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Synthesis of Cyclopropane Isosteres of the Antiepilepsy Drug Vigabatrin and Evaluation of their Inhibition of GABA Aminotransferase

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The antiepilepsy drug vigabatrin (1; 4-aminohex-5-enoic acid; γ-vinyl GABA) is a mechanism-based inactivator of the pyridoxal 5′-phosphate (PLP)-dependent enzyme γ-aminobutyric acid aminotransferase (GABA-AT). Inactivation has been shown to proceed by two divergent mechanisms (Nanavati, S. M. and Silverman, R. B. (1991) *J. Am. Chem. Soc.* 113, 9341–9349), a Michael addition pathway (Scheme 2, pathway a) and an enamine pathway (Scheme 2, pathway b). Analogs of vigabatrin with a cyclopropyl or cyanocyclopropyl functionality in place of the vinyl group (2–5) were synthesized as potential inactivators of GABA-AT that can inactivate the enzyme only through a Michael addition pathway, but they were found to be only weak inhibitors of the enzyme.

Keywords: GABA; GABA-AT; Vigabatrin; γ-Aminobutyric acid aminotransferase; γ-Aminobutyric acid

INTRODUCTION

γ-Aminobutyric acid aminotransferase[1] (GABA-AT, E.C. 2.6.1.19) is the enzyme responsible for the degradation of γ-aminobutyric acid (GABA), one of the major inhibitory neurotransmitters in the mammalian central nervous system,[2] to succinic semialdehyde. The enzyme utilizes pyridoxal 5′-phosphate (PLP) as its cofactor, which is converted to pyridoxamine 5′-phosphate (PMP) when GABA is degraded and restored back to PLP by transamination with α-ketoglutarate (α-KG), generating the excitatory neurotransmitter L-glutamate (Scheme 1). Inhibition of this enzyme results in an increased concentration of GABA in the brain and could have therapeutic applications in neurological disorders including epilepsy,[3] Parkinson's disease,[4] Huntington's chorea,[5] and Alzheimer's disease.[6] It has also been found that an increase in availability of GABA blocks the effects of drug addiction.[7,8]

The selective GABA-AT inactivator, vigabatrin (1, 4-aminohex-5-enoic acid; γ-vinyl GABA),[9] a mechanism-based inactivator[10] of the enzyme, is already successfully utilized as an anticonvulsant[11] and has been in clinical trials for the treatment of drug addiction.[12] Studies in our group have revealed that it inactivates the enzyme via two divergent mechanisms.[13] The major mechanism, a Michael addition pathway (Scheme 2, pathway a), involves γ-proton removal, tautomerization into the PLP ring, followed by Michael addition of an active site lysine residue at the conjugated vinyl group to give a stable covalent adduct with the protein. The minor inactivation mechanism, the enamine pathway (Scheme 2, pathway b), also involves γ-proton removal, but tautomerization occurs through the vinyl group, followed by an enamine rearrangement that leads to attachment of the inactivator to the PLP, which is bound to the protein. The Michael addition mechanism accounts for 70–75% of the total activation.

Often a cyclopropane is considered to be an isostere of an alkene, only less reactive. Whereas α, β-unsaturated carbonyl compounds are highly electrophilic, cyclopropylcarbonyl compounds are only weakly electrophilic unless a strongly activating, resonance-stabilizing group is attached to the cyclopropane.[14] The PLP system is strongly

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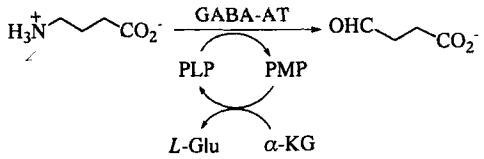


SCHEME 1 Reaction catalyzed by GABA-AT.

electron-stabilizing and may be sufficiently stabilizing to permit nucleophilic attack if a cyclopropane were adjacent to it. If PLP alone is not strong enough to activate the cyclopropane, then the attachment of an additional electron-withdrawing group would provide the additional electrophilic activation.

Four analogs of vigabatrin that contain a cyclopropane functionality (Figure 1, **2**–**5**) were designed as potential inactivators of GABA-AT that can only inactivate the enzyme through the Michael addition pathway (Scheme 3). Compounds **2** and **4** were designed because they contain the β-alanine structure, another natural substrate of GABA-AT. The exchange of the vinyl group of vigabatrin for a cyclopropyl group may also increase the lipophilicity of the molecule, making it easier to cross the blood–brain barrier[15] for greater *in vivo* potency.

Here we wish to report the synthesis of **2**–**5** and the results of their GABA-AT inhibitory evaluation.

