Design, Synthesis and Evaluation of Substituted Triarylnipecotic Acid Derivatives as GABA Uptake Inhibitors: Identification of a Ligand with Moderate Affinity and Selectivity for the Cloned Human GABA Transporter GAT-3

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γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. Molecular biology has revealed the presence of four high-affinity GABA transporters in the brain, GAT-1, GAT-2, GAT-3, and BGT-1, the latter transporting both GABA and the osmolyte Betaine. We have shown that known GABA uptake inhibitors such as SK&F 89976-A, CI-966, and Tiagabine exhibit high affinity and selectivity for GAT-1. In the present paper we describe the design and synthesis of a novel series of triarylnipecotic acid derivatives for evaluation as GABA uptake inhibitors. The design lead for this series of compounds was the nonselective GABA uptake inhibitor EGYT-3886, [(−)-2-phenyl-2-[(dimethylamino)ethoxy]-(1*R*)-1,7,7-trimethylbicyclo[2.2.1]heptane]. From this series of compounds (*S*)-1-[2-[tris(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic acid, **4(*S*)** was identified as a novel ligand with selectivity for GAT-3. **4(*S*)** displayed an IC_{50} of 5 μM at GAT-3, 21 μM at GAT-2, >200 μM at GAT-1, and 140 μM at BGT-1. This compound will be an important tool for evaluating the role of GAT-3 in neural function.

Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. For most neurotransmitters, including GABA, neurotransmission is terminated by the rapid uptake of neurotransmitter via specific, high-affinity transporters located in the presynaptic terminal and/or surrounding glial cells.[1] Since inhibition of uptake by pharmacologic agents increases the levels of neurotransmitter in the synapse, and thus enhances synaptic transmission, neurotransmitter transporters provide important targets for therapeutic intervention. Inhibitors of GABA transport have anticonvulsant activity in laboratory animals;[2] one such compound, Tiagabine, has displayed anticonvulsant activity in humans in early clinical trials.[3]

The first neurotransmitter transporter to be cloned was a high-affinity GABA transporter, termed GAT-1, which was obtained from rat brain.[4] Subsequently we cloned and pharmacologically characterized two novel rat GABA transporters, termed GAT-2 and GAT-3.[5] An additional member of the GABA transporter family was cloned from dog kidney that transports both the osmolyte betaine and GABA and was thus referred to as BGT-1.[6] It should be noted that a rat clone identical to GAT-3, termed GAT-B, was described by Clark *et al.*[7] and that clones for all four GABA transporters have been identified in mice, although a different nomenclature was employed.[8] We have also cloned the human homologs of GAT-3 (hGAT-3)[9] and BGT-1 (hBGT-1).[10] Because of possible species differences in transporter pharmacology, we use the human homologs of GABA transporters in our drug design program.

Conformationally restrained GABA analogs such as nipecotic acid and guvacine are known to be potent GABA uptake inhibitors.[11] However, determining the efficacy of such compounds *in vivo* has been hampered by their poor penetration of the blood–brain barrier, a property attributable to their high degree of hydrophilicity. In an effort to overcome this problem, Ali *et al.*[12] examined the effect of adding lipophilic side chains to the nitrogen atom of various GABA transport blockers. Surprisingly, the addition of 4,4-diphenyl-3-butenyl side chains to nipecotic acid and guvacine (SK&F 89976-A and SK&F 100330-A, respectively) resulted in a 20-fold increase in potency when tested in brain synaptosomes. Since this report, other groups have synthesized similar derivatives such as CI-966[13] and Tiagabine.[14]

Determining the site of action of transport inhibitors is complicated by the heterogeneity of GABA transporters. We recently examined the potency of the lipophilic inhibitors described above and found that they are all highly selective for GAT-1.[15] These data suggest that their anticonvulsive activity is mediated via inhibition of GABA transport by GAT-1. Due to their specificity for GAT-1, the lipophilic compounds described above are important tools for the study of this transporter but offer little insight into the functions of the other GABA transporters.

In contrast to GAT-1, little is known regarding the functional role(s) of GAT-2 and GAT-3, due in part to the lack of selective probes. We have designed and synthesized a series of triarylnipecotic acid derivatives from which compound **4(*S*)** was identified as a novel compound with selectivity for GAT-3.

Results

To design inhibitors of GAT-2, GAT-3, or BGT-1, we began by examining the potency of known GABA transport inhibitors at the four cloned GABA transporters. As will be described elsewhere[15] and as shown in

