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Conformationally Restricted GABA with Bicyclo[3.1.0]hexane Backbone as the First Highly Selective BGT-1 Inhibitor

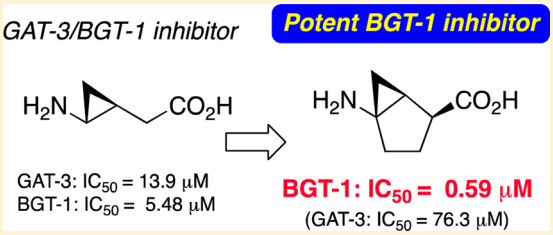
Takaaki Kobayashi,[†] Akihiro Suemasa,[†] Arisa Igawa,[†] Soichiro Ide,[†] Hayato Fukuda,[†] Hiroshi Abe,[†] Mitsuhiro Arisawa,[†,§] Masabumi Minami,[†] and Satoshi Shuto*[,†,‡]

[†]Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

[‡]Center for Research and Education on Drug Discovery, Hokkaido University, Kita-ku, Sapporo 060-0812, Japan

Supporting Information

ABSTRACT: On the basis of the three-dimensional diversity-oriented conformational restriction strategy using key chiral cyclopropane units, we previously identified **3** ((2*S*,3*R*)-4-amino-3,4-methanobutyric acid) with a chiral *trans*-cyclopropane structure as a γ-aminobutyric acid (GABA) transporter inhibitor selective for GABA transporter (GAT) subtypes GAT-3 and BGT-1 (betaine/GABA transporter-1). Further conformational restriction of **3** with the rigid bicyclo[3.1.0]hexane backbone led to the successful development of the first highly potent and selective BGT-1 inhibitor **4** (IC_{50} = 0.59 μM). The bioactive conformation of **3** for BGT-1 was also identified.



KEYWORDS: *BGT-1, cyclopropane, conformational restriction, GABA, GAT*

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system, whose activity in the synaptic cleft is terminated upon its reuptake by the transporters of GABAergic neuronal cells and astroglial cells.[1−3] Inhibition of GABA reuptake by its transporters is thought to be an effective drug development strategy targeting the GABAergic neuronal system.[4−7] Four GABA transporter (GAT) subtypes, i.e., GAT-1, GAT-2, GAT-3, and betaine/GABA transporter-1 (BGT-1) are expressed in the brain.[8] The representative GAT inhibitors are shown in Figure 1. Tiagabine, a selective GAT-1 inhibitor, is clinically effective for the treatment of epilepsy.[9,10] Successful development of potent and highly selective inhibitors of GABA transporters other than GAT-1, however, has not been achieved. EF1502,[11−13] NNC05-2090,[14,15] and tiagabine analogue **A**[16] are selective inhibitors for BGT-1; however, the selectivity is not high enough for BGT-1. Here we report the successful development of the first potent GABA transporter inhibitor highly selective for BGT-1.

Although transporters, including GABA transporters, are important targets for drug development, their structural analysis is often hampered by the membranous nature of these proteins, compared with that of proteins soluble in blood or cytosol. Therefore, structural data on the target transporters are generally poor, and an effective method to identify compounds targeting these proteins without knowledge of their structural data is needed for drug development. Therefore, we previously developed the four types of chiral cyclopropane units bearing two adjacent substituents in a trans or a cis relationship, namely, **1** and **2**, and their enantiomers ***ent*-1** and ***ent*-2** (Figure 2).[17] These units are effectively used for three-dimensionally diverse conformational restriction of biologically active

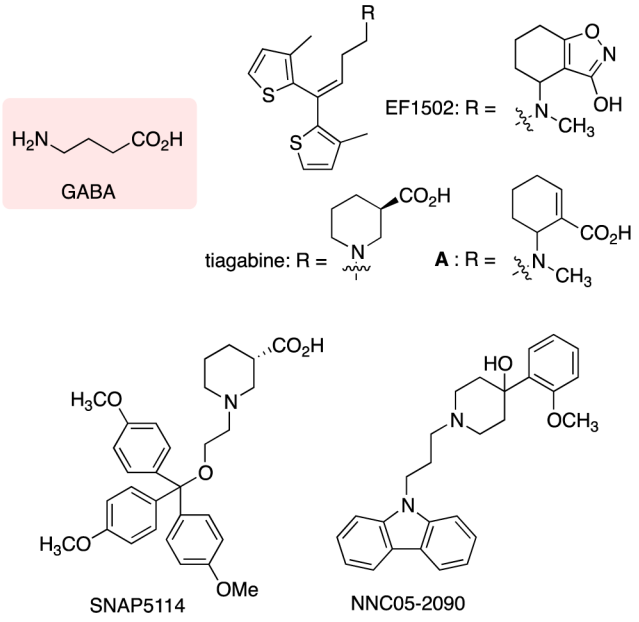


Figure 1. GABA and representative GAT inhibitors.