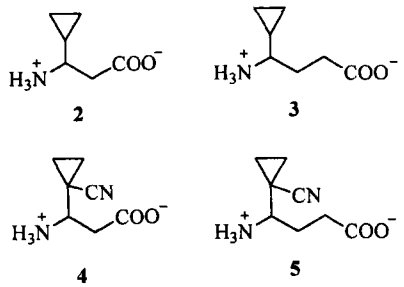
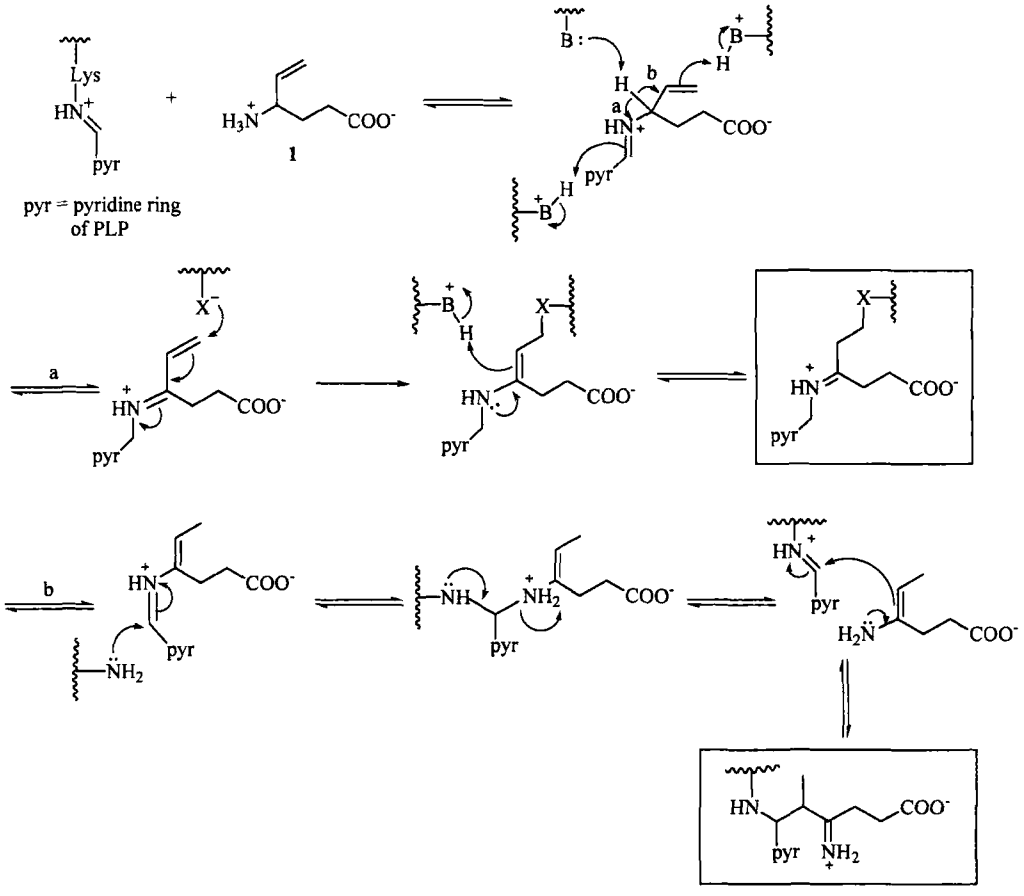


FIGURE 1 Structures of **2**–**5**.

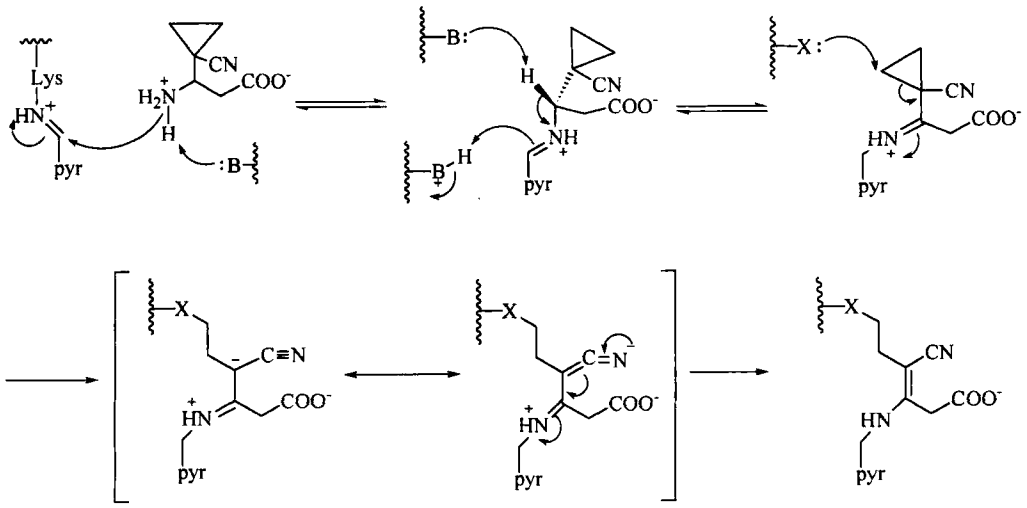
MATERIALS AND METHODS

Chemistry

The first target molecule, 3-amino-3-cyclopropylpropanoic acid (**2**), was synthesized by a Mannich reaction following a patent procedure (Scheme 4).[16] The amino group was then Boc protected, and the product was subjected to an Arndt-Eistert reaction,[17] in which cyclization occurred and the pyrrolidinone **7** was formed. Deprotection followed by hydrolytic ring-opening afforded 4-amino-4-cyclopropylbutyric acid (**3**) as a cyclopropane isostere of vigabatrin (Scheme 4).



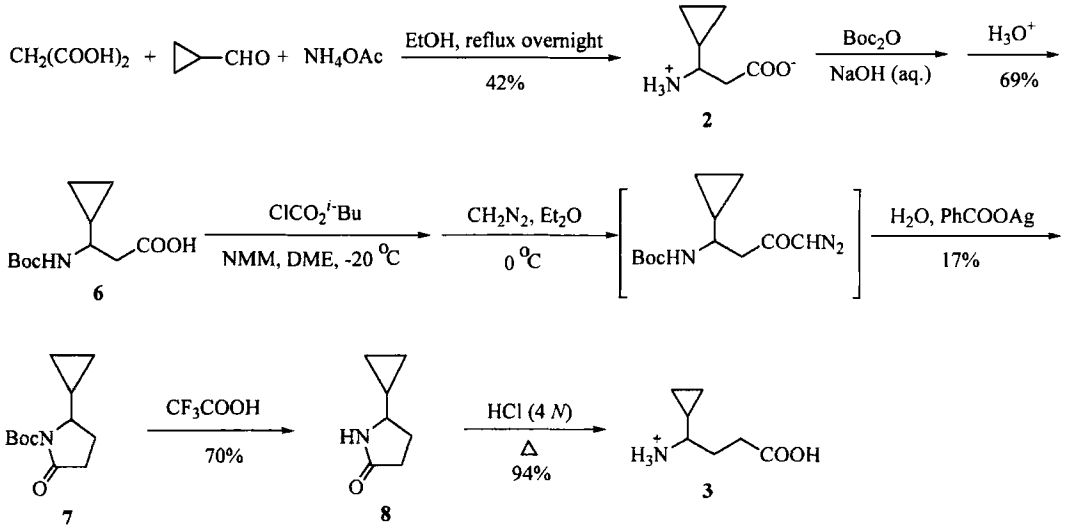
SCHEME 2 Inactivation mechanism of GABA-AT by vigabatrin.



