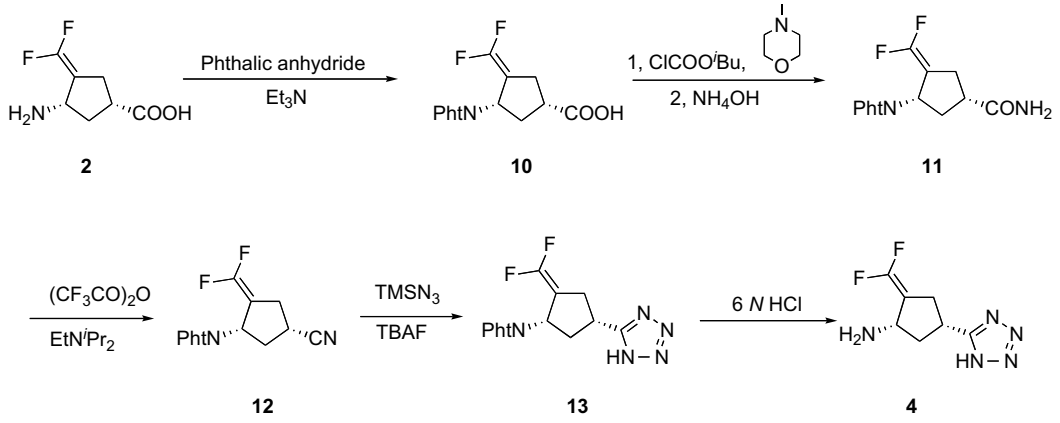
Compound **3** was tested as a time-dependent inhibitor of GABA-AT. GABA-AT, however, showed no significant decrease in activity after up to 74 min of incubation with 1.7 mM concentration of **3**, which indicates that **3** is not a potent inactivator. In addition, it was shown that the enzyme retains approximately 58% of its activity in the presence of 10 mM of **3**, which suggests that the IC_{50} value for **3** is greater than 10 mM, and **3** is only a weak reversible inhibitor of GABA-AT. The decrease in activity of **3** compared with the corresponding difluoro compound **2** is quite dramatic and indicates that the chlorine atoms in **3** might be too bulky for the enzyme active site.

Compound **4** showed time- and concentration-dependent inhibition of GABA-AT. The kinetic constants for **4** at pH 8.5, the optimal pH for the enzyme, were determined using a Kitz and Wilson replot (Fig. 2).[33] The k_{inact} and K_I values, however, could not be determined accurately because the intercepts were very close to the origin. Instead, the k_{inact}/K_I value, which is in proportion to the reciprocal of the slope of the plot, was calculated to be 2.48 min^{-1} mM^{-1}. At pH 6.5, the k_{inact} and K_I values for **4** were determined to be 1.05 min^{-1} and 3.75 mM, respectively, and the k_{inact}/K_I value was calculated to be 0.28 min^{-1} mM^{-1}. According to the k_{inact}/K_I values, **4** is less potent than its parent compound, **2** (k_{inact}/K_I = 5.7 min^{-1} mM^{-1}, pH 6.5),[18] but it is a better inactivator of GABA-AT than *S*-vigabatrin (k_{inact}/K_I = 1.7 min^{-1} mM^{-1} at pH 8.5, 0.11 min^{-1} mM^{-1} at pH 6.5).

Despite the decrease in activity, the replacement of a carboxylic group with a tetrazole increases the lipophilicity of the molecule and may potentially improve the in vivo bioavailability.[34] The Clog *P* values for **2**, **4**, and vigabatrin were calculated to give an estimate of their lipophilicity. The Clog *P* for **4** (−0.48) indicated that it is approximately 69 times more lipophilic than **2** (−2.32) and 55 times more lipophilic than vigabatrin (−2.22). The considerably increased lipophilicity may



Scheme 1. Synthesis of **3**.



Scheme 2. Synthesis of **4**.

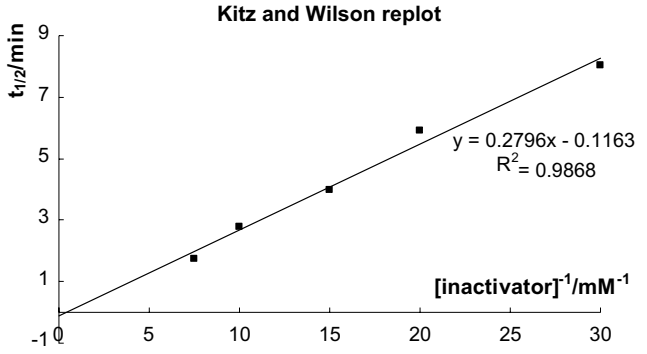


Figure 2. Kitz and Wilson replot for **4** (pH 8.5, 25 °C).

result in improved permeability of the blood-brain barrier, and therefore, may compensate for the decrease in in vitro activity.

In summary, two analogues of **2**, a potent inactivator of GABA-AT, were synthesized. Dichloro analogue **3** is only a weak reversible inhibitor of GABA-AT, which, presumably, is the result of the larger size of the chlorine atoms. Tetrazole analogue **4** was shown to be a time- and concentration-dependent inhibitor of GABA-AT. It is less potent than parent compound **2**, although its in vitro potency is higher than that for the antiepilepsy drug vigabatrin (**1**). The increased lipophilicity of **4** relative to **2** and to vigabatrin makes it desirable.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.119.

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