compounds,[18] and therefore ,we previously designed a series of cyclopropane-based conformationally restricted GABA analogues with stereochemical diversity to develop useful GABA transporter inhibitors.[19] Although some of these

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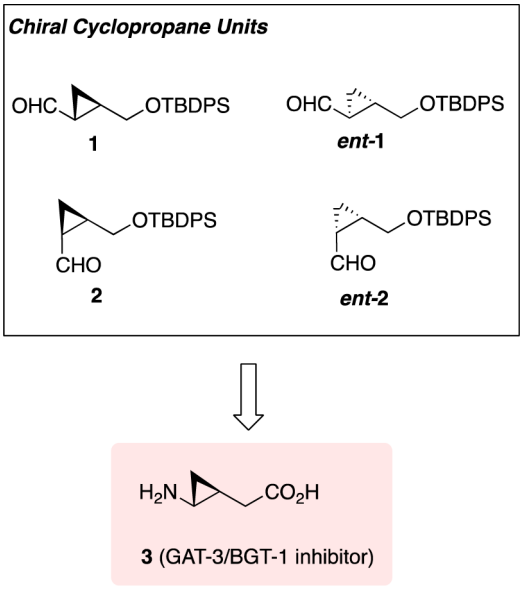

Figure 2. Chiral cyclopropane units and the conformationally restricted analogue **3** with GAT-3/BGT-1 inhibitory activity derived therefrom.

cyclopropane-based conformationally restricted GABA analogues have been synthesized by other groups,[20−27] we systematically synthesized all of the 2,3-methano- and 3,4-methano stereoisomers of GABA from the chiral cyclopropane units **1** and **2**, and their enantiomers ***ent*-1** and ***ent*-2**. As a result, we identified **3** ((2*S*,3*R*)-4-amino-3,4-methanobutyric acid) with a chiral *trans*-cyclopropane structure as a selective inhibitor for GAT-3 and BGT-1, although its potency and selectivity might not be high enough.[19] Although these units were shown to be actually useful,[18] they are still too flexible to fully restrict movement of the $-CH_2-$ moiety adjacent to the cyclopropane ring.

A characteristic structural feature of cyclopropane is that cis-oriented adjacent substituents on the ring exert significant mutual steric repulsion because they are fixed in an eclipsed orientation, which we previously termed cyclopropylic strain.[27] Conformation of the substituents on a cyclopropane can be restricted so that steric repulsion due to the strain is minimal, as shown in Figure 3. Accordingly, in inhibitor **3**, bond rotation

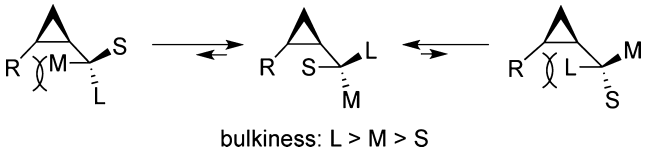

Figure 3. Cyclopropylic strain-based conformational restriction.

between the cyclopropane (C1) and its adjacent carbon (C1′) would be restricted by the strain as shown in Figure 4. Therefore, the two conformers **3** (syn, the cyclopropane ring "up"/the side chain $-CO_2H$ "up") and **3** (anti, the cyclopropane ring "up"/the side chain $-CO_2H$ "down") would be preferable to the conformer **3** (eclipse) due to the significant steric repulsion between the $-CO_2H$ and the adjacent cis-oriented H-2 in conformer **3** (eclipse). Importantly, the restriction of the special positioning of the CO_2H group differs in the preferred conformers **3** (syn) and **3** (anti).