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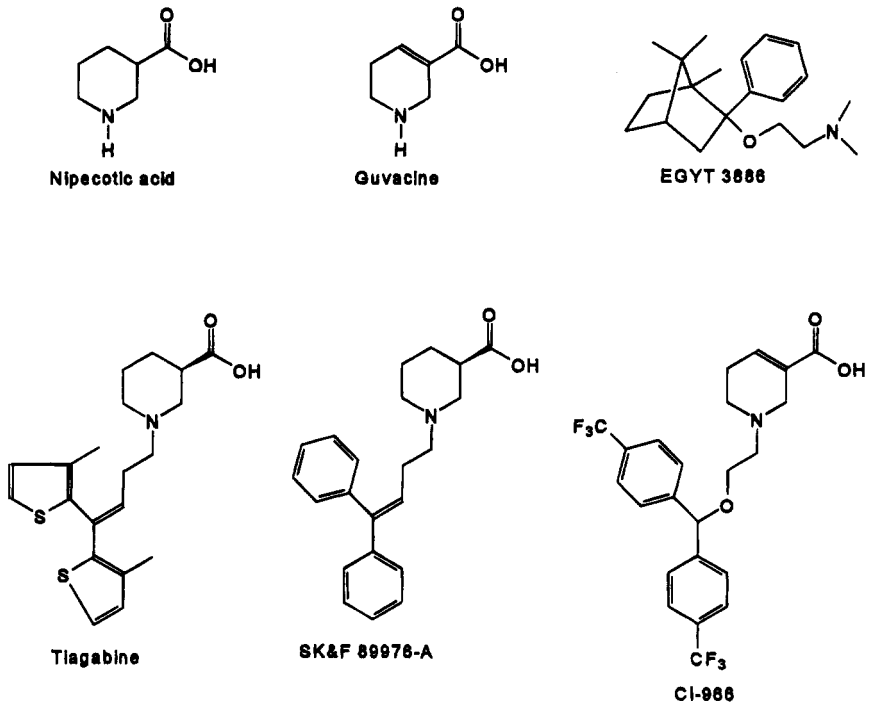
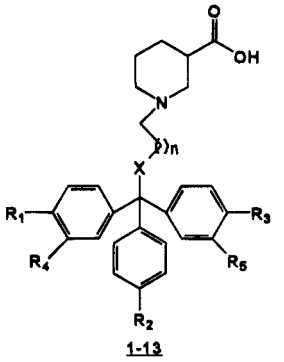


Figure 1. Structures of known GABA transport inhibitors.

Table 1. Method of Synthesis and Physical Data for Compounds **1–17**[a]



compd	R_1	R_2	R_3	R_4	R_5	X	*n*	method of synthesis	HRFABMS (M + 1)[b]/elemental anal.	R_f[c]
1	H	H	H	H	H	O	1	A	415.2142	0.30 (d)
2	H	H	OCH_3	H	H	O	1	A	446.2341	0.32 (d)
3	H	OCH_3	OCH_3	H	H	O	1	A	476.2446	0.36 (d)
4(*R,S*)	OCH_3	OCH_3	OCH_3	H	H	O	1	A	506.2544	0.40 (d)
4(*R*)	OCH_3	OCH_3	OCH_3	H	H	O	1	A	506.2579	0.40 (d)
4(*S*)	OCH_3	OCH_3	OCH_3	H	H	O	1	A	506.2534	0.40 (d)
5	OCH_3	CH_3	CH_3	H	H	O	1	B	474.2650	0.40 (e)
6	F	F	F	H	H	O	1	B	$C_{27}H_{26}NO_3F_3 \cdot 1.2CH_2Cl_2$	0.30 (e)
7	H	H	OCH_3	CF_3	CF_3	O	1	B	$C_{30}H_{29}NO_4 \cdot 3.0CH_2Cl_2$	0.34 (e)
8	H	H	OCH_3	F	F	O	1	B	482.2162	0.33 (e)
9	Cl	Cl	OCH_3	H	H	O	1	B	514.1540	0.42 (f)
10	F	F	OCH_3	H	H	O	1	B	482.2144	0.42 (g)
11	OCH_3	OCH_3	OCH_3	H	H	O	2	A	542.2543 (M + Na)	0.40 (d)
12	H	H	H	H	H	S	1	C	$C_{27}H_{29}NO_2S \cdot 0.75H_2O$	0.32 (d)
13	OCH_3	OCH_3	OCH_3	H	H	S	1	D	522.2291	0.43 (f)
14						O	1	B	444.2180	0.39 (f)
15						O	1	B	414.2098	0.33 (e)
16						O	1	B	430.2015	0.34 (e)
17						O	1	A	$C_{30}H_{33}NO_6 \cdot 0.84CCl_4$	0.29 (e)

[a] For structures **14–17**, refer to Figure 4. [b] Experimental (M + 1) value. [c] TLC system: d = CH_2Cl_2–MeOH–$MeOHNH_3$ (2.0 M), 8:1:1; e = CH_2Cl_2–MeOH–$MeOHNH_3$ (2.0 M), 7.4:1.4:1.2; f = CH_2Cl_2–MeOH–$MeOHNH_3$ (2.0 M), 7.0:1.6:1.4; g = CH_2Cl_2–MeOH–$MeOHNH_3$ (2.0 M), 3:1:1.

Table 2, (±)-nipecotic acid and guvacine display modest selectivity for GAT-1 (IC_{50} = 4–39 μM) as compared to GAT-2 and GAT-3 (IC_{50} = 102–378 μM), whereas both compounds have low affinity at hBGT-1 (IC_{50} > 1 mM). The IC_{50} values of the lipophilic compounds at GAT-1 range between 26 and 260 nM at GAT-1 (Table 1); these compounds are thus 50–200-fold more potent at this transporter than are (±)-nipecotic acid and guvacine.

