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New substrates and inhibitors of γ-aminobutyric acid aminotransferase containing bioisosteres of the carboxylic acid group: Design, synthesis, and biological activity

Hai Yuan and Richard B. Silverman*

Department of Chemistry, Department of Biochemistry, Molecular Biology, and Cell Biology, and the Center for Drug Discovery and Chemical Biology, Northwestern University, Evanston, IL 60208-3113, USA

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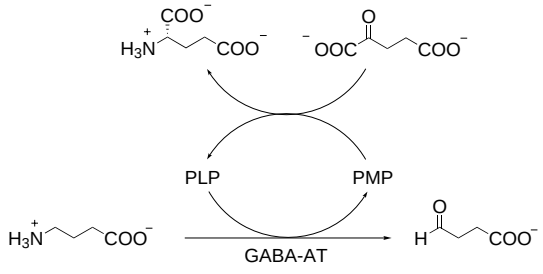
Abstract—A series of potential substrates of γ-aminobutyric acid aminotransferase (GABA-AT) with lipophilic bioisosteres of the carboxylic acid group (**2**–**7**) were synthesized and tested. Most of the synthesized compounds showed substrate activities with GABA-AT; 1*H*-tetrazole-5-propanamine (**6**) was the best of those tested. The potential time-dependent inhibitor of GABA-AT, 1*H*-tetrazole-5-(α-vinyl-propanamine) (**8**), was designed based on the structures of **6** and the antiepilepsy drug vigabatrin (4-amino-hex-5-enoic acid, **1**). The synthesized compound **8** showed time-dependent inhibition of GABA-AT, but its potency is lower than that of vigabatrin. Methylation of the tetrazole group in **8** resulted in loss of time-dependent activity, suggesting that the tetrazole ring, the carboxylate bioisostere, exists in its deprotonated form in the enzyme active site.

1. Introduction

γ-Aminobutyric acid aminotransferase[1] (GABA-AT) is a pyridoxal-5′-phosphate (PLP)-dependent enzyme that degrades the major inhibitory neurotransmitter γ-aminobutyric acid[2] (GABA) in the central nervous system (CNS, Scheme 1). GABA is important to several neurological disorders, including Parkinson's disease,[3] Huntington's chorea,[4] Alzheimer's disease,[5] and epilepsy,[6] a central nervous system disease characterized by recurring convulsive seizures. A deficiency of GABA in the brain has been implicated as one cause for convulsions.[7] In an effort to raise the concentration of GABA in the brain, both direct injection and oral administration of GABA have been studied. It was shown that injection of GABA into the brain has an anticonvulsant effect, but it is obviously not a practical method.[8] Taking GABA orally, however, is not effective because GABA cannot cross the blood–brain barrier, a membrane protecting the CNS from xenobiotics in the blood.[9]

To correct the deficiency of brain GABA and therefore stop convulsions, an important approach is to use an inhibitor of GABA-AT that is able to cross blood–brain barrier.[10] Inhibition of this enzyme increases the concentration of GABA in the brain and could have therapeutic applications in epilepsy as well as other neurological disorders. One of the most effective in vivo time-dependent inhibitors of GABA-AT is 4-amino-5-hexenoic acid[11] (Fig. 1, vigabatrin, **1**), an anticonvulsant drug marketed all over the world except in the U.S.

Previously, our group reported various analogues of vigabatrin as inhibitors of GABA-AT.[12–14] All of these inhibitors contain the hydrophilic carboxylic acid group. However, lipophilicity is an important factor that



Scheme 1. GABA degradation by GABA-AT.

Keywords: GABA-AT; Vigabatrin; Bioisostere; Tetrazole.
* Corresponding author. Tel.: +1 847 491 5653; fax: +1 847 491 7713; e-mail: Agman@chem.northwestern.edu

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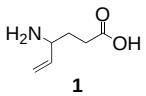


1
Vigabatrin

Figure 1. Vigabatrin.

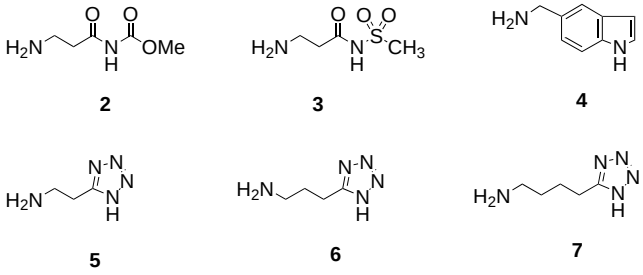


Figure 2. Designed GABA-AT substrates.

influences the ability of a compound to permeate the blood–brain barrier.[15] Therefore, we designed a series of potential substrates of GABA-AT by replacing the carboxylic acid group with more lipophilic bioisosteres (Fig. 2, **2**–**5**). In order to fit the potential substrates containing bioisosteric groups larger than the carboxylic acid group of GABA into the small and constricted active site of the enzyme,[16] β-alanine, another natural substrate of GABA-AT containing one less methylene group than GABA,[17] was selected as the parent structure.

Compound **2** was selected because it contains an isosteric functionality that is less acidic (pK_a ~8) than that of a carboxylic group; compound **3** has a pK_a value comparable to that of a carboxylic acid. Compound **4** contains an indole ring, which may be able to participate in a π-cation interaction with Arg-192, the residue to which the carboxylic acid group of GABA binds.[16] Compound **5** was also considered because of the biological compatibility of its tetrazole group, which is a well-known bioisostere of a carboxylic acid but with higher lipophilicity.[18] To optimize the carbon chain length, tetrazole derivatives **6** and **7** with one and two additional methylenes, respectively, were also made.