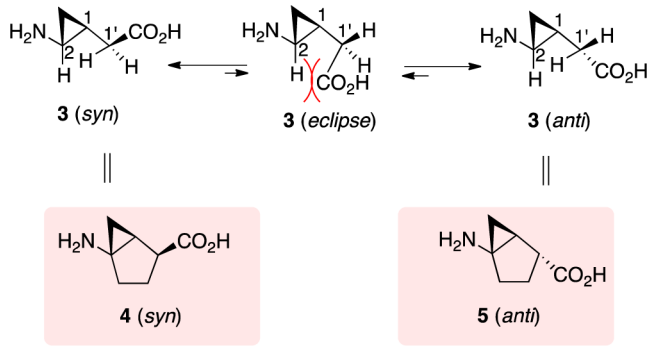

Figure 4. Conformational restriction of **3** in the syn- or anti-form due to the cyclopropylic strain and its analogues **4** and **5** rigidly restricted in the syn- or anti-form, respectively, by the two-ring system.

We computationally analyzed the conformations of **3** by density functional theory (B3LYP/6-31G*) using Spartan '08 software (full edition for Windows OS, Wave Function Inc.), and the results are shown in Figure 5. As we expected, two

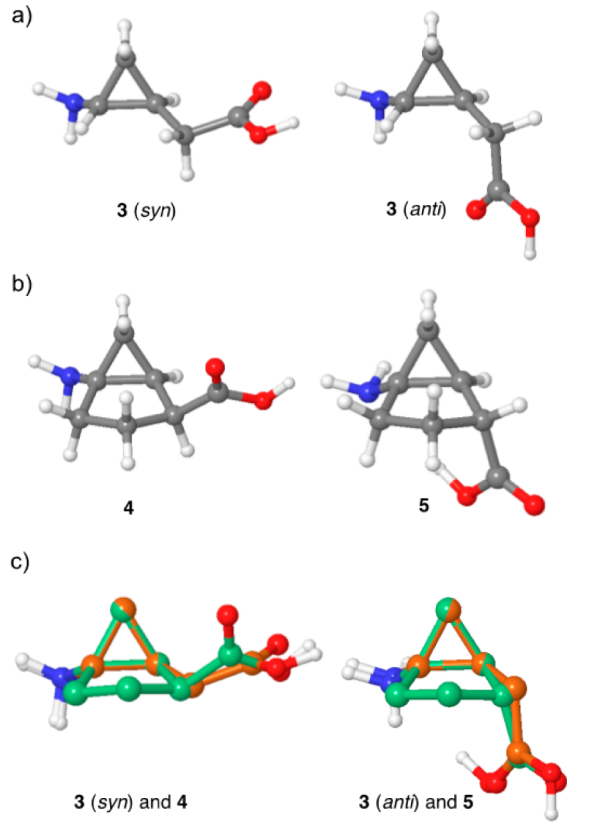

Figure 5. Stable conformation of **3**, **4**, and **5** by conformational analysis of compounds by density functional theory: (a) **3** (syn) and **3** (anti); (b) **4** and **5**; (c) superimposition of the stable conformation of compounds, left [**3** (syn) and **4**] and right [**3** (anti) and **5**].

significantly stable structures, corresponding to the syn- and the anti-forms in Figure 5a, respectively, were obtained. The two conformers are nearly equally stable, where the anti-conformer is slightly more stable than the syn-conformer (ΔE < 1 kcal/mol) and the eclipse conformer is significantly less stable (ΔE > 5 kcal/mol) than the two conformers. Thus, the results of the calculations are consistent with the above hypothesis for the conformation of **3** based on the cyclopropylic strain (Figure 4),

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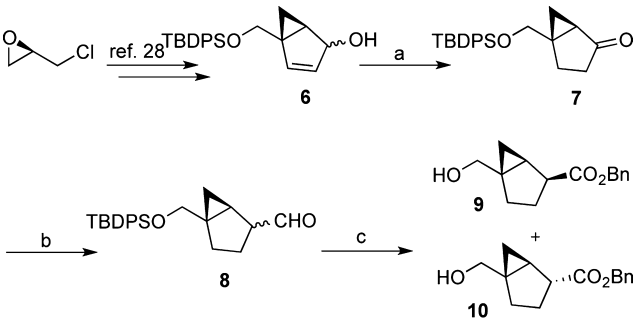
which suggests that the bioactive conformations binding to the GABA transporters are analogous to either the syn-form or the anti-form.

On the basis of these results, we designed other conformationally restricted analogues **4** and **5** (Figure 4). Because of the rigid bicyclo[3.1.0]hexane ring system, in analogues **4** and **5**, the side-chain moiety is restricted rigidly in the syn-form and the anti-form, respectively.