In contrast to GAT-1, the lipophilic compounds all display low affinity at GAT-2, GAT-3, and BGT-1. As

Table 2. Potency of Compounds at Cloned GABA Transporters

compound	IC_{50}[a] or % inhibition[b]			
	hGAT-1	rGAT-2	hGAT-3	hBGT-1
GABA	5 ± 1 (3)	5 ± 2 (3)	7 ± 1 (4)	36 ± 3 (4)
(*R,S*)-nipecotic acid	8 ± 0.4 (3)	39 ± 4 (4)	106 ± 13 (3)	2370 ± 617 (4)
(*R*)-nipecotic acid	5.9 ± 0.8 (3)	19 ± 2 (3)	51 ± 6 (4)	2310 ± 114 (3)
(*S*)-nipecotic acid	116 ± 8 (3)	763 ± 55 (3)	2320 ± 86 (4)	6410 ± 213 (3)
guvacine	14 ± 3 (3)	58 ± 5 (4)	119 ± 24 (3)	1870 ± 387 (3)
Tiagabine	0.07 ± 0.01 (7)	1410 ± 250 (3)	917 ± 193 (3)	1670 ± 722 (3)
SK&F 89976-A	0.13 ± 0.01 (3)	550 ± 225 (4)	1990 ± 1060 (4)	7210 ± 3630 (4)
CI-966	0.26 ± 0.05 (4)	1280 ± 980 (3)	333 ± 76 (3)	300 ± 10 (3)
EGYT-3886	26 ± 5 (2)	30 ± 4 (2)	46 ± 1 (2)	39 ± 6 (3)
1	1.4 ± 0.06 (3)	11 ± 7% (3)	17 ± 10% (3)	28 ± 4% (3)
2	6.9 ± 4 (3)	137 ± 34 (3)	178 ± 45 (3)	655 ± 82 (3)
3	43 ± 4 (3)	62 ± 8 (3)	25 ± 6 (3)	382 ± 61 (3)
4(*R,S*)	233 ± 62 (4)	34 ± 8 (5)	10 ± 4 (5)	208 ± 65 (3)
4(*R*)	439 ± 160 (3)	240 ± 21 (3)	86 ± 18 (4)	630 ± 49 (3)
4(*S*)	388 ± 92 (3)	21 ± 3 (3)	5 ± 1 (4)	140 ± 30 (3)
5	142 ± 46 (3)	45 ± 15 (3)	20 ± 3 (3)	109 ± 3 (4)
6	58 ± 9 (3)	3670 ± 1800 (3)	6740 ± 5200 (3)	1270 ± 238 (5)
7	73 ± 22 (3)	177 ± 5 (3)	212 ± 49 (3)	201 ± 65 (3)
8	10 ± 2 (3)	417 ± 149 (3)	198 ± 92 (3)	27 ± 5% (4)
9	122 ± 44 (3)	39 ± 3 (3)	29 ± 3 (3)	88 ± 33 (4)
10	19 ± 5 (3)	49 ± 14 (3)	25 ± 8 (3)	151 ± 18 (4)
11	121 ± 26 (3)	103 ± 24 (3)	169 ± 19 (4)	18 ± 6% (3)
12	43 ± 5 (4)	85 ± 27 (4)	91 ± 20 (4)	435 ± 58 (4)
13	78 ± 3 (3)	84 ± 21 (3)	142 ± 26 (3)	12 ± 8% (3)
14	133 ± 50 (3)	51 ± 4 (3)	142 ± 21 (3)	27 ± 10% (3)
15	3 ± 0.1 (3)	23 ± 13% (3)	0 ± 1% (3)	23 ± 10% (3)
16	5 ± 1 (3)	24 ± 14% (3)	10 ± 4% (3)	920 ± 358 (3)
17	268 ± 97 (3)	2800 ± 1900 (3)	1080 ± 153 (3)	2660 ± 1550 (3)

[a] Data show the IC_{50} for inhibition of [^{3}H]GABA uptake, in μM, and represent means ± SEM; the values in parentheses indicate the number of experiments. [b] Data show percent inhibition at 100 μM and represent ± SEM; the values in parentheses indicate the number of experiments.

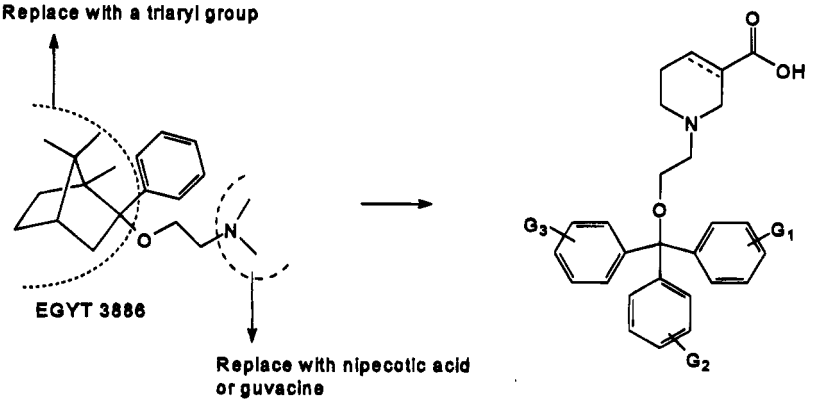


Figure 2. Rationale for the synthesis of triarylnipecotic acid derivatives.

shown in Table 2, the IC_{50} values at these transporters range from about 300 μM to greater than 1 mM. Thus, all four lipophilic compounds display specificity for GAT-1 of nearly 3 orders of magnitude when compared to the other cloned GABA transporters.