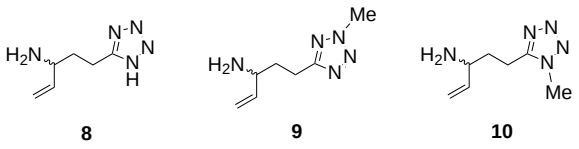


Figure 3. Designed GABA-AT inhibitors.

Based on the structure of **6**, compound **8**, a tetrazole bioisostere of the antiepilepsy drug vigabatrin, was synthesized. *N*-Methyl tetrazole derivatives **9** and **10** were also made and tested to determine the form of the tetrazole ring in the active site of the enzyme (Fig. 3). Herein we report the syntheses and the enzymatic results with these compounds.

2. Results

2.1. Search for efficient bioisosteres of the carboxylic acid group with higher lipophilicities

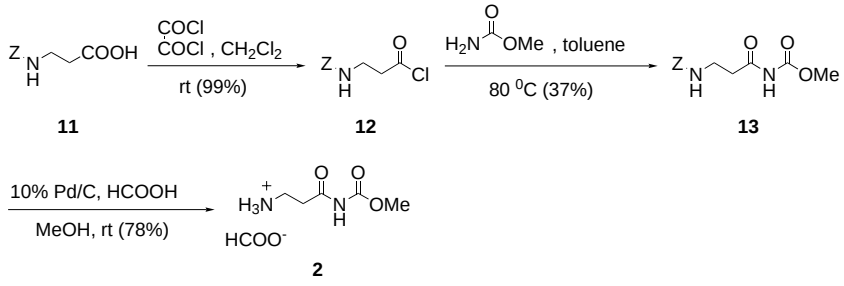
Methyl β-alanylcarbamate (**2**) was made from *N*-Cbz-β-alanine (**11**) as shown in Scheme 2. Compound **11** was treated with oxalyl chloride to give acyl chloride **12**, which was allowed to react with methyl carbamate to give methyl *N*-Cbz-β-alanylcarbamate (**13**).[19] Catalytic transfer hydrogenation[20] using formic acid and 10% palladium on active carbon gave **2** in the form of a formate salt.

Methyl β-alanylsulfonamide (**3**) was synthesized as shown in Scheme 3. Protected β-alanine **11** was treated with carbonyldiimidazole to give **14**, which was allowed to react with methanesulfonamide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford **15**.[21] Deprotection of the Cbz group with 30% HBr in acetic acid provided the desired **3** in the form of a hydrobromide salt.

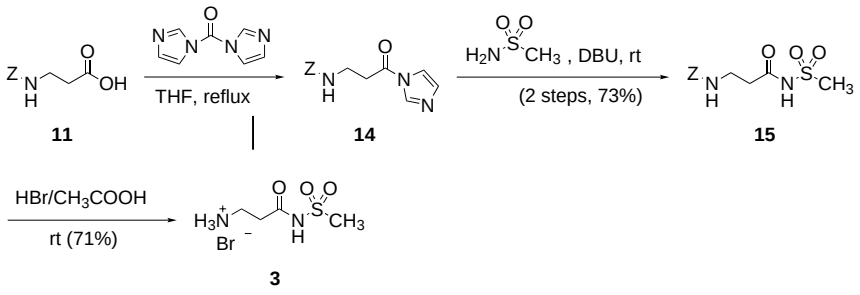
Indole-5-methanamine (**4**) was prepared from 5-cyanoindole (**16**) by reduction with $LiAlH_4$ (Scheme 4).[22]

The synthesis of 1*H*-tetrazole-5-ethanamine (**5**) is shown in Scheme 5. 3-Aminopropionitrile (**17**) was treated with benzyl chloroformate and sodium hydroxide to give *N*-Cbz-3-aminopropionitrile (**18**). The reaction of **18** with sodium azide in the presence of triethylammonium hydrochloride afforded *N*-Cbz-aminoethyltetrazole (**19**).[23] Deprotection provided desired compound **5**.

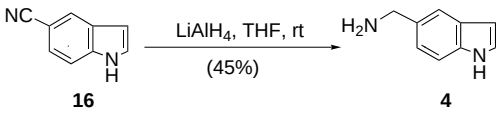
Preliminary substrate activity tests using [^{14}C]-labeled α-ketoglutarate (α-KG) showed that compounds **2**, **3**, and **5** are substrates for GABA-AT; **4**, however, showed an



Scheme 2. Synthesis of **2**.



Scheme 3. Synthesis of **3**.



Scheme 4. Synthesis of **4**.

α-KG conversion of only 0.1%, indicating that **4** has little or no substrate activity (Table 1). Tetrazole derivative **5** was the most efficient of the synthesized substrates. The tetrazole group was the best bioisostere for the carboxylic acid group in this series.