The conformation of **4** and **5** was analyzed by the above-described computational method, and one significantly stable structure was obtained for the two compounds (Figure 5b). The most stable conformers of **4** and **5** are well-superimposable onto the *syn*-form of **3** and the *anti*-form of **3**, respectively (Figure 5c). Accordingly, biological evaluation of the two compounds **4** and **5** might provide information on the bioactive conformation of **3** and also allow us to develop a potent GAT inhibitor highly selective for GAT-3 or BGT-1.

The key chiral units **9** and **10** with the bicyclo[3.1.0]hexane backbone, which had all of the asymmetric centers in the targets **4** and **5**, respectively, were prepared as shown in Scheme 1. We

Scheme 1. Synthesis of the Key Units 9 and 10[a]

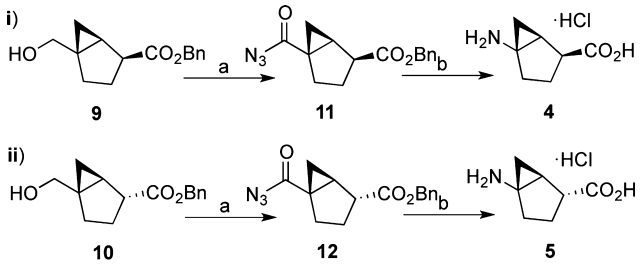


[a]Conditions: (a) $Ru(CO)HCl(PPh_3)_3$, toluene, reflux, 94%; (b) (1) $MeOCH_2PPh_3Cl$, NaHMDS, THF, 0 °C, (2) aq. HCl, THF, 0 °C; (c) (1) NaClO, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH, H_2O, 0 °C, (2) benzyl chloroformate, Et_3N, DMAP, CH_2Cl_2, 0 °C to r.t., (3) 3HF·Et_3N, THF, r.t., 16% (**9**) from **7**, 13% (**10**) from **7**.

selected a diastereomeric mixture of alcohol **6**[28] as an intermediate, which was obtained from (*R*)-epichlorohydrin by the procedure reported by Jeong and co-workers.[28,29] Isomerization of **6** effectively occurred with $Ru(CO)HCl(PPh_3)_3$ as a catalyst to give the ketone **7**. One carbon elongation of **7** by Wittig reaction, followed by acidic treatment, gave the aldehyde **8** as a diastereomeric mixture. Oxidation and benzylation of **8** and subsequent removal of the *O*-silyl protecting group afforded a mixture of the 1′(*S*)-isomer **9** and the 1′(*R*)-isomer **10**, which was obtained in a diastereomerically pure form (de > 99%), respectively, after silica gel column chromatography.

The conformationally restricted analogues **4** and **5** were synthesized as shown in Scheme 2. Successive oxidation under Dess–Martin oxidation and Pinnick oxidation conditions of **9** gave the corresponding carboxylic acid, which was then treated with DPPA, and Et_3N in CH_2Cl_2 produced the acid azide **11**. Pyrolyzation of **11** by refluxing in toluene afforded the corresponding amine, the benzyl group of which was removed with aqueous HCl to give the desired target amino acid **4**[30] as its hydrochloride. Similarly, the diastereomeric amino acid **5**[30] was prepared from **10**. Configurations of the 1′-position in **4**

Scheme 2. Synthesis of the Target Compounds 4 and 5[a]



[a]Conditions i and ii: (a) (1) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0 °C to r.t., (2) NaClO, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH, H_2O, 0 °C, (3) DPPA, Et_3N, CH_2Cl_2, r.t.; (b) (1) toluene, reflux, (2) HCl aq., r.t., 80% (**4**) from **9**, 60% (**5**) from **10**.

and **5** were determined based on their $J_{1,1'}$ values (**4**, $J_{1,1}$ = 4.6 Hz; **5**, $J_{1,1}$ = 0 Hz).[31]

The inhibitory effects of 100 μM GABA analogues on GABA uptake in GAT-1/CHO, GAT-2/CHO, GAT-3/CHO, and BGT-1/CHO cells were examined according to a previously reported method.[19] Of the two newly synthesized analogues, analogue **4** completely inhibited GABA uptake via BGT-1 (97.6%) and moderately inhibited GABA uptake via GAT-1 (12.2%), GAT-2 (29.0%), and GAT-3 (48.2%). Analogue **5** moderately inhibited the GABA uptake via BGT-1 (63.9%) and weakly inhibited GABA uptake via the other subtypes. The parent compound **3** inhibited the GABA uptake via GAT-3 and BGT-1 almost completely consistent with the previous results.[19]