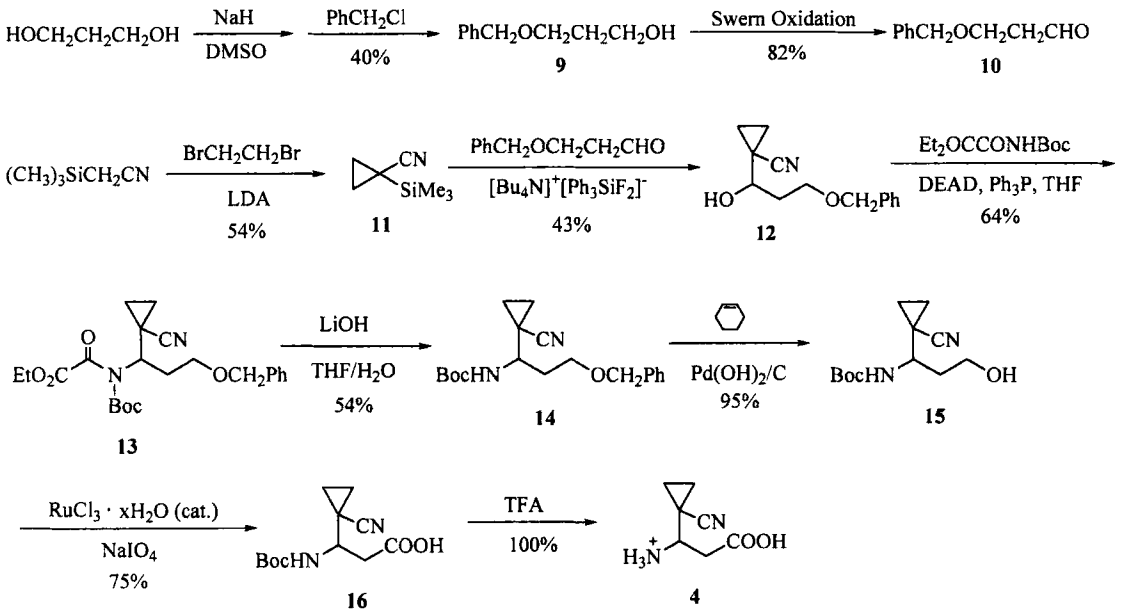
SCHEME 3 Possible inactivation mechanism of GABA-AT by **4**.

An attempt to prepare 3-amino-3-(1′-cyanocyclopropyl)propanoic acid (**4**), by a similar reaction from 1-cyano-1-cyclopropanecarboxaldehyde, ammonium acetate, and malonic acid failed. Possibly an imine was generated from the interaction of 1-cyano-1-cyclopropanecarboxaldehyde with ammonium acetate, and the combined activating effects of both the imine and the cyano groups were so strong that the cyclopropane ring was opened by malonic acid. Therefore a different approach was taken (Scheme 5). The aldol reaction between 1-(trimethylsilyl)cyclopropanenitrile and 3-benzyloxypropanal was effected by tetrabutylammonium triphenyldifluorosilicate (TBAT).[18] Although the fluoride salt can be used catalytically, the yield was better if a stoichiometric amount of the reagent was employed (30% vs. 43% yield). The alcohol (**12**) was converted into the corresponding Boc-protected amine (**14**) by the procedure of Berree *et al.*[19] The benzyl group was deprotected by transfer hydrogenation,[20] and the resulting primary alcohol (**15**) was oxidized to the carboxylic acid **16** by catalytic ruthenium (III) chloride with sodium periodate as the cooxidant.[21] Final deprotection of the Boc group afforded **4**.

A similar synthetic route was adopted for the synthesis of **5** (Scheme 6). First we tried the deprotection of the ethoxyoxoacetyl group on the nitrogen of **18**, and the resulting alkene was subjected to oxidation. The cyclized counterpart of **19**, *N-tert*-butoxycarbonyl-5-(1′-cyanocyclopropyl)-pyrrolidin-2-one, however, was obtained in twice the amount as **19**. The oxidation is likely stepwise, and an aldehyde intermediate might be involved,[21] which becomes extremely electrophilic by complexation with the ruthenium and cyclizes with the Boc-protected amino group to make the corresponding pyrrolidinone. Therefore oxidation



SCHEME 4 Syntheses of **2** and **3**.



SCHEME 5 Synthesis of **4**.

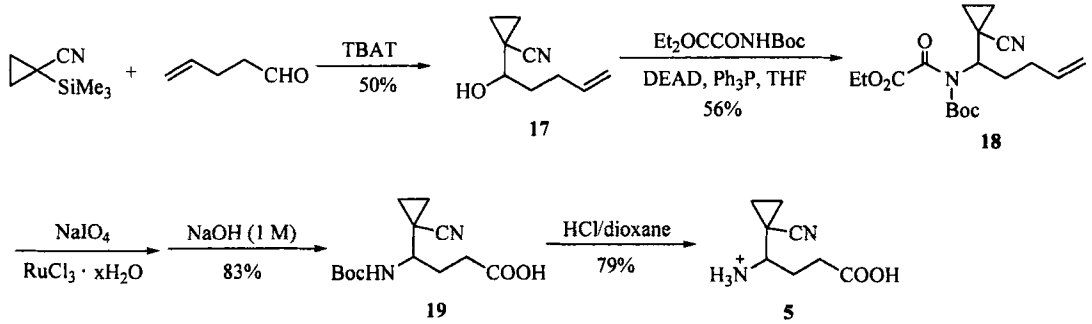
was performed directly on **18**, and it was found that the ethoxyoxoacetyl group could be efficiently removed during work-up by simple washing with 1 M aqueous sodium hydroxide solution, affording pure **19** in one pot.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 400 MHz and Inova 500 MHz NMR spectrometers. Chemical shifts are reported as δ values in parts per million (ppm) as referenced to chloroform (7.27 ppm for ^{1}H and 77.23 ppm for ^{13}C) or to methanol (4.87 ppm for CD_3OH and 49.15 ppm for ^{13}C). Mass spectra were obtained on Finnigan MAT900XL (EI) and VG70-250SE (ESI) mass spectrometers in the Analytical Service Laboratory at Northwestern University, and on a 70-SE-4F mass spectrometer (FAB) in the Mass Spectrometry Laboratory at University of Illinois. Flash column chromatography was carried out with Merck silica gel 60 (230–400 mesh). TLC was run with EM Science silica gel 60 F254 precoated glass plates. Cation exchange chromatography was performed on Dowex 50 resin (BioRad AG50W-X8, 100–200 mesh). Melting points were measured on a Fisher-Johns melting point apparatus and are uncorrected. All reactions involving moisture sensitive reagents were conducted in oven-dried glassware under a nitrogen atmosphere. 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.

