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Synthesis and evaluation of novel aromatic substrates and competitive inhibitors of GABA aminotransferase

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Abstract—The design, synthesis, and evaluation of novel γ-aminobutyric acid aminotransferase (GABA-AT) inhibitors and inactivators can lead to the discovery of new GABA-related therapeutics. To this end, a series of aromatic amino acid compounds was synthesized to aid in the design of new inhibitors and inactivators of GABA-AT. All compounds were tested as competitive inhibitors of GABA-AT. The amino acids with benzylic amines were also tested as substrates for GABA-AT. It was found that these compounds were all poor competitive inhibitors of GABA-AT, but some were substrates of the enzyme, suggesting their utility as scaffolds for potential GABA-AT mechanism-based inactivators. Computer modeling was used to rationalize the substrate activity of the various compounds.

γ-Aminobutyric acid aminotransferase (GABA-AT) catalyzes the degradation of γ-aminobutyric acid (GABA) to succinic semialdehyde (SSA). Control of GABA levels in the body has numerous therapeutic benefits. Depleted levels of GABA, a major inhibitory neurotransmitter,[1] have been shown to cause convulsions.[2] Raising GABA levels in the brain has an anticonvulsant effect;[3–5] however, direct GABA administration is not effective, as GABA cannot cross the blood brain barrier.[6] Disruption of GABA levels has also been implicated in numerous neurological disorders, such as Alzheimer's disease,[7] Parkinson's disease,[8] Huntington's disease,[9] and senile dementia.[10] Numerous strategies exist to elevate GABA levels in the brain. The strategy that we have taken involves inhibition or inactivation of GABA-AT, the enzyme that is responsible for the degradation of GABA into an inactive form.

To enhance the lipophilicity of GABA analogues for more favorable bioavailability, a series of aromatic compounds, inspired by the anticonvulsant drug vigabatrin (**1**) and by **2**, a potent conformationally restricted analogue of **1**[11] (Fig. 1), was designed, synthesized, and evaluated. The results of these studies will aid in the design of future GABA-AT inhibitors and inactivators. Compound **2** is a potent irreversible inactivator of GABA-AT, showing a potency that is 186 times greater than that of **1.**

The syntheses of the three 1,3-disubstituted aromatic amino acids (**8**, **10**, and **14**), that were not commercially available, are outlined in Scheme 1.

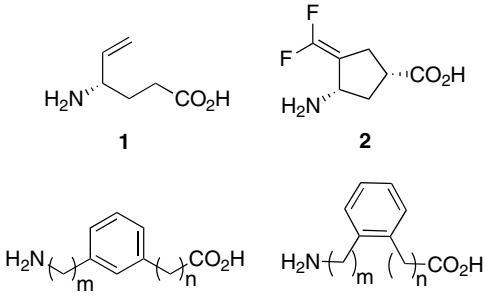

Figure 1. Previously described GABA-AT inactivators (**1** and **2**) and the new series of aromatic analogues investigated ($m = 0, 1$ and $n = 0, 1$).