Table 2 shows that EGYT-3886[16] a bicycloheptane derivative, shows moderate potency at all four cloned GABA transporters, but lacks selectivity. Examination of the structure of EGYT-3886 reveals the presence of a (dimethylamino)ethanol moiety attached to a quaternary carbon atom. An unusual structural feature of EGYT-3886 is that it lacks a carboxylic acid unit or an isostere thereof, which is unlike other known GABA uptake inhibitors. Comparison of the structures of EGYT-3886 with the GAT-1 inhibitor CI-966, revealed that (a) both have an aminoethanol unit in common, (b) the oxygen atom of EGYT-3886 is linked to a quaternary carbon atom whereas that of CI-966 is linked to a tertiary carbon atom, and (c) CI-966 has a guvacine moiety whereas EGYT-3886 has a (dimethylamino)-ethanol unit.

On the basis of these observations, we designed a series of triarylnipecotic acid derivatives which mimic key structural features of EGYT-3886 and CI-966. This is illustrated schematically in Figure 2. We chose to link a triaryl moiety to the quaternary carbon atom because of the general observation that for SK&F 89976-A, CI-966, and Tiagabine, aromatic substituents not only increase the potency but also confer the ability of the compound to cross the blood–brain barrier.

Chemistry

The compounds listed in Table 1 were synthesized using general methods illustrated in Figure 3. The structures for compounds listed in Table 1 are noted in Figure 4.

The trityl derivatives were prepared according to the general sequence shown in method A. Ethyl nipecotate

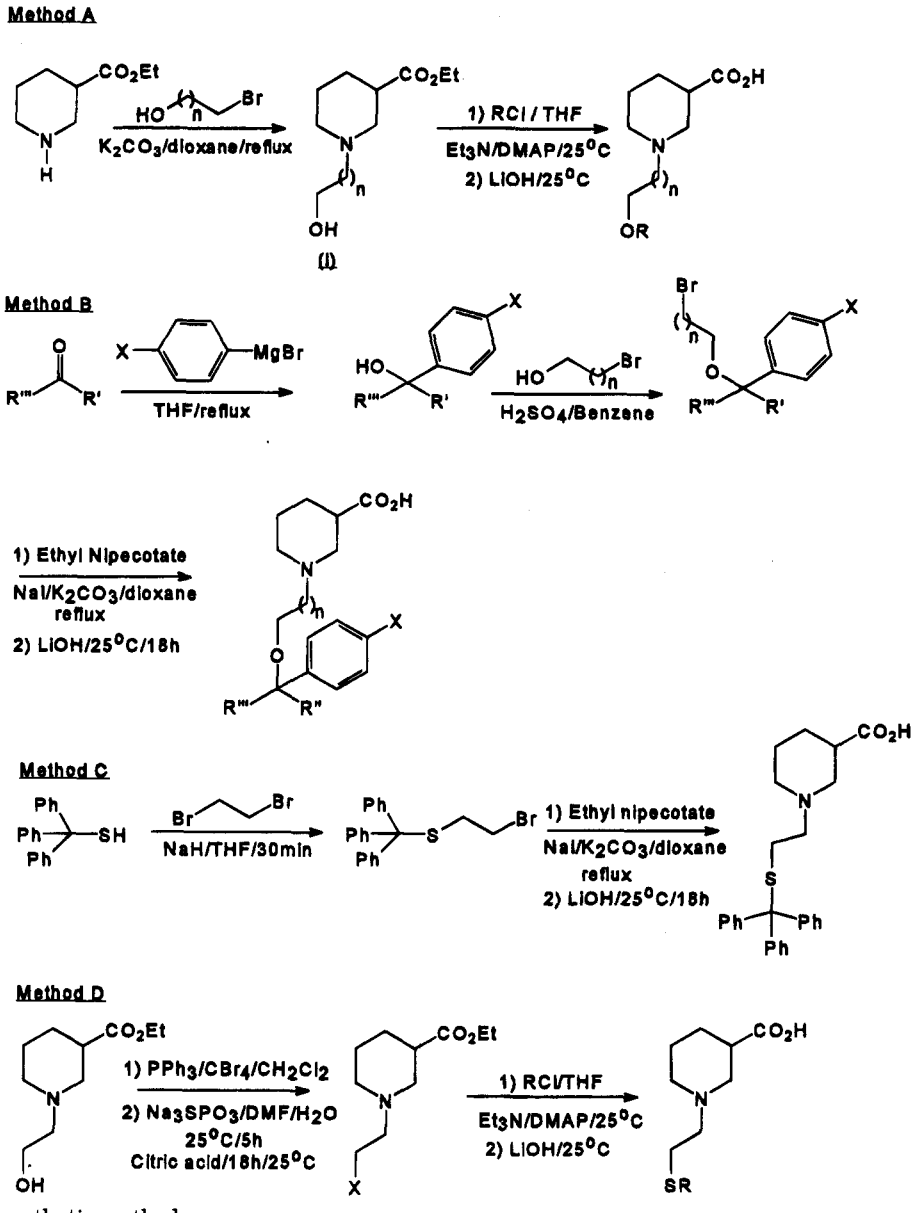


Figure 3. General synthetic methods.