Diethyl azodicarboxylate (DEAD) was purchased from Lancaster Synthesis, Inc. All other reagents and solvents were purchased from Aldrich Chemical Co. or Fisher Scientific without further purification except anhydrous ether and tetrahydrofuran, which were distilled over sodium metal under nitrogen, and anhydrous dichloromethane, which was distilled over calcium hydride.

3-Amino-3-cyclopropylpropanoic acid (**2**)[16] and 1-(trimethylsilyl)cyclopropane-nitrile (**11**)[22] were prepared according to the literature procedures.



SCHEME 6 Synthesis of **5**.

3-(*tert*-Butoxycarbonylamino)-3-cyclopropylpropanoic Acid (6)

To a solution of **2** (0.22 g, 1.7 mmol) and sodium hydroxide (0.10 g, 2.5 mmol) in 10 mL of water was added a solution of di-*tert*-butyl dicarbonate (1.12 g, 5.2 mmol) in 7 mL of ethanol. After the reaction mixture was heated at 60°C overnight, it was extracted with ethyl acetate (3 × 30 mL) to remove excess di-*tert*-butyl dicarbonate. The pH of the aqueous solution was adjusted to about pH 3, and it was extracted with ethyl acetate (3 × 30 mL), washed with brine (30 mL), dried over sodium sulfate, and evaporated under reduced pressure to afford a white solid (0.27 g, 69%); mp 107–108°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.27–0.53 (m, 4 H, H-2′, H-3′), 1.04 (m, 1 H, H-1′), 1.45 (s, 9 H, $COOC(CH_3)_3$), 2.69 (m, 2 H, H-2), 3.24 (m, 1 H, H-3), 5.13 (bs, NH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 3.68 (C-2′), 3.92 (C-3′), 15.79 (C-1′), 28.55 ($(CH_3)_3$), 39.74 (C-2), 52.28 (C-3), 79.86 ($OCMe_3$), 155.74 (*t*-BuOOC), 177.11 (C-1).

N-*tert*-Butoxycarbonyl-5-cyclopropylpyrrolidin-2-one (7)

To a solution of **6** (1.14 g, 5.0 mmol) in ethylene glycol dimethyl ether (anhydrous, 40 mL), cooled to −20°C, was added with stirring 4-methylmorpholine (0.60 mL, 5.5 mmol), followed by isobutyl chloroformate (0.70 mL, 5.4 mmol). After 15 min the white precipitated salt was removed by filtration and washed with anhydrous ether. A diazomethane solution in ether (ca. 22.6 mmol), which was prepared from Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide, 6.8 g, 32.0 mmol) in an Aldrich mini Diazald apparatus and dried with potassium hydroxide for 1 h prior to usage, was added to the filtrate via a cannula, and the solution was stirred at 0°C for 1 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in water (10 mL) and treated with silver benzoate (0.23 g, 1.0 mmol). After 1 h stirring at room temperature, the dark mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (100 mL), and the insoluble material was removed by filtration. The filtrate was washed with brine (4 × 30 mL), dried over sodium sulfate, and concentrated. Chromatography (ethyl acetate/hexane, 1:5) afforded the title compound as a clear oil (0.21 g, 17%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.17–0.63 (m, 4 H, H-2′, H-3′), 0.99–1.06 (m, 1 H, H-1′), 1.54 (s, 9 H, $COOC(CH_3)_3$), 1.85–1.88 (m, 1 H, H-4a), 2.08–2.19 (m, 1 H, H-4b), 2.39–2.46 (m, 1 H, H-3a), 2.62–2.71(m, 1 H, H-3b), 3.68–3.71 (m, 1 H, H-5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 2.04 (C-2′), 5.26 (C-3′), 15.89 (C-1′), 24.55 (C-4), 28.34 ($(CH_3)_3$), 32.02 (C-3), 62.00 (C-5), 82.91 ($OCMe_3$), 150.30 (*t*-BuOOC), 174.53 (C-2). HRMS (ESI): calculated for $C_{12}H_{19}NO_3Na$ $(M + Na)^+$ 248.1263, found 248.1279.

5-Cyclopropylpyrrolidin-2-one (8)

To a solution of **7** (0.21 g, 0.86 mmol) in 5 mL of dichloromethane was added 5 mL of trifluoroacetic acid. The solution was stirred at room temperature for 0.5 h, and the solvent was removed. Water (10 mL) was added, and the solution was extracted with ethyl acetate (3 × 20 mL). The phases were separated, and the organics were washed with brine (2 × 20 mL), dried with sodium sulfate, and evaporated in vacuo to afford a white solid (75 mg, 70%), mp 85–87°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.18–0.26 (m, 2 H, H-2′ a, H-3′ a), 0.49–0.56 (m, 2 H, H-2′ b, H-3′ b), 0.84–0.88 (m, 1 H, H-1′), 1.90–1.93 (m, 1 H, H-4a), 2.29–2.42 (m, 3 H, H-4b, H-3), 2.97–3.01 (m, 1 H, H-5), 6.55 (bs, 1 H, NH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 2.04 (C-2′), 3.10 (C-3′), 16.52 (C-1′), 27.60 (C-4), 30.07 (C-3), 59.81 (C-5), 178.46 (C-2). HRMS (EI): calculated for $C_7H_{11}NO$ M^+125.0835, found 125.0835.