Keywords: γ-Aminobutyric acid; γ-Aminobutyric acid aminotransferase; GABA-AT; Substrate of GABA-AT; Inhibitor; Computer modeling; Aromatic amino acid.
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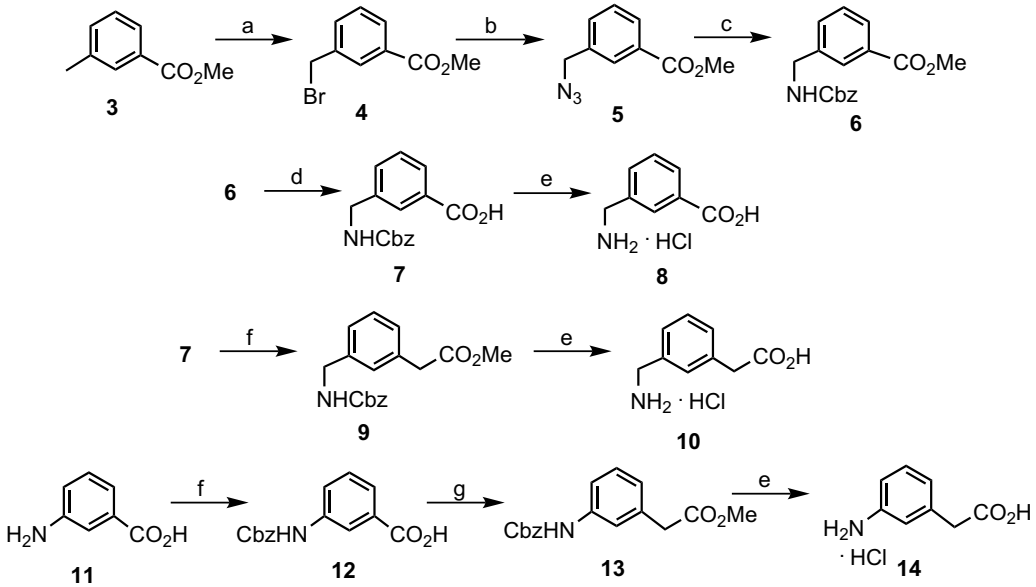
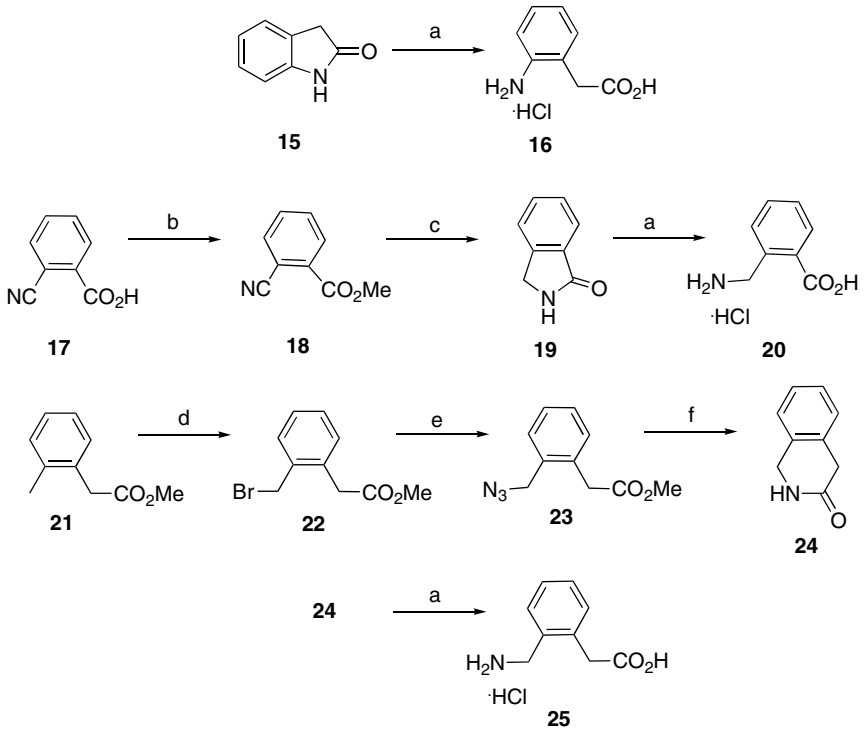


Scheme 1. Synthesis of the 1,3-disubstituted aromatic amino acids. Reagents and conditions: (a) NBS, *hv*, MeCN; (b) NaN_3, EtOH, reflux; (c) i—H_2, Pd–C, MeOH; ii—CbzCl, TEA, THF, 0 °C to rt; (d) NaOH, MeOH; (e) 6 M HCl, reflux; (f) CbzCl, H_2O, Na_2CO_3, 0 °C to rt; (g) i—$SOCl_2$, reflux; ii—CH_2N_2, TEA, ether, 0 °C; iii—cat. AgOBz, TEA, MeOH.