or guvacine ethyl ester were N-alkylated with either bromoethanol or bromopropanol in refluxing 1,4-dioxane with potassium carbonate as the base to yield the amino alcohol, which after O-tritylation and saponification using 1 N LiOH gave the corresponding amino acids (method A, compounds **1**–**4**, **11**, and **17** respectively). Compounds **5**–**10** and **14**–**16** were prepared by acid-catalyzed O-alkylation of the corresponding tertiary alcohols with bromoethanol followed by N-alkylation with ethyl nipecotate and saponification with 1 N LiOH (method B). The tertiary alcohols were either obtained from commercial sources or synthesized by Grignard addition to commercially available ketones. Compound **12** was synthesized by S-alkylation of triphenylmethyl mercaptan with 1,2-dibromoethane followed by N-alkylation and saponification (method C). Thioether **13** was made starting from the amino alcohol **I** obtained in method A, which was converted to the thiol according to the protocol developed by Bieniarz *et al.*[17] (method D). The thiol was coupled to *p*-trimethoxytrityl chloride and hydrolyzed according to conditions described in method A. Nipecotic acid ethyl ester was resolved into its *R* and *S* isomers according to a literature method[18] and used to synthesize the enantiomers of compound **4** [Table 1, compounds **4**(***R***) and **4**(***S***)].

Discussion

The methods of synthesis and potency of compounds **1**–**17** at cloned GABA transporters are illustrated in Tables 1 and 2, respectively.

The parent trityl derivative[13] (**1**) had a profile similar to CI-966 and was actually more potent at GAT-1 than at GAT-2, GAT-3, or BGT-1. However, substituting the para position of the phenyl rings with methoxy groups led to a gradual increase in affinity for GAT-2 and GAT-3 with a concomitant loss of affinity at GAT-1 (compounds **2** and **3**). The trimethoxytrityl derivative **4** showed a clear preference for GAT-3 over GAT-1, GAT-2, and BGT-1. Compound **4** displayed an IC_{50} at hGAT-3 of 10 μM and is thus nearly as potent as GABA itself. It is 4.5-fold and 33-fold more potent at human GAT-3 than the parent compounds EGYT-3886 and CI-966, respectively, and is 20-fold less potent at human GAT-1 and human BGT-1 (IC_{50} values >200 μM) as compared to GAT-3.

Increasing the carbon chain length between a nitrogen and oxygen atom or replacing the oxygen atom with

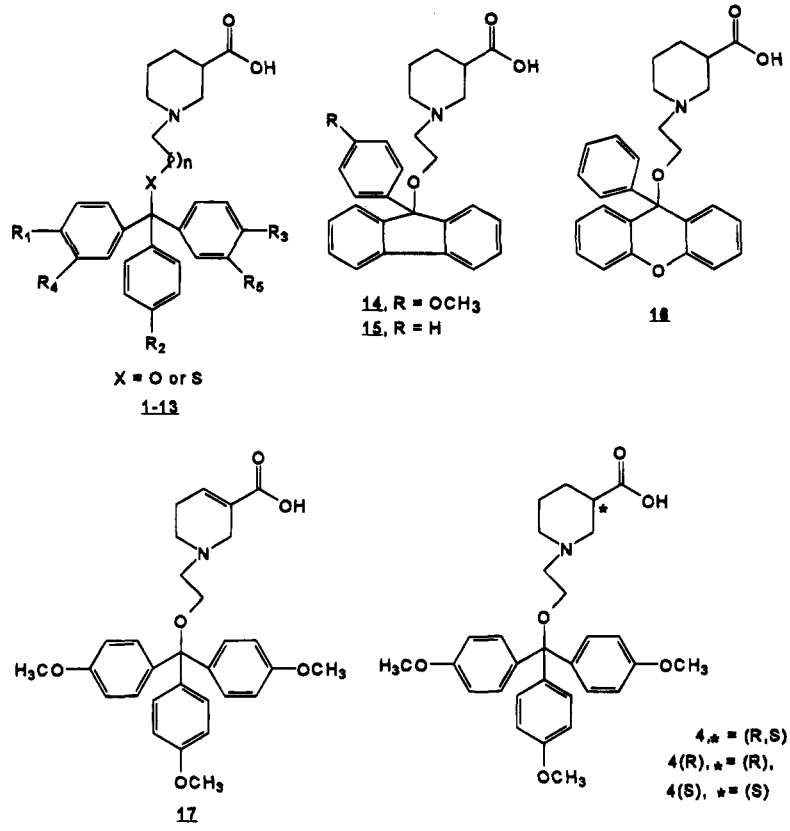


Figure 4. General structures of triarylnipecotic acid derivatives.

a sulfur bridge eliminates the selectivity and decreases the affinity at GAT-3. meta disubstituted derivatives **7** and **8** had diminished affinity and selectivity for GAT-2 and GAT-3. A general observation is that compounds having lipophilic moieties such as methoxy, chlorine, or methyl groups at the para position of the phenyl ring (compounds **4**, **5**, and **9**) displayed higher affinity for GAT-3. Cyclic fluorene or xanthene analogs such as **15** and **16**, where the tertiary carbon atom was part of the ring system, had very low affinities for GAT-2, GAT-3, and BGT-1, although they displayed high affinity for GAT-1. Interestingly, the cyclic fluorene analog **14** where one of the phenyl groups was substituted by a *p*-methoxy group displayed moderate affinity for GAT-2 and was 3-fold selective for this site, as compared to GAT-1 and GAT-3. This compound thus represents a design lead for GAT-2-selective GABA transport inhibitors.