4-Amino-4-cyclopropylbutyric Acid (3)

A solution of **8** (43 mg, 0.3 mmol) in 6 mL of hydrochloric acid (4 N) was heated at 100°C for 4 h. The solution was washed with methylene chloride (4 × 20 mL), and it was loaded onto 2 g of BioRad AG50W-X8 ion-exchange resin. The column was washed with water until the eluent was neutral, then it was eluted with 0.2 N ammonium hydroxide to give a yellow solid. Recrystallization with ethanol afforded a white solid (46 mg, 94%), mp 187–188°C. ^{1}H NMR (CD_3OD, 400 MHz) δ 0.38–0.43 (m, 2 H, H-2′), 0.60–0.73 (m, 2 H, H-3′), 0.84–0.92 (m, 1 H, H-1′), 1.93–2.06 (m, 2 H, H-3), 2.43–2.56 (m, 3 H, H-2, H-4). ^{13}C NMR (CD_3OD, 100 MHz) δ 3.76 (C-2′), 5.34 (C-3′), 14.62 (C-1′), 30.22 (C-3), 31.00 (C-2), 58.16 (C-4), 176.12 (C-1). HRMS (ESI): calculated for $C_7H_{14}NO_2$ $(M + H)^+$ 144.1021, found 144.1024.

3-Benzyloxypropanol (9)

3-Benzyloxypropanol was prepared from the monobenzylation of 1,3-propanediol by the procedure of Wei *et al.*[23] Sodium hydride (60% dispersion in mineral oil, 1.6 g, 40 mmol) was dissolved in 25 mL of anhydrous DMSO, and to this solution was added 1, 3-propanediol (2.9 mL, 40 mmol) at room temperature. The mixture was stirred for 30 min, and benzyl chloride (4.6 mL, 40 mmol) was added dropwise over a period of 10 min. After stirring further for 3 h, the reaction was quenched with saturated ammonium chloride solution (20 mL), and the organics were diluted with ether (200 mL), washed with water (7 × 30 mL), dried with sodium sulfate, and evaporated to leave a yellow oil. Chromatography (ethyl acetate/hexanes 1:3) afforded the title compound as a yellow oil (2.56 g, 40%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.85–1.91 (quintet, 2 H, J = 5–6 Hz, H-2), 2.27–2.30 (t, 1 H, J = 5–6 Hz, OH), 3.67–3.70 (t, 2 H, J = 5–6 Hz, H-3), 3.78–3.42 (q, 2 H, J = 5–6 Hz, H-1), 4.54 (s, 2 H, OCH_2Ph), 7.30–7.38 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 32.40 (C-2), 62.04 (C-3), 69.54 (C-1), 73.45

(OCH_2Ph), 127.71 (Ph), 127.77 (Ph), 128.50 (Ph), 138.10 (Ph).

3-Benzyloxypropanal (10)

The compound was prepared by a Swern oxidation of **9**.[24,25] To a Dry Ice-acetone cooled solution of oxalyl chloride in dichloromethane (2.0 M, 7.5 mL, 15.0 mmol) was added dropwise a solution of anhydrous methyl sulfoxide (2.6 mL, 36.0 mmol) in dry dichloromethane (10 mL) during 15 min, and the mixture was stirred for 15 min. A solution of **9** (1.19 g, 7.2 mmol) in dry dichloromethane (10 mL) was added dropwise over a period of 15 min, followed by the addition of triethylamine (5.0 mL, 36.0 mmol) during 10 min after the mixture had been stirred for an additional 15 min. The mixture was slowly warmed to room temperature over 1 h, and the reaction was quenched with water (20 mL) and extracted with methylene chloride. The organics were washed with water (3 × 20 mL), dried with sodium sulfate, concentrated and chromatographed (ethyl acetate/hexanes 1:7) to afford the desired product as a colorless oil (0.97 g, 82%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.70–2.73 (m, 2 H, H-2), 3.82–3.84(t, 2 H, J = 6.2 Hz, H-3), 4.55 (s, 2 H, OCH_2Ph), 7.29–7.38 (m, 5 H, Ph), 9.81 (s, 1 H, CHO). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 44.05 (C-2), 64.03 (C-3), 73.46 (OCH_2Ph), 127.91 (Ph), 127.98 (Ph), 128.65 (Ph), 138.04 (Ph), 201.24 (CHO).

3-Benzyloxy-1-(1′-cyanocyclopropyl)propanol (12)

To a Dry Ice-acetone cooled solution of **10** (0.45 g, 2.7 mmol) and **11** (0.14 g, 1.0 mmol) in anhydrous THF (10 mL) was added dropwise over a period of 10 min a solution of tetrabutylammonium triphenyldifluorosilicate (0.65 g, 1.2 mmol) in THF (15 mL). The reaction mixture was allowed to warm up slowly to room temperature and was stirred overnight, then it was diluted with ether. The solution was chilled to remove most of the solid, and the filtrate was washed with 3 N hydrochloric acid and brine. After being dried with magnesium sulfate, the solvent removed under reduced pressure, and purification by column chromatography (ethyl acetate/hexanes, 1:3), the title compound was obtained as a yellow oil (0.10 g, 43%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.98–1.26 (m, 4 H, H-2′, H-3′), 2.00–2.16 (m, 2 H, H-2), 3.43–3.44 (d, 1 H, J = 3.2 Hz, OH), 3.49–3.52 (m, 1 H, H-1), 3.67–3.84 (m, 2 H, H-3), 4.53 (s, 2 H, OCH_2Ph), 7.31–7.36 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.15 (C-2′), 11.79 (C-3′), 15.97 (C-1′), 34.85 (C-2), 68.69 (C-1), 72.95 (C-3), 73.70 (OCH_2Ph), 121.98 (CN), 127.87 (Ph), 128.06 (Ph), 128.62 (Ph), 137.40 (Ph). HRMS (EI): calculated for $C_{14}H_{17}NO_2$ M^+ 231.1254, found 231.1256.