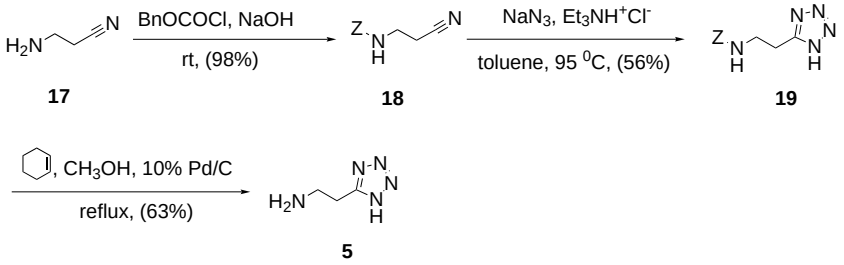
2.2. Optimization of the carbon chain length of the tetrazole derivatives

To determine the optimal carbon chain length of the tetrazole derivatives, compounds **6** and **7** with one and two additional methylene groups, respectively, than **5** were synthesized. 1*H*-Tetrazole-5-propanamine (**6**) was synthesized from 4-bromobutyronitrile (**20**) as shown in Scheme 6. Compound **20** was treated with sodium azide to give azide **22**, which was then reduced to 4-aminobutyronitrile (**24**). The preparation of **6** from **24** was similar to that of **5** from **17**. Compound **7** was synthesized in a similar manner (Scheme 6).

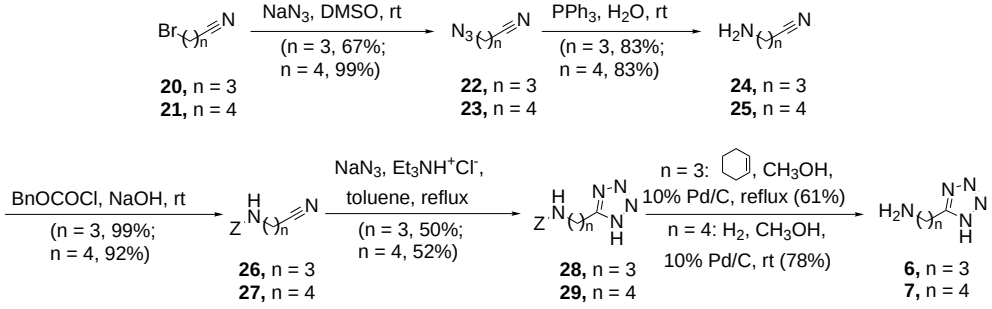
The substrate kinetic constants K_m and k_{cat} for the three tetrazoles (**5**–**7**) were determined by Hanes and Woolf plots.[24] Compound **6**, containing three methylene groups, has the highest k_{cat}/K_m value, indicating that **6** is the most efficient GABA-AT substrate with the optimal carbon chain length (Table 2).

2.3. GABA-AT inhibitors based on the structure of the most efficient substrate

Based on the structure of **6**, a potential time-dependent inhibitor of GABA-AT (**8**) was designed and synthesized. The synthesis of α-vinyl-1*H*-tetrazole-5-propanamine (**8**) from 4,4-diethoxybutanenitrile (**30**) is shown in Scheme 7. Deprotection of **30** gave the 4-oxobutanenitrile (**31**), which was treated with vinylmagnesium bromide to give **32**. The hydroxyl group in **32** was then



Scheme 5. Synthesis of **5**.



Scheme 6. Syntheses of **6** and **7**.

Table 1. Preliminary substrate activity test results

Compound	GABA	**2**	**3**	**4**	**5**
Converted α-KG[a]	25%	1.3%	6.4%	0.10%	20%

[a] Incubation time: 48 h, GABA-AT: 0.7 μM, substrate: 2.5 mM, α-KG: 2.9 mM.

Table 2. Kinetic constants for substrates **5**–**7**

Compound	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (mM^{-1} min^{-1})
GABA	2.4	49	20.4
5	2.3	15.9	6.9
6	2.4	28.6	11.7
7	8.0	41.6	5.2

converted to the phthalimide-protected amino group. The reaction of nitrile **33** with sodium azide resulted in tetrazole **34**. Deprotection of **34** with 6 N HCl gave the desired compound **8**.

As expected, **8** showed time-dependent inhibition of GABA-AT, and its kinetic constants k_{inact} and K_I were determined by a Kitz and Wilson replot[25] to be 0.73 min^{-1} and 5.6 mM, respectively (Table 3). The k_{inact} and K_I values of racemic vigabatrin were determined to be 2.2 min^{-1} and 2.6 mM, respectively. Therefore, **8** is 6.6 times less efficient (k_{inact}/K_I) than vigabatrin.

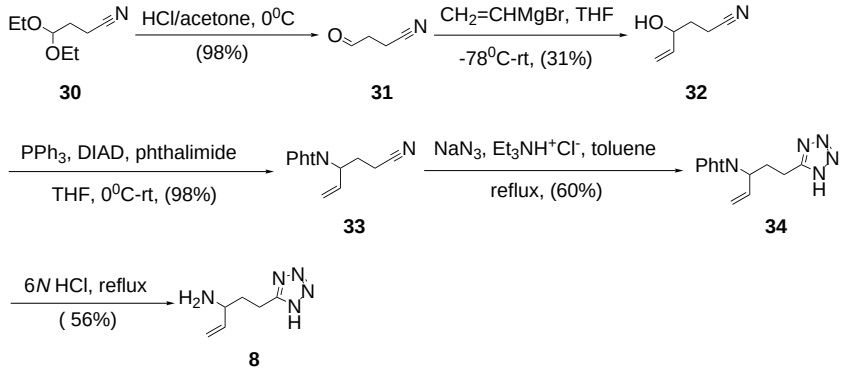
Table 3. Kinetic constants for the time-dependent inhibitor **8** and vigabatrin

Compound	K_I (mM)	k_{inact} (min^{-1})	k_{inact}/K_I (mM^{-1} min^{-1})
Vigabatrin	2.6	2.2	0.86
8	5.6	0.73	0.13