To examine whether there was any stereochemical bias in the binding of these compounds to the transporter, the enantiomers of compound **4** were evaluated for their potency at the cloned GABA transporters. Table 2 reveals that (*R*)-nipecotic acid was more potent than the *S* isomer at GAT-3. In contrast, **4(*S*)** is more potent than the *R* isomer. **4(*S*)** displays an IC_{50} of 5 μM at GAT-3, 21 μM at GAT-2, >200 μM at GAT-1, and 140 μM at BGT-1. Most of the activity of the racemate resides in the *S* isomer. To our knowledge, **4(*S*)** is the first lipophilic inhibitor with both moderate affinity and selectivity for human GAT-3. Since very little is known about the tertiary structure of these transporter proteins, it is not yet possible to identify the regions of the transporter with which these molecules interact. We speculate that binding of the *p*-trimethoxytrityl moiety with an unidentified region of the transporter forces the carboxylic acid residue of (*S*)-nipecotic acid to interact favorably (either via an ion pair interaction with a basic residue or a hydrogen bond interaction) with a region of the transporter which is unfavored for the *R* isomer. The orientation of the carboxylic acid could be important for this class of GAT-3 uptake inhibitors since the guvacine analog of compound **4** (compound **17**) has very low affinity for GAT-3. Future site-directed mutagenesis studies or the use of chimeric transporters may indicate the region of the transporter where this interaction occurs.[19]

Summary

From a novel series of triaryl-substituted nipecotic acid derivatives, we have identified compound **4(*S*)** as an inhibitor with moderate affinity and selectivity for the cloned human GABA transporter hGAT-3. On the basis of the evaluation of the compounds *in vitro*, the following structural features for affinity and selectivity appear to be necessary for this class of GAT-3 inhibitors: (a) a two-carbon chain length between nitrogen and oxygen atoms is preferred, (b) an oxygen atom linking the carbon chain to the tertiary carbon atom provides increased GAT-3 affinity, and (c) the presence of lipophilic groups at the para position of the three phenyl groups are required, with the *p*-methoxy group being preferred for greater selectivity. Although we have not measured the degree of lipophilicity of **4(*S*)**, it is expected to cross the blood–brain barrier because of its structural similarity with CI-966.[13] This compound will be a useful tool in evaluating the role of GAT-3 in neural function.

Experimental Section

All compounds were characterized by ^1H NMR spectra run in $CDCl_3$ or CD_3OD on a Ge QEplus 300-MHz instrument. Chemical shifts are expressed in parts per million (δ units)

relative to tetramethylsilane (δ 0.0) or $CHCl_3$ (δ 7.26). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, bd = broad doublet), coupling constant(s), and integration. Flash chromatography was carried out using silica gel (230-400 mesh, 60 Å) purchased from Baxter Scientific Products. High-resolution mass spectra were obtained using a JEOL 303DXHF instrument using either a EI or FAB mode with either poly(ethylene glycol) or perfluorokerosene as the reference peaks. In the FAB mode the sample was evaluated in 3-nitrobenzyl alcohol. Elemental analyses were obtained from Robertson Microlit Laboratories, Inc., Madison, NJ. All compounds were found to be homogeneous by TLC analysis. The methanolic ammonia solution used for TLC analysis was a 2.0 M solution of NH_3 in methyl alcohol. Melting points were recorded on a Mel-Temp II instrument. SK&F-89976A,[12] CI-966,[13] Tiagabine,[14] EGYT-3886,[16] (*R*)- and (*S*)-ethyl nipecotate,[18] and (*R*)- and (*S*)-nipecotic acids[18] were synthesized according to published methods.

The following is a representative example for the compounds synthesized using method A.

(±)-1-[2-(Triphenylmethoxy)ethyl]-3-piperidinecarboxylic Acid (1). Step a. (±)-1-(2-Hydroxyethyl)-3-piperidinecarboxylic Acid Ethyl Ester. A mixture of ethyl nipecotate (2.48 mL, 16 mmol) in 1,4-dioxane (20 mL), bromoethanol (1.13 g, 16 mmol), 30 mg of sodium iodide, and potassium carbonate (6.63 g, 48 mmol) was refluxed overnight. After concentration of the reaction mixture, it was extracted with ethyl acetate (30 mL), washed with water (15 mL), dried over sodium sulfate, and filtered, and the solvent was removed under reduced pressure. Purification of the crude product by flash chromatography (2:1, ethyl acetate–hexane) produced (±)-1-(2-hydroxyethyl)-3-piperidinecarboxylic acid ethyl ester. Yield: 3.1 g, 96%. ^{1}H NMR ($CDCl_3$): 1.25 (t, J = 7.8 Hz, 3H), 1.53–2.56 (m, 8H), 2.54 (t, J = 6 Hz, 2H), 2.67–2.72 (m, 1H), 2.85–2.89 (m, 1H), 3.59 (t, J = 6.0 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H).