3-Benzyloxy-*N*-*tert*-butoxycarbonyl-*N*-ethoxyoxoacetyl-1-(1′-cyanocyclopropyl)propylamine (13)

Diethyl azodicarboxylate (0.16 mL, 1.0 mmol) was added to a solution of **12** (0.10 g, 0.4 mmol), triphenylphosphine (0.27 g, 1.0 mmol) and ethyl *N*-*tert*-butoxycarbonyloxamate[19] (0.21 g, 1.0 mmol) in 10 mL of anhydrous THF, and the solution was refluxed for 9 h. The solvent was removed at reduced pressure, and the residual oil was chromatographed (ethyl acetate/hexanes, 1:5) to afford the title compound as a yellow oil (0.12 g, 64%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.07–1.30 (m, 4 H, H-2′, H-3′), 1.35–1.38 (t, 3 H, J = 7.2 Hz, OCH_2CH_3), 1.55 (s, 9 H, $COOC(CH_3)_3$), 2.28–2.33 (m, 1 H, H-2a), 2.51–2.55 (m, 1 H, H-2b), 3.44–3.62 (m, 2 H, H-3), 4.20–4.23 (t, 1 H, J = 8.0 Hz, H-1), 4.32–4.36 (q, 2 H, J = 7.2 Hz, OCH_2CH_3), 4.40–4.42 (d, 1 H, J = 12.0 Hz, OCH_2Ph), 4.48–4.50 (d, 1 H, J = 12.0 Hz, OCH_2Ph), 7.26–7.35 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.03 (C-2′), 13.96 (C-3′), 14.04 (C-1′), 18.52 (OCH_2CH_3), 27.88 ($(CH_3)_3$), 31.40 (C-2), 55.00 (C-1), 62.51 (OCH_2CH_3), 66.14 (C-3), 73.36 (OCH_2Ph), 87.10 ($OCMe_3$), 122.16 (CN), 127.83 (Ph), 127.97 (Ph), 128.52 (Ph), 138.03 (Ph), 151.89 (*t*-BuOOC), 161.47 (EtOCOCO), 164.37 (EtOCOCO).

3-Benzyloxy-*N*-*tert*-butoxycarbonyl-1-(1′-cyanocyclopropyl)propylamine (14)

To a solution of **13** (0.60 g, 1.4 mmol) in THF (10 mL) was added a solution of lithium hydroxide (0.24 g, 10.0 mmol) in water (5 mL), and the mixture was stirred at room temperature overnight. Water (15 mL) was added, and the organics were extracted with ethyl acetate (4 × 20 mL), washed with brine (2 × 20 mL), dried with sodium sulfate, concentrated and chromatographed (ethyl acetate/hexanes, 1:8) to afford a white solid (0.25 g, 54%); mp 78–79°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91–1.32 (m, 4 H, H-2′, H-3′), 1.44 (s, 9 H, $COOC(CH_3)_3$), 1.97–2.09 (m, 2 H, H-2), 3.37–3.42 (dd, 1 H, J = 14.0 Hz, 8.8 Hz, H-1), 3.55–3.65 (m, 2 H, H-3), 4.45–4.48 (d, 1 H, J = 12.0 Hz, OCH_2Ph), 4.49–4.52 (d, 1 H, J = 12.0 Hz, OCH_2Ph), 5.07–5.09 (d, 1 H, J = 7.2 Hz, NH), 7.31–7.36 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz) 12.76 (C-2′), 12.84 (C-3′), 15.88 (C-1′), 28.48 ($(CH_3)_3$), 33.86 (C-2), 52.44 (C-1), 66.67 (C-3), 73.38 (OCH_2Ph), 79.87 ($OCMe_3$), 122.00 (CN), 127.79 (Ph), 128.51 (Ph), 137.99 (Ph), 155.54 (t-BuOOC). HRMS (ESI): calculated for $C_{19}H_{26}N_2O_3Na$ $(M + Na)^+$ 353.1841, found 353.1848.

N-*tert*-Butoxycarbonyl-1-(1′-cyanocyclopropyl)-3-hydroxypropylamine (15)

Palladium hydroxide, 20 wt. % on carbon (40 mg), was added to a mixture of **14** (0.17 g, 0.51 mmol) and cyclohexene (5 mL) in ethanol (10 mL), and the suspension was refluxed overnight. The mixture was filtered through a pad of Celite, and the residue was washed with ethyl acetate. The filtrate was dried

with sodium sulfate and evaporated to give the title compound as a colorless oil (0.12 g, 95%), which solidified upon standing; mp 97–99°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.99–1.35 (m, 4 H, H-2′, H-3′), 1.46 (s, 9 H, $COO(CH_3)_3$), 1.78–2.06 (m, 2 H, H-2), 2.67 (bs, 1 H, OH), 3.24–3.30 (dt, 1 H, J = 9.8 Hz, 3.6 Hz, H-1), 3.71 (m, 2 H, H-3), 4.96-4.98 (d, 1 H, J = 9.2 Hz, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.63 (C-2′), 13.68 (C-3′), 15.98 (C-1′), 28.45 ($(CH_3)_3$), 36.94 (C-2), 52.26 (C-1), 58.57 (C-3), 80.75 ($OCMe_3$), 121.70 (CN), 156.49 (*t*-BuOOC).

3-(*tert*-Butoxycarbonylamino)-3-(1′-cyanocyclopropyl)propanoic Acid (16)

A flask equipped with a magnetic stirrer was charged with **15** (0.12 g, 0.5 mmol), carbon tetrachloride (2.0 mL), acetonitrile (2.0 mL), water (3.0 mL), sodium periodate (0.22 g, 1.0 mmol), and ruthenium (III) chloride hydrate (11 mg, 50 μmol as the monohydrate). The mixture was stirred at room temperature for 10 h, then the reaction was quenched with aqueous sodium hydroxide solution (0.5 M, 20 mL). The aqueous solution was first washed with methylene chloride (4 × 20 mL), and the organic solution was discarded. The aqueous layer was acidified to about pH 3 by the addition of 3 N hydrochloric acid and was extracted with methylene chloride (4 × 20 mL). The combined organics were washed with brine once (30 mL), dried with sodium sulfate, and evaporated *in vacuo* to afford the title compound as a white solid (96 mg, 75%); mp 167–169°C. ^{1}H NMR (CD_3OD, 400 MHz) δ 0.98–1.24 (m, 4 H, H-2′, H-3′), 1.39 (s, 9 H, $COOC(CH_3)_3$), 2.58–2.73 (m, 2 H, H-2), 3.58–3.64 (dt, 1 H, J = 8.8 Hz, 5.2 Hz, H-3), 7.12–7.14 (d, 1 H, J = 9.0 Hz, NH). ^{13}C NMR (CD_3OD, 125 MHz) δ 13.18 (C-2′), 13.50 (C-3′), 17.03 (C-1′), 28.79 ($(CH_3)_3$), 39.01 (C-2), 52.69 (C-3), 80.66 ($OCMe_3$), 122.76 (CN), 157.91 (*t*-BuOOC), 173.50 (COOH).