Thus, we next investigated the GABA uptake inhibitory effect of **4** in detail. The potency of analogue **4** (hydrochloride) for the four cloned GAT subtypes was compared with those of the parent compound **3** and some representative GAT inhibitors (Figure 1) with IC_{50} values as summarized in Table 1. Analogue **4** showed high affinity for BGT-1 (IC_{50} = 0.59 μM) and weak affinity for GAT-3 (IC_{50} = 76.3 μM), while it was inactive for GAT-1 and GAT-2 (IC_{50} > 100 μM). To our knowledge, **3** (IC_{50} = 5.48 μM) and NNC 05-2090 (IC_{50} = 5.10 μM) are the two most potent BGT-1 inhibitors reported in the literature,[14,15,19] and therefore, the potency of **4** (IC_{50} = 0.59 μM) is markedly stronger compared with these previous inhibitors. However, a tiagabine analogue **A** was most recently reported as a selective inhibitor for BGT-1 (IC_{50} = 45 μM),[16] but its selectivity index is moderate (GAT-3/BGT-1 = 6.4). The selectivity index of **4** (GAT-3/BGT-1 = 129) clearly surpasses that of tiagabine analogue **A**. Accordingly, **4** was identified as the most potent and selective BGT-1 inhibitor reported to date.

In summary, using a GAT-3/BGT-1 nonselective inhibitor **3**, which is the conformationally restricted analogue of GABA with a chiral *trans*-cyclopropane structure, as a lead compound, we have successfully developed the first highly potent and selective BGT-1 inhibitor **4** using the bicyclo[3.1.0]hexane backbone as the key conformationally restricting ring system. Importantly, these results demonstrate that the bioactive conformation of **3** at BGT-1 would be its syn-form. The inhibitor **4** may be useful for investigating the physiological function of BGT-1 and as a lead structure for developing drugs targeting BGT-1, while further studies to clarify its effects on GABA receptors and transporters are needed. Thus, this study provides important information for medicinal chemical studies to develop useful GAT inhibitors.

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Table 1. IC_{50} Values (μM) of Analogues 3 and 4 and Representative GABA Ligands on GABA Uptake in GAT Subtypes[a]

	GAT-1	GAT-2	GAT-3	BGT-1	GAT-3/BGT-1
3		36.9	13.9	5.48	
	>100	(28.2−48.4)	(3.62−53.4)	(3.77−7.97)	2.54
4			76.3	0.59	
	>100	>100	(44.4−131.2)	(0.28−1.23)	129
SNAP5114		11.9	1.12	71.7	
	>100	(2.00−71.2)	(0.64−1.94)	(22.6−228)	0.015
β-alanine			41.2		
		>100	(10.0−169)		
NNC05-2090	11.4	6.20	8.92	5.10	
	(2.13−60.9)	(3.86−9.96)	(4.30−18.5)	(1.96−13.1)	1.75
A[b]	>100	>100	286	45	6.4

[a]Data are expressed as means ± SEM. [b]Data were taken from ref 16.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details of the synthesis of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(S.S.) E-mail: shu@pharm.hokudai.ac.jp.

Present Address

§(M.A.) Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

BGT-1, betain/GABA transporter-1; CHO, Chinese hamster ovary; DMAP, 4-dimethylaminopyridine; DPPA, diphenylphosphoryl azide; GABA, γ-aminobutylic acid; GAT, GABA transporter; NaHMDS, sodium hexamethyldisilazide; TBDPS, *tert*-butyldiphenylsilyl

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(30) The optical purity was more than 94%ee, see Supporting Information.
(31) Similar determination of configuration in a bicyclo[3.1.0] hexanes ring system, see: Shuto, S.; Ono, S.; Hase, Y.; Kamiyama, N.; Takada, H.; Yamashita, K.; Matsuda, A. Conformational restriction by repulsion between adjacent substituents on a cyclopropane ring: Design and enantioselective synthesis of 1-phenyl-2-(1-aminoalkyl)-cyclopropane-*N*,*N*-diethylcarboxamides as potent NMDA receptor antagonists. *J. Org. Chem.* **1996**, *61*, 915−923.