Step b. (±)-1-[2-(Triphenylmethoxy)ethyl]-3-piperidinecarboxylic Acid Ethyl Ester. To a solution of 1-(2-hydroxyethyl)-3-piperidinecarboxylic acid ethyl ester (1.63 g, 8.1 mmol) in dry THF (10 mL) were sequentially added triethylamine (2 mL, 18.6 mmol), DMAP (98 mg), and triphenylmethyl chloride (2.93 g, 10.5 mmol). The reaction mixture was stirred at room temperature for 18 h and partitioned between methylene chloride (50 mL) and water (10 mL). The methylene chloride layer was dried over sodium sulfate, filtered, concentrated, and purified using hexane–ethyl acetate (7:3) to yield (±)-1-[2-(triphenylmethoxy)ethyl]-3-piperidinecarboxylic acid ethyl ester as a syrup. Yield: 2.87 g, 80%. ^{1}H NMR ($CDCl_3$): 1.21 (t, J = 7.1 Hz, 3H), 1.45–2.1 (m, 5H), 2.20 (t, J = 10.4 Hz, 1H), 2.49–2.56 (m, 1H), 2.64 (t, J = 6.1 Hz, 2H), 2.7–2.71 (m, 1H), 2.9–3.02 (m, 1H), 3.2 (t, J = 6.1 Hz, 2H), 4.09 (q, J = 7.1 Hz, 2H), 7.19–7.5 (m, 15H).

Step c. (±)-1-[2-(Triphenylmethoxy)ethyl]-3-piperidinecarboxylic Acid (1). To a solution of the ester obtained in step b (0.6 g, 1.35 mmol) in ethanol (6 mL) was added 1 N LiOH (2 mL, 2.7 mmol), and the contents were stirred at room temperature for 18 h. The reaction mixture was adjusted to pH 6 using 5% NaH_2PO_4 and partitioned between methylene chloride (30 mL) and water (10 mL). The methylene chloride layer was dried over sodium sulfate, filtered, and concentrated to yield (±)-1-[2-(triphenylmethoxy)ethyl]-3-piperidinecarboxylic acid as a foamy solid. Yield: 0.55 g, 89%. Mp: 80–83 °C. ^{1}H NMR (CD_3OD): 1.2–3.35 (m, 13H), 7.2–7.5 (m, 15H).

(±)-1-[2-[(4-Methoxyphenyl)diphenylmethoxy]ethyl]-3-piperidinecarboxylic Acid (2). Yield: 0.457 g, 81%. Mp: 45–47 °C. ^{1}H NMR (CD_3OD): 1.65–3.5 (m, 13H), 3.8 (s, 3H), 6.85–7.49 (m, 14H).

(±)-1-[2-[Bis(4-Methoxyphenyl)phenylmethoxy]ethyl]-3-piperidinecarboxylic Acid (3). Yield: 1.21 g, 80%. Mp: 48–52 °C. ^{1}H NMR (CD_3OD): 1.65–3.5 (m, 13H), 3.8 (s, 6H), 6.85 (d, J = 8.7 Hz, 4H), 7.18–7.3 (m, 3H), 7.34 (d, J = 8.7 Hz, 4H), 7.49 (m, 2H).

(±)-1-[2-[Tris-(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (4). Yield: 1.12 g, 80%. Mp: 58–62 °C; ^{1}H NMR (CD_3OD): 1.65–3.5 (m, 13H), 3.8 (s, 9H), 6.8 (d, J = 8.7 Hz, 4H), 7.35 (d, J = 8.7 Hz).

(*R*)-(+)-1-[2-[Tris(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid [4(*R*)]. This compound was synthesized starting from (*R*)-ethyl nipecotate, which was obtained from (±)-ethyl nipecotate based on a published procedure.[18] Yield: 1.475 g, 74%. The ^{1}H NMR in $CDCl_3$ was identical to that of compound **4**. $[\alpha]^{25}_D$: +9.5° (c = 1.2, $CHCl_3$).

(*S*)-(−)-1-[2-[Tris(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid 4(*S*). This compound was synthesized starting from (*S*)-ethyl nipecotate, which was obtained from (±)-ethyl nipecotate based on a published procedure.[18] Yield: 2.12 g, 72%. The ^{1}H NMR in $CDCl_3$ was identical to that of compound **4**. $[\alpha]^{25}_D$: −9.1° (c = 1.21, $CHCl_3$).

(±)-1-[2-[Tris(4-methoxyphenyl)methoxy]propyl]-3-piperidinecarboxylic Acid (11). Yield: 0.819 g, 82%. Mp: 110–112 °C. ^{1}H NMR (CD_3OD): 1.65–3.5 (m, 15H), 3.78 (s, 9H), 6.79 (d, J = 8.7 Hz, 6H), 7.26 (d, J = 8.7 Hz, 6H).

1-[2-[Tris(4-methoxyphenyl)methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic Acid (17). This compound was synthesized using method A, except that guvacine ethyl ester was employed instead of ethyl nipecotate in step a. Yield: 0.212 g, 95%. Mp: 96–100 °C. ^{1}H NMR ($CDCl_3$): 2.28 (br s, 2H), 2.54 (t, J = 5.8 Hz, 2H), 2.73 (t, J = 5.8 Hz, 2H), 3.19–3.24 (m, 4H), 3.74 (s, 9H), 4.9–5.0 (bs, 1H), 6.78 (d, J = 8.8 Hz, 6H), 6.9 (br s, 1H), 7.29 (d, J = 8.8 Hz, 6H).