3-Amino-3-(1′-cyanocyclopropyl)propanoic Acid (4)

To a solution of **16** (20 mg, 79 μmol) in methylene chloride (5 mL) was added an equal volume of trifluoroacetic acid, and the solution was stirred at room temperature for 5 h. The solvent was removed, and the residue was redissolved in water and loaded onto 1 g of BioRad AG50W-X8 ion-exchange resin. The column was washed with water until the eluent was neutral, and it was eluted with 0.1 N hydrochloric acid to give a colorless oil, which was crystallized with methanol/ethyl acetate to give a white solid as the hydrochloric acid salt of the title compound(15 mg, 100%), mp 168–170°C. ^{1}H NMR (CD_3OD, 500 MHz) δ 1.01–1.47 (m, 4 H, H-2′, H-3′), 2.83–2.87 (dd, 1 H, J = 17.0 Hz, 3.7 Hz, H-2a), 2.95–2.98 (dd, 1 H, J = 9.5 Hz, 3.7 Hz, H-2b), 3.07–3.12 (dd, 1 H, J = 17.0 Hz, 9.5 Hz, H-1). ^{13}C NMR (CD_3OD, 125 MHz) δ 13.60 (C-2′ and C-3′), 16.90 (C-1′), 36.36 (C-2), 56.06 (C-1), 121.87 (CN), 178.28 (COOH). HRMS (EI): calculated for $C_7H_{10}N_2O_2$ M^+ 154.0737, found 154.0732.

1-(1′-Cyanocyclopropyl)-4-pentenol (17)

A solution of tetrabutylammonium triphenyldifluorosilicate (5.4 g, 10.0 mmol) in anhydrous THF (50 mL) was added dropwise over a period of 15 min to a mixed solution of 1-(trimethylsilyl)cyclopropanenitrile (**11**, 1.3 g, 9.4 mmol) and 4-pentenal (1.7 g, 20.0 mmol) in THF (30 mL). The reaction mixture quickly turned yellow and was stirred at room temperature overnight. Following the same work-up procedure as in the preparation of **12** the title compound was obtained as a clear oil (0.70 g, 50%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91–1.26 (m, 4 H, H-2′, H-3′), 1.80–1.86 (q, 2 H, J = 7.2 Hz, H-2), 2.14–2.26 (m, 2 H, H-3), 2.66 (bs, 1 H, OH), 3.06–3.09 (t, 1 H, J = 6.4 Hz, H-1), 4.98–5.01 (d, 1 H, J = 10.4 Hz, *cis*-H-5), 5.03–5.08 (d, 1 H, J = 16.8 Hz, *trans*-H-5), 5.75–5.85 (m, 1 H, H-4). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.18 (C-2′), 12.26 (C-3′), 16.78 (C-1′), 30.01 (C-2), 34.77 (C-3), 73.70 (C-1), 115.60 (C-5), 121.75 (CN), 137.47 (C-4).

N-*tert*-Butoxycarbonyl-*N*-ethoxyoxoacetyl-1-(1′-cyanocyclopropyl)-4-pentenyl-amine (18)

The same procedure for the preparation of **13** was followed starting with **17** (2.5 g, 16.6 mmol) and 1.2 equivalent of all the other reactants, affording a colorless oil (3.2 g, 56%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.05-1.45 (m, 4 H, H-2′, H-3′), 1.36–1.38 (t, 3 H, J = 7.2 Hz, OCH_2CH_3), 1.58 (s, 9 H, $COOC(CH_3)_3$), 2.04–2.43 (m, 4 H, H-2, H-3), 3.93–3.96 (dd, 1 H, J = 9.2 Hz, 5.7 Hz, H-1), 4.32–4.37 (q, 2 H, J = 7.2 Hz, OCH_2CH_3), 5.02–5.04 (d, 1 H, J = 10.0 Hz, *cis*-H-5), 5.05–5.08 (d, 1 H, J = 17.0 Hz, *trans*-H-5), 5.72–5.80 (m, 1 H, H-4). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.02 (C-2′), 14.01 (C-3′), 14.39 (C-1′), 18.67 (OCH_2CH_3), 27.97 ($(CH_3)_3$), 30.32 (C-2), 32.36 (C-3), 57.26 (C-1), 62.61 (OCH_2CH_3), 87.30 ($OCMe_3$), 116.55 (C-5), 122.14 (CN), 136.57 (C-4), 151.90 (*t*-BuOOC), 161.48 (EtOCOCO), 164.47 (EtOCOCO).

4-(*tert*-Butoxycarbonylamino)-4-(1′-cyanocyclopropyl)butyric Acid (19)

Following the same procedure as described above for the preparation of **16**, compound **18** (1.40 g, 4.0 mmol) was oxidized to give a red solid, which was flash chromatographed (ethyl acetate/hexanes, 1:5 to pure ethyl acetate) to afford a white solid as the title compound (0.89 g, 83%), mp 113–115°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.98–1.28 (m, 4 H, H-2′, H-3′), 1.43 (s, 9 H, $COOC(CH_3)_3$), 1.93–2.14 (m, 2 H, H-3), 2.44–2.47 (t, 2 H, J = 6.8 Hz, H-2), 3.16–3.21 (dt, 1 H, J = 9.6 Hz, 3.9 Hz, H-4), 4.94–4.96 (d, 1 H, J = 9.6 Hz, NH), 10.25 (bs, 1 H, COOH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.10 (C-2′), 13.40 (C-3′), 16.62 (C-1′), 28.44 ($(CH_3)_3$), 28.77 (C-3), 30.51 (C-2), 54.40

(C-4), 80.53 ($OCMe_3$), 121.55 (CN), 155.80 (*t*-BuOOC), 177.62 (COOH). HRMS (ESI): calculated for $C_{13}H_{20}N_2O_4Na$ $(M + Na)^+$ 291.1321, found 291.1329.