The commercially unavailable 1,2-disubstituted aromatic amino acids (**16**, **20**, and **25**) were synthesized as shown in Scheme 2. The chemistry applied to the synthesis of intermediate **6** proved applicable to the synthesis of amino acid **25**, but different synthetic strategies were required in the cases of **16** and **20**.

All of the compounds were tested as competitive inhibitors of GABA-AT using a coupled enzyme assay,[12] except for **16**. Compound **16** underwent cyclization to **15** under the conditions of the assays and was, therefore, omitted from testing. It was found that **8**, **10**, **11**, **14**, **20**, **25**, and **26** (Fig. 2) all showed IC_{50} values that ex-



Scheme 2. Synthesis of the 1,2-disubstituted aromatic amino acids. Reagents and conditions: (a) 6 M HCl, reflux; (b) MeI, $NaHCO_3$, DMF; (c) H_2, Pd–C, AcOH/MeOH (1:1), then TEA, MeOH; (d) NBS, *hv*, MeCN; (e) NaN_3, EtOH, reflux; (f) H_2, Pd–C, MeOH, then TEA, MeOH.

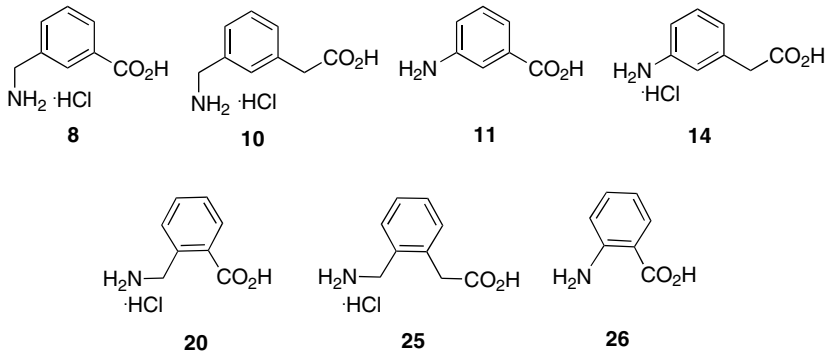


Figure 2. Amino acids that were tested as inhibitors and substrates of GABA-AT.

Table 1. Substrate activities of compounds shown in Fig. 2 relative to GABA

Compound	Relative substrate activity (%)
GABA	100.00
8	3.42 (±0.82)
10	0.78 (±0.06)
11	0.00
14	0.00
20	0.00[a]
25	5.65 (± 0.86)
26	0.00

Numbers represent relative rates of turnover. Standard deviations are also shown.

[a] Transamination was observed, but it was found to be insignificant.

ceeded 5.0 mM concentrations. Because of the relatively poor potency of these compounds, the exact IC_{50} values were not determined.

All of the compounds were subsequently tested as substrates for GABA-AT. The substrate activities (relative to the rate of GABA turnover) for all of the compounds are shown in Table 1. The fact that **11**, **14**, and **26** showed no transamination was anticipated, as these compounds lack protons alpha to the amino group and, therefore, cannot undergo oxidation at the necessary position. It is clear that the two best substrates are **8** and **25**. This most likely results from their similarity to the structure of bound GABA in terms of the relative positions of the acid and amine functionalities. The automated flexible docking program FlexX was employed to perform docking calculations for **8**, **10**, **20**, and **25** using the crystallographic structure of the homodimers of pig liver GABA-AT in complex with vigabatrin **1** (PDB code: 1OHW).[13] The FlexX docking models show that none of carboxylic groups of these compounds can exactly mimic the binding mode of the carboxylic group of vigabatrin, as illustrated in Fig. 3. However, compared with **10** and **20**, the carboxylic groups of **8** and **25** are more similar to that of bound vigabatrin. The poor turnover of **10** may result from the fact that there are too many carbon atoms between the polar groups and, therefore, the carboxylic acid group of **10** cannot be accommodated by the active site of GABA-AT (Fig. 3a).