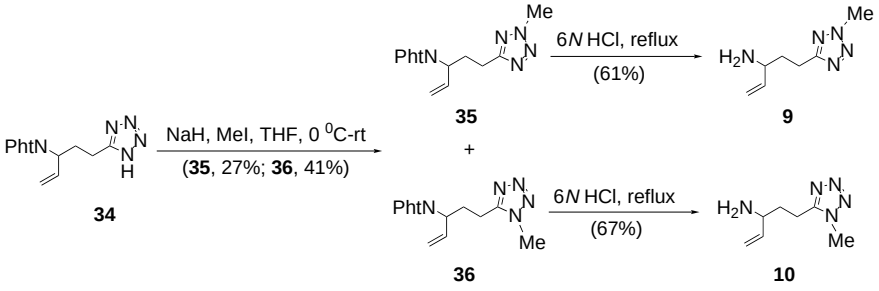
The in vivo potency of enzyme inhibition, however, strongly depends on the efficiency of the inhibitor to permeate the blood–brain barrier, which is related to the lipophilicity of the molecule.[15] To determine an estimate of lipophilicity of these compounds, the log *P* values were calculated using Clog *P* software. The log *P* values calculated for **8** and vigabatrin are −0.47 and −2.217, respectively, which indicates that **8** has considerably higher lipophilicity and, therefore, higher potential permeability of the blood–brain barrier compared to vigabatrin.

The tetrazole ring of **8** may exist either in a protonated or deprotonated form in the active site of GABA-AT; the deprotonated form mimics the carboxylate anion. To determine the existence of the deprotonated form of the tetrazole ring in the enzyme active site methyl tetrazole derivatives **9** and **10**, which cannot exist in a deprotonated form, were synthesized as shown in Scheme 8. The previously made compound **34** was treated with sodium hydride and iodomethane to give a mixture of **35** and **36**, which were separated by column chromatography.[26] Deprotection with 6 N HCl gave the desired compounds **9** and **10**.

Neither **9** nor **10** showed time-dependent inhibition of GABA-AT in comparison with the corresponding tetrazole derivative **8** at the same concentration. Instead, both **9** and **10** were found to be weak time-independent inhibitors of GABA-AT with estimated IC_{50} values for



Scheme 7. Synthesis of **8**.



Scheme 8. Syntheses of **9** and **10**.

both greater than 10 mM. If the tetrazole ring of **8** were active in its protonated form in the active site of the enzyme, **9** and **10** would have inhibited the enzyme to a similar extent to that of **8**. The significantly decreased activities of **9** and **10** caused by methylation of the tetrazole, therefore, suggest that the tetrazole ring of **8** exists in the deprotonated form in the enzyme active site.

2.4. Conclusion

In summary, a series of substrates of GABA-AT containing bioisosteres of the carboxylic acid group with higher lipophilicity were synthesized and tested. Most of the synthesized compounds were shown to be substrates of GABA-AT; the tetrazole derivative was the most efficient. The tetrazole group, therefore, was selected as the carboxylate bioisostere for a potential time-dependent inhibitor of the enzyme related to the structure of vigabatrin. The optimal carbon chain length of the tetrazole derivatives was determined. Compound **8**, a more lipophilic analogue of the antiepilepsy drug vigabatrin, showed time-dependent inhibition of GABA-AT. Its potency is similar to that of vigabatrin (one-sixth the potency), and it is more lipophilic (Clog *P* value). Therefore, it is potentially more easily able to cross the blood–brain barrier, although this potential has yet to be determined. *N*-Methyl tetrazole derivatives **9** and **10** were found to be only weak time-independent inhibitors of GABA-AT, indicating that deprotonation of the tetrazole ring may be important for the molecule to bind in the enzyme active site.

3. Experimental

3.1. General

All chemicals, reagents and solvents were purchased from commercial sources (e.g., Sigma–Aldrich, Fisher Scientific, etc.) where available. Tetrahydrofuran was distilled over sodium metal under N_2, and dichloromethane was distilled over calcium hydride under N_2. Moisture sensitive reactions were carried out in oven-dried glassware, cooled under a N_2 atmosphere. Flash chromatography was performed with Merck silica gel 60 (230–400 mesh). Cation-exchange chromatography was performed on Dowex 50 resin (BioRad AG50W-X8, 100–200 mesh). ^{1}H and ^{13}C NMR spectra were collected on Varian Mercury 400 MHz and Inova 500 MHz NMR spectrometers in the Analytical Service Laboratory at Northwestern University. High resolution mass spectra were obtained on a Finnigan MAT900XL mass spectrometer (EI) in the Analytical Services Laboratory at Northwestern University and on Micromass 70-VSE (EI) and Micromass Q-Tof Ultima (ESI) mass spectrometers in the Mass Spectrometry Laboratory at the University of Illinois. Elemental analyses were obtained from Atlantic Microlab, Inc. (Norcross, GA). Enzyme assays were recorded on a Perkin–Elmer Lambda 10 UV–vis spectrophotometer. Radioactivity was measured by liquid scintillation counting using a Packard Tri-Carb 2100TR counter and Packard Ultima Gold XR scintillation cocktail.

3.2. Chemistry

3.2.1. *N*-Cbz-β-alanyl chloride (12). To a solution of *N*-Cbz-β-alanine (**11**, 1.0 g, 4.5 mmol) in dry methylene chloride (10 mL) was added 2.0 M oxalyl chloride solution in methylene chloride (20 mL, 40 mmol). The mixture was stirred at 25 °C for 4 h. Evaporation of the solvents gave **12** as a yellow oil (1.07 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4 (s, 5H), 5.1 (s, 2H), 3.5 (t, 2H, J = 6.0 Hz), 3.2 (t, 2H, J = 5.2 Hz).