4-Amino-4-(1′-cyanocyclopropyl)butyric Acid (**5**)

Hydrogen chloride in 1,4-dioxane (4.0 M, 10 mL) was added to **19** (0.73 g, 2.7 mmol), and the solution was stirred at room temperature overnight. Filtration afforded a white powder, which was washed with methylene chloride and purified by cation-exchange chromatography as described for the preparation of **3** to give a white solid (0.36 g, 79%), mp 204–205°C. ^1H NMR (CD_3OD, 400 MHz) δ 1.22–1.52 (m, 4 H, H-2′, H-3′), 2.15–2.21 (ddd, 2 H, $J = 14.5$ Hz, 7.3 Hz, 2.4 Hz, H-3), 2.54–2.59 (m, 2 H, H-2), 2.84–2.88 (t, 1 H, $J = 7.2$ Hz, H-4). ^{13}C NMR (CD_3OD, 100 MHz) δ 14.07 (C-2′), 14.43 (C-3′), 16.09 (C-1′), 28.88 (C-3), 30.64 (C-2), 56.55 (C-4), 120.34 (CN), 175.31 (COOH). HRMS (FAB): calculated for $C_8H_{13}N_2O_2$ $(M + H)^+$ 169.0977, found 169.0975.

Enzyme and Assays

GABA aminotransferase was isolated from pig brain by the published procedure.[26] Succinic semialdehyde dehydrogenase (SSDH) was isolated from GABAse, a commercially available mixture of SSDH and GABA-AT, using the method of Jeffery *et al.*[27] GABA-AT activity was assayed using a modification of the coupled assay of Scott and Jakoby.[28] The assay solution had final concentrations of 11 mM GABA, 1.1 mM $NADP^+$, 5.3 mM α-ketoglutarate, 2 mM β-mercaptoethanol, and excess SSDH in 50 mM potassium pyrophosphate buffer at pH 8.5. With this assay, the change in absorbance at 340 nm, corresponding to the formation of NADPH from $NADP^+$ at 25°C is proportional to the GABA-AT activity.

Time-Dependent Inactivation Test of GABA-AT by 2–5

GABA aminotransferase (16.7 μM, 20 μL) was added to solutions of **2–5** (100 μL final volume, 10 mM) in 50 mM potassium pyrophosphate buffer, pH 8.5, containing 15 mM α-ketoglutarate and 1 mM β-mercaptoethanol at 25°C. At timed intervals, aliquots (20 μL) were withdrawn and added to the assay solution (575 μL) followed by the addition of SSDH (5 μL, excess amount), and reaction rates were measured spectrophotometrically at 340 nm. Unfortunately, with incubation times of more than 24 h, none of the compounds exhibited time-dependent inhibition of enzyme activity.

Competitive Inhibition of GABA-AT by 2–5

The activity of GABA-AT (16.7 μM, 4 μL) at 25°C in 600 μL of 50 mM potassium pyrophosphate buffer (pH 8.5) containing excess SSDH, 5 mM α-ketoglutarate, and 1 mM $NADP^+$ was determined upon the introduction of varying concentrations of **2–5** at different GABA concentrations. The percentage of inhibition was obtained by comparison to an untreated enzyme control. It was found that **2** was not an inhibitor of GABA-AT, whereas the other three compounds were poor competitive inhibitors of the enzyme (data from Cornish–Bowden plot[29] for the determination of the type of inhibition not shown). When the GABA concentration were equal to the concentrations of **3–5** (10 mM), the enzyme still retained 88%, 82% and 76% of it's original activity, respectively. Based on the following equation: % Inhibition $= 100[I]/\{[I] + K_i(1 + [GABA]/K_m)\}$,[30] the K_i values of **3–5** were estimated to be more than 10 mM.

Substrate Activity Test of 2–5

Compounds **2–5** (2.5 mM) were individually incubated for 48 h with GABA-AT (16.7 μM, 3 μL) in 50 mM potassium pyrophosphate buffer, pH 8.5, containing 2.0 mM β-mercaptoethanol in a total volume of 100 μL in the presence of 2.8 mM [^{14}C]-α-ketoglutarate at 25°C. A control containing the above but without the expected substrate was run in parallel. No [^{14}C]-L-glutamate was detected.

RESULTS AND DISCUSSION

The four cyclopropane-containing analogs of vigabatrin are neither inactivators nor substrates of GABA-AT. Compounds **3–5** are weak reversible inhibitors of GABA-AT, whereas **2** has no effect on the activity of the enzyme, even at a concentration of 10 mM.

Both of the compounds containing a cyano group (**4** and **5**) are more potent than those without (**2** and **3**), suggesting some additional binding interaction of the cyano group with the enzyme. Also, the GABA analogs (**3** and **5**) are more potent than their corresponding β-alanine analogs (**2** and **4**) probably because GABA is a better substrate for GABA-AT than is β-alanine.

Because none of these compounds inactivates GABA-AT, their substrate activity was determined. None of the compounds is a substrate, which accounts for why they do not inactivate the enzyme. In order for inactivation to occur, the proton adjacent to the amino group has to be removed. It is not known if Schiff base formation occurs, but if it does, it appears that the proton that needs to be removed is

misoriented, so that Lys329 is not able to remove it or, if it does, tautomerization, leading to transamination cannot occur. Apparently, the cyclopropane ring is interfering with this process.

In conclusion, a new class of cyclopropane-containing compounds was synthesized as potential mechanism-based inactivators of GABA-AT, based on the well-known mechanism of inactivation of GABA-AT by the corresponding vinyl analog, vigabatrin. Despite the mechanistic analogy between activated cyclopropanes and activated alkenes, these compounds are neither inactivators nor substrates. However, we have shown that they are competitive inhibitors, suggesting that they bind in the substrate binding site. Possibly the cyclopropane ring is sterically responsible for the lack of catalytic activity.

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