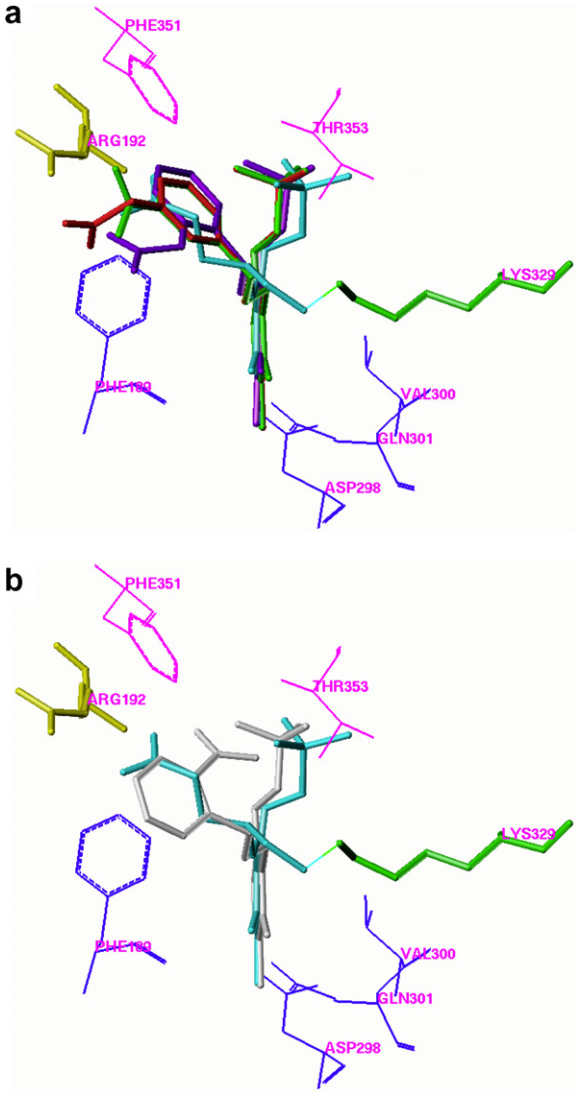


Figure 3. (a) The superimposition of the FlexX docking conformations of **8**, **10**, and **25** and the binding conformation of vigabatrin from the crystallographic structure (PDB code: 1OHW). The **8**–PLP adduct, the **10**–PLP adduct, and the **25**–PLP adduct are shown in green, red, and purple, respectively. (b) The superimposition of the FlexX docking conformations of **20** and the binding conformation of vigabatrin from the crystallographic structure (PDB code: 1OHW). Lys329 is shown in green. Phe351 and Thr353, which are from the other monomer of the homodimer, are shown in magenta. All of the hydrogen atoms were omitted for clarity. Vigabatrin and PLP from the crystal structure are shown in cyan. The **20**–PLP adduct is shown in gray.

Compound **20** has the same chain length as GABA so it is surprising that it lacks substrate proficiency. Docking analysis indicates that the rigidity of the aromatic ring prevents the carboxylate from engaging in a critical salt bridge interaction with Arg-129[13] present in the active site (Fig. 3b). This could explain why **25** undergoes transamination and **20** does not; the additional methylene unit in **25** allows the carboxylate to assume an appropriate conformation that facilitates binding by GABA-AT and subsequent turnover.[14]

The results described here suggest that the aromatic scaffold of these GABA analogues may be useful in the design of potential mechanism-based inactivators of GABA-AT having increased lipophilicity. It appears that compounds with the general structures related to **8** and **25** show the most promise for further investigations.

Acknowledgements

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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.2007.10.060.

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14. The 3D coordinates of the active site of GABA-AT (PDB code: 1OHW) in complex with the docking ligands (the **8**–PLP adduct, the **10**–PLP adduct, the **20**–PLP adduct, and the **25**–PLP adduct) and the crystallographic vigabatrin–PLP adduct are accessible from the supporting information.