3.2.2. *N*-Cbz-methyl-β-alanylcarbamate (13). Methyl carbamate (0.68 g, 9 mmol) was added to a solution of **12** (1.0 g, 4.4 mmol) in dry toluene (5 mL) at room temperature. The mixture was heated at 80 °C for 6 h, cooled, diluted with ethyl acetate (35 mL), and washed with water (2×30 mL) and brine (1×30 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated. The product was crystallized from ethyl acetate/hexanes to afford **13** as a white solid (0.46 g, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 (s, 5H), 5.1 (s, 2H), 3.8 (s, 3H), 3.5 (t, 2H, J = 5.6 Hz), 3.0 (t, 2H, J = 5.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 73.6, 156.5, 152.2, 136.6, 128.7, 128.3, 128.2, 66.9, 53.4, 36.9, 36.1.

3.2.3. Methyl β-alanylcarbamate (2). A mixture of methyl *N*-Cbz-β-alanylcarbamate (**13**, 0.056 g, 0.2 mmol), 10% Pd/C (0.05 g), formic acid (88%, 0.15 mL), and methanol (7 mL) was stirred for 2 h at room temperature. The catalyst was removed by filtration through a Celite bed. The filtrate was concentrated to give the formate salt of **2** as a white solid (0.030 g, 78%). ^{1}H NMR (400 MHz, D_2O) δ 8.4 (s, 1H), 3.8 (s, 3H), 3.2 (t, 2H, J = 6.0 Hz), 3.0 (t, 2H, J = 5.6 Hz). ^{13}C NMR (100 MHz, D_2O) δ 172.7, 169.2, 153.4, 53.3, 34.7, 33.1. HRMS (ESI): calculated for $C_5H_{11}N_2O_3$ $(M+H)^+$: 147.0770. Found: 147.0773.

3.2.4. Methyl *N*-Cbz-β-alanylsulfonamide (15). A solution of **11** (2.23 g, 10 mmol) in dry THF (20 mL) was added dropwise to a stirred solution of carbonyldiimidazole (1.62 g, 10 mmol) in dry THF (20 mL) under N_2. The mixture was stirred for 30 min, refluxed for 30 min, and allowed to cool to room temperature. Methyl sulfonamide (0.95 g, 10 mmol) was added in one portion, and the mixture was stirred for 10 min before a solution of DBU (1.52 g, 10 mmol) in dry THF (10 mL) was added dropwise. The resulting mixture was stirred overnight and poured into ice-cold 1 N HCl (200 mL). The formed precipitate was filtered, washed with water, and dried to give **15** as a white solid (2.2 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4 (s, 5H), 5.1 (s, 2H), 3.5 (tetra, 2H, J = 6.0 Hz), 3.3 (s, 3H), 2.6 (t, 2H, J = 4.8 Hz).

3.2.5. Methyl β-alanylsulfonamide (3). To **15** (1.0 g, 3.3 mmol) was added a solution of hydrogen bromide in acetic acid (30%, 10 g) with stirring. After 20 min, the mixture was slowly diluted to 100 mL with diethyl ether, and the liquids were decanted. The solid was resuspended in ether (100 mL) and stirred, and the suspension filtered and washed with ether to give **3** as a white solid (0.54 g, 71%). ^{1}H NMR (400 MHz, D_2O) δ

3.33 (s, 3H), 3.29 (t, 2H, $J = 6.0$ Hz), 2.85 (t, 2H, $J = 6.4$ Hz). ^{13}C NMR (100 MHz, D_2O) δ 171.8, 40.8, 34.5, 32.5. HRMS (ESI): calculated for $C_4H_{11}N_2O_3S$ $(M+H)^+$: 167.0490. Found: 167.0497.

3.2.6. Indole-5-methanamine (4). To an ice-cold 1.0 M solution of $LiAlH_4$ in THF (18 mL, 0.018 mol) was added dropwise under N_2 a solution of 5-cyanoindole (**16**, 1.56 g, 0.011 mol) in dry THF (25 mL). After the addition was complete, the mixture was allowed to warm to room temperature and was stirred overnight. The resulting mixture was cooled in an ice bath, and excess $LiAlH_4$ was quenched with 10% NaOH. The product was extracted with ethyl acetate and dried over anhydrous magnesium sulfate. The solvent was removed by rotary evaporation to give the crude product (1.1 g), which was recrystallized from ethyl acetate/hexanes to give crystalline **5** (0.7 g, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.0 (s, 1H), 7.5 (s, 1H), 7.3 (d, 2H, $J = 8.4$ Hz), 7.0 (d, 1H, $J = 8.4$ Hz), 6.4 (s, 1H), 3.8 (s, 2H), 2–3 (br, 2H). HRMS (EI): calculated for $C_9H_{10}N_2$ (M^+): 146.0838. Found: 146.0835.

3.2.7. *N*-Cbz-3-aminopropionitrile (18). 3-Aminopropionitrile **17** (0.56 g, 8.0 mmol) was suspended in water (10 mL) and THF (10 mL). The pH was adjusted to 9.0 by addition of NaOH (0.2 g, 5 mmol). Benzyl chloroformate (1.7 g, 10 mmol) was added dropwise over 2 h at 20–25 °C to the resulting clear solution, and the pH was kept constant at 9.0 by addition of aqueous NaOH (4 M, 2.5 mL). The mixture was stirred for 1 h at pH 9.0, extracted with ethyl acetate, and dried with Na_2SO_4. The solvents were removed by rotary evaporation to give crude **18** (1.6 g, 98%) as an oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.3 (s, 5H), 5.0 (s, 2H), 3.22 $\sim$ 3.27 (m, 2H), 2.6 (t, 2H, $J = 6.4$ Hz).

3.2.8. *N*-Cbz-1*H*-tetrazole-5-ethanamine (19). The mixture of *N*-Cbz-3-aminopropionitrile **18** (0.26 g, 1.3 mmol), triethylamine hydrochloride (0.38 g, 4 mmol), and sodium azide (0.26 g, 4 mmol) in toluene (10 mL) was heated to 95–100 °C for 24 h. After cooling, the product was extracted with water (20 mL). The separated aqueous layer was acidified with 1 N HCl to pH 1.5 to precipitate the produced tetrazole. The formed precipitate was filtered, washed with 1 N HCl, and dried under reduced pressure to give **19** (0.17 g, 56%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.3 (s, 5H), 5.0 (s, 2H), 3.5 (t, 2H, $J = 4.8$ Hz), 3.1 (d, 2H, $J = 5.6$ Hz).

3.2.9. 1*H*-Tetrazole-5-ethanamine (5). A mixture of **19** (0.17 g, 0.7 mmol), 10% Pd/C (0.10 g), cyclohexene (4 mL), and methanol (6 mL) was refluxed overnight. The catalyst was removed by filtration through a Celite bed. The solvent was removed by rotary evaporation to give crude **5** as a white solid, which was purified by cation-exchange chromatography (AG® 50W-X8, eluting with 0.15 N HCl) to give pure **5** in the form of a hydrochloride salt (0.05 g, 63%). ^{1}H NMR (400 MHz, D_2O) δ 3.3 (t, 2H, $J = 6.0$ Hz), 3.1 (t, 2H, $J = 6.0$ Hz). ^{13}C NMR (100 MHz, D_2O) δ 159.0, 38.3, 22.7. Anal. Calcd for $C_3H_8ClN_5 \cdot 0.2H_2O$: C, 23.52; H, 5.53; N, 45.73. Found: C, 23.93; H, 5.42; N, 45.52.

3.2.10. 4-Azidobutanenitrile (22). Sodium azide (1.2 g, 18.5 mmol) was added to a solution of 4-bromobutyronitrile (**20**, 1.8 g, 12.0 mmol) in DMSO (20 mL). After 18 h of stirring at room temperature, water (40 mL) was added, and the solution was extracted with diethyl ether. Evaporation of the solvent gave **20** as a light yellow oil (0.9 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.5 (t, 2H, $J = 6.0$ Hz), 2.5 (t, 2H, $J = 7.2$ Hz), 1.90 $\sim$ 1.95 (m, 2H).

3.2.11. 4-Aminobutanenitrile (24). Triphenylphosphine (2.11 g, 8.0 mmol) and water (0.2 mL) were added to **22** (0.9 g, 8.0 mmol) dissolved in THF (10 mL). After 18 h at room temperature, the solvent was removed by rotary evaporation. Ethyl acetate (30 mL) was added to the crude product, and the desired compound was extracted with 1 N HCl (30 mL). The aqueous phase was basified to pH 12 with 10% NaOH and was extracted with ethyl acetate (2×30 mL). Evaporation of the solvent gave **24** as an oil (0.56 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.9 (t, 2H, $J = 6.8$ Hz), 2.4, (t, 2H, $J = 7.2$ Hz), 1.75–1.82 (m, 2H).

3.2.12. 1*H*-Tetrazole-5-propanamine (6). The synthetic procedure from **24** to **6** (4 mmol scale, 30% for three steps) is similar to that from **17** to **5**. ^{1}H NMR (400 MHz, D_2O): δ 2.91–2.99 (m, 4H), 2.02–2.10 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 161.9, 38.9, 25.9, 21.5. Anal. Calcd for $C_4H_{10}ClN_5 \cdot 0.4H_2O$: C, 28.13; H, 6.37; N, 41.00. Found: C, 28.53; H, 6.02; N, 40.81.

3.2.13. 1*H*-Tetrazole-5-butanamine (7). The synthetic procedure from **21** to **7** (12 mmol scale, 31% for five steps) is similar to that from **20** to **6**. ^{1}H NMR (500 MHz, D_2O) δ 2.93–2.98 (m, 2H), 2.84–2.88 (m, 2H), 1.72–1.78 (m, 2H), 1.57–1.62 (m, 2H). ^{13}C NMR (126 MHz, D_2O) δ 163.1, 39.3, 26.2, 24.9, 23.6. Anal. Calcd for $C_5H_{12}ClN_5 \cdot 0.5H_2O$: C, 32.18; H, 7.02; N, 37.52. Found: C, 32.39; H, 6.76; N, 37.29.

3.2.14. 4-Oxobutanenitrile (31). A mixture of 4,4-diethoxybutanenitrile (**30**, 0.95 g, 6.0 mmol), acetone (30 mL), and 6 N HCl (12 mL) was stirred at 0 °C for 9 h. After the reaction was complete, the mixture was concentrated to approximately 2 mL and was extracted with chloroform (4×10 mL). The combined organic phase was dried with sodium sulfate. The solvent was removed by rotary evaporation to give crude **31** as an oil (0.49 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.8 (s, 1H), 2.9 (t, 2H, $J = 7.2$ Hz), 2.6 (t, 2H, $J = 7.2$ Hz).

3.2.15. 4-Hydroxy-5-hexenenitrile (32). A 1.0 M solution of vinylmagnesium bromide (5.9 mL, 5.9 mmol) in THF was added dropwise to a solution of crude **31** (0.49 g, 5.9 mmol) in dry THF (10 mL) at −78 °C. The mixture was stirred at −78 °C for 1 h and was further stirred at room temperature overnight. Saturated aqueous NH_4Cl (15 mL) was added with stirring to the turbid solution chilled in an ice bath. The aqueous phase was extracted with ethyl acetate (3×15 mL), and the combined organic extracts were washed with water (10 mL) and brine (2×10 mL), dried with sodium sulfate, and concentrated under vacuum to give crude **32** as a yellow oil. The

crude product was purified by chromatography on silica gel (ethyl acetate/hexanes, 4:6) to give a colorless oil (0.20 g, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82–5.90 (m, 1H), 5.3 (d, 1H, J = 17.6 Hz), 5.2 (d, 1H, J = 10.0 Hz), 2.46–2.57 (m, 2H) 1.78–1.95 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.5, 116.5, 71.3, 32.3, 13.6.

3.2.16. 4-Phthalimido-5-hexenenitrile (33). A solution of **32** (0.35 g, 3.1 mmol), triphenylphosphine (0.87 g, 3.3 mmol), and phthalimide (0.50 g, 3.3 mmol) in dry THF (15 mL) was stirred at 0 °C under N_2 for 10 min. A solution of diisopropyl azodicarboxylate (DIAD, 0.66 g, 3.3 mmol) in THF (8 mL) was added dropwise over 20 min. The mixture was stirred at room temperature for 3 h. After the solvent was removed by rotary evaporation, the crude product was purified by chromatography on silica gel (ethyl acetate/hexanes, 1:9) to give a mixture of **33** and diisopropyl hydrazodicarboxylate, a by-product formed from DIAD (1.19 g, ~3:2 m/m, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72–7.85 (m, 4H), 6.14–6.23 (m, 1H), 5.34 (d, 1H, J = 17.2 Hz), 5.26 (d, 1H, J = 10.4 Hz) 4.8 (tetra, 1H, J = 5.6 Hz), 2.27–2.48 (m, 4H).

3.2.17. 5-(3-Phthalimido-4-pantenyl)-1*H*-tetrazole (34). The mixture of **33** and diisopropyl hydrazodicarboxylate prepared above (0.88 g, 2.6 mmol) was added to a solution of triethylamine hydrochloride (0.76 g, 8 mmol) and sodium azide (0.52 g, 8 mmol) in toluene (15 mL). After 18 h of stirring at 95–100 °C, the cooled product was extracted with water (20 mL). The separated aqueous layer was acidified with 10% HCl to pH 1.5 to salt out the produced tetrazole. The formed precipitate was filtered and dried to give **34** as a light brown solid (0.44 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.8 (dd, 4H, J = 35.2 Hz, 2.4 Hz) 6.22–6.29 (m, 1H), 5.27 (d, 1H, J = 4.4 Hz), 5.24 (d, 1H, J = 3.2 Hz) 4.72 (s, 1H), 3.19–3.22 (m, 1H), 2.78–2.84 (m, 1H), 2.62–2.71 (m, 1H), 2.18–2.22 (m, 1H).

3.2.18. 1*H*-Tetrzole-5-(α-vinyl-propanamine) (8). To a solution of 6 N HCl (20 mL) was added **34** (0.2 g, 1 mmol), and the mixture was refluxed for 6 h. The mixture was washed with ethyl acetate (2 × 20 mL). Evaporation of the solvent gave crude **8** as a yellow oil. To remove the trace amount of phthalic acid, the crude product was purified by cation-exchange chromatography (AG® 50W-X8, eluting with 0.2 N HCl) to give **8** in the form of a hydrochloride as a colorless oil. The hydrochloride was loaded on a second cation-exchange column, eluted with water followed by 0.15 N ammonium hydroxide to give the free amine form of **8** as a white solid (0.086 g, 56%). ^{1}H NMR (400 MHz, D_2O) δ 5.72–5.81 (m, 1H), 5.38–5.45 (m, 2H), 3.80 (br s, 1H), 3.00–3.07 (m, 2H), 2.24–2.32 (m, 1H), 2.09–2.19 (m, 1H). ^{13}C NMR (400 MHz, D_2O) δ 155.1, 131.5, 122.3, 53.2, 29.1, 19.0. Anal. Calcd for $C_6H_{11}N_5 \cdot 0.4H_2O$: C, 44.93; H, 7.42; N, 43.66. Found: C, 44.93; H, 7.38; N, 43.56.

3.2.19. 2-Methyl-2*H*-tetrazole-5-(α-vinyl-*N*-phthaloylpropanamine) (35) and 1-methyl-1*H*-tetrazole-5-(α-vinyl-*N*-phthaloylpropanamine) (36). A solution of **34** (0.167 g, 0.6 mmol) in dry THF (10 mL) was cooled to 0 °C. NaH (60% in mineral oil, 0.034 g, 0.75 mmol) dissolved in dry THF (5 mL) was added dropwise over 20 min. The mixture was stirred for an additional 10 min, and iodomethane (0.11 g, 0.75 mmol) was added. After the mixture was stirred at room temperature for 2 h, H_2O (20 mL) was added, and the resulting mixture was extracted with EtOAc (2 × 20 mL). The combined organic phases were washed with H_2O (2 × 20 mL) and brine (2 × 20 mL), dried with Na_2SO_4, and the solvents were removed by rotary evaporation. The resulting mixture of **35** and **36** was separated by column chromatography on silica gel (EtOAc/hexanes, 4:6). Evaporation of the faster eluting fractions gave **35** as an oil (0.047 g, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73–7.85 (m, 4H), 6.22–6.28 (m, 1H), 5.30 (d, 1H, J = 17.2 Hz), 5.23 (d, 1H, J = 10.4 Hz), 4.82 (tetra, 1H, J = 8.0 Hz), 4.25 (s, 3H), 2.91 (tetra, 2H, J = 8.0 Hz), 2.56–2.61 (m, 1H), 2.45–2.55 (m, 1H). Evaporation of the slower eluting fractions gave **36** as an oil (0.071 g, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74–7.86 (m, 4H), 6.21–6.30 (m, 1H), 5.32 (d, 1H, J = 17.6 Hz) 5.27 (d, 1H, J = 10.0 Hz), 4.86 (tetra, 1H, J = 5.6 Hz), 3.97 (s, 3H), 2.84–2.90 (m, 2H), 2.66–2.74 (m, 1H), 2.51–2.55 (m, 1H).

3.2.20. 2-Methyl-2*H*-tetrazole-5-(α-vinyl-propanamine) (9) and 1-methyl-1*H*-tetrazole-5-(α-vinyl-propanamine) (10). The deprotection procedures from **35** and **36** to **9** and **10**, respectively, are similar to that from **34** to **8**. Compound **9** (0.016 g, 61%). ^{1}H NMR (400 MHz, D_2O) δ 5.74 ~ 5.81 (m, 1H), 5.35–5.40 (m, 2H), 4.25 (s, 3H), 3.76 (s, 1H), 2.87–2.91 (m, 2H), 2.14–2.22 (m, 1H), 2.02 ~ 2.10 (m, 1H). ^{13}C NMR (400 MHz, D_2O) δ 165.1, 132.2, 122.1, 53.5, 33.6, 30.1, 20.9. HRMS (EI): calculated for $C_7H_{13}N_5$ (M^+): 167.1171. Found: 167.1160. Compound **10** (0.027 g, 67%). ^{1}H NMR (400 MHz, D_2O) δ 5.63–5.71 (m, 1H), 5.29–5.33 (m, 2H), 3.84 (s, 3H), 3.76–3.79 (m, 1H), 2.80–2.84 (m, 2H), 2.13–2.17 (m, 1H), 2.00–2.05 (m, 1H). ^{13}C NMR (100 MHz, D_2O) δ 155.2, 131.9, 122.5, 53.4, 33.6, 28.6, 19.0. HRMS (EI): calculated for $C_7H_{13}N_5$ (M^+): 167.1171. Found: 167.1158.

3.3. Enzymatic tests

3.3.1. Enzyme and assays. GABA-AT (1.88 mg/mL, specific activity 2.73 unit/mg) was purified from pig brain by the procedure described by Churchich and Moses.[27] Succinic semialdehyde dehydrogenase (SSDH) was purified from GABAse, a commercially available mixture of SSDH and GABA-AT, using the procedure of Jeffery et al.[28] GABA-AT activity was assayed using a published method with modifications.[29] The final assay solution consists of 11 mM GABA, 1.1 mM $NADP^+$, 5.3 mM α-KG, 2 mM β-mercaptoethanol, and excess SSDH in 50 mM potassium pyrophosphate buffer, pH 8.5. The change in UV absorbance at the wavelength of 340 nm caused by the formation of NADPH is proportional to the GABA-AT activity.

3.3.2. Substrate activities of 2–7. Potential substrates **2**–**7** of varying concentrations (1–5 mM) were incubated with GABA-AT (17.1 μM, 5–7 μL) at 25 °C in 50 mM potassium pyrophosphate buffer, pH 8.5, containing 2 mM β-

mercaptoethanol and 2.9 mM [5-^{14}C]2-ketoglutarate (0.1 mCi/mmol) in a total volume of 100 μL. After incubation (48 h for the preliminary test, 1 h for determination of kinetic constants), the mixture was quenched with trichloroacetic acid. The resulting [^{14}C]glutamate was isolated by cation-exchange chromatography, and the DPM (disintegration per minute) value was measured. Controls consisted of the entire incubation mixture with substrates omitted. The substrate kinetic constants k_{cat} and K_m were determined by the method of Hanes and Woolf.[24]

4. Time-dependent inhibition of GABA-AT by 8

GABA-AT (17.1 μM, 25 μL) was incubated with **8** (120 μL final volume, 1–2 mM) at 25 °C in 50 mM potassium pyrophosphate buffer solution, pH 8.5, containing 2 mM α-ketoglutarate and 2 mM β-mercaptoethanol. Aliquots (20 μL) were withdrawn at timed intervals and were added immediately to the assay solution (575 μL) followed by the addition of excess SSDH (5 μL). The reaction rates were measured by a UV–vis spectrophotometer at 340 nm. Racemic vigabatrin was tested under the same conditions. A Kitz and Wilson replot[25] was used to determine the kinetic constants k_{inact} and K_I.

5. Time-independent inhibition of GABA-AT by 9 and 10

GABA-AT (17.1 μM, 5 μL) was assayed for its activity at 25 °C with varying concentrations (1–10 mM) of **9** and **10**. The percentage of remained enzyme activity was obtained by comparison to that of an untreated enzyme control. The logarithm of the percentage of remained activity is plotted versus the concentration of the inhibitors to calculate IC_{50} values.

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