The following are two representative examples for the compounds synthesized using method B.

(±)-1-[2-[Bis(4-methylphenyl)(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (5). Step a. Bis-(4-methylphenyl)methanol. To magnesium turnings (0.419 g, 17.4 mmol) in a flame-dried flask under nitrogen were added 15 mL of THF and 4-bromotoluene (3.07 mL, 25 mmol), and the solution was heated at reflux for 20 min. The reaction mixture was then cooled to 0 °C, and *p*-tolualdehyde (1.96 g, 16.6 mmol) in THF was added in a dropwise fashion. The solution was refluxed for 2 h followed by quenching with a saturated solution of ammonium chloride at 0 °C. The reaction mixture was concentrated, extracted with ethyl acetate (30 mL), washed with brine (20 mL), dried over Na_2SO, filtered, and concentrated under reduced pressure. Purification of the crude product by flash chromatography (18:1 hexane–ethyl acetate) produced bis(4-methylphenyl)methanol as a syrup. Yield: 2.1 g, 75%. ^{1}H NMR ($CDCl_3$): 2.4 (s, 6H), 2.58 (s, 1H), 5.78 (s, 1H), 7.18 (d, J = 8.1 Hz, 4H), 7.28 (d, J = 8.1 Hz, 4H).

Step b. 4,4′-Dimethylbenzophenone. To bis(4-methylphenyl)methanol (2.61 g, 12.3 mmol) in dichloromethane (8 mL), was added pyridinium chlorochromate (5.33 g, 24.7 mmol), and the solution was heated at reflux for 30 min. The reaction mixture was concentrated and repeatedly extracted with ether. The ethereal solution was dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purification of the crude product by flash chromatography (18:1 hexane–ethyl acetate as eluant) produced 4,4′-dimethylbenzophenone. Yield: 2.2 g, 86%. ^{1}H NMR ($CDCl_3$): 2.42 (s, 6H), 7.25 (d, J = 8.1 Hz, 4H), 7.68 (d, J = 8.1 Hz, 4H).

Step c. Bis(4-methylphenyl)(4-methoxyphenyl)methanol. To magnesium turnings (266 mg, 11.1 mmol) in a flame-dried flask under nitrogen were added 15 mL of THF and 4-bromoanisole (1.98 g, 16 mmol), and the solution refluxed for 20 min. The reaction mixture was then cooled to 0 °C, and 4,4′-dimethylbenzophenone (2.21 g, 10.5 mmol) in ether was added in a dropwise fashion. The solution was refluxed for 2 h and upon completion was quenched with a saturated solution of ammonium chloride at 0 °C. It was then concentrated, extracted with ethyl acetate (30 mL), washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the crude product by flash chromatography (18:1 hexane–ethyl acetate) produced bis(4-methylphenyl)(4-methoxyphenyl)methanol. Yield: 2.52 g, 75%. ^{1}H NMR ($CDCl_3$): 2.32 (s, 6H), 2.67 (s, 1H), 3.78 (s, 3H), 6.80 (d, J = 8.8 Hz, 2H), 7.06–7.16 (m, 10H).

Step d. (±)-1-[2-[Bis(4-methylphenyl)(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid Ethyl Ester. To solution of bis(4-methylphenyl)(4-methoxyphenyl)-

methanol (2.52 g, 7.9 mmol) in benzene (10 mL) was added 117 μL of concentrated sulfuric acid, and the solution was heated at 65 °C for 5 min. After addition of bromoethanol (0.84 mL, 11.9 mmol), the reaction mixture was stirred at room temperature for 20 min. It was partitioned between benzene (30 mL) and water (15 mL), dried over calcium chloride, filtered, concentrated, and used as such for the subsequent step. Yield: 0.68 g, 20%.

A mixture of ethyl nipecotate (0.25 mL, 1.6 mmol) in 1,4-dioxane (20 mL), the bromo compound obtained above (0.68 g, 1.6 mmol), potassium carbonate (0.66 g, 4.8 mmol), and sodium iodide (20 mg) was refluxed overnight. After concentration of the reaction mixture, it was extracted with ethyl acetate (30 mL), washed with water (15 mL), dried over sodium sulfate, and filtered, and the solvent was removed under reduced pressure. Purification of the crude product by flash chromatography (3:1 hexane–ethyl acetate) produced (±)-1-[2-[bis(4-methylphenyl)(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic acid ethyl ester as a syrup. Yield: 0.59 g, 74%. ^{1}H NMR ($CDCl_3$): 1.21 (t, J = 7.8 Hz, 3H), 1.45–2.1 (m, 5H), 2.2 (t, J = 10.6 Hz, 1H), 2.32 (s, 6H), 2.49–2.56 (m, 1H), 2.64 (t, J = 6.1 Hz, 2H), 2.70–2.71 (m, 1H), 2.9–3.02 (m, 1H), 3.2 (t, J = 6.1 Hz, 2H), 3.78 (s, 3H), 4.09 (q, J = 7.1 Hz, 2H), 6.78 (d, J = 8.8 H, 2H), 7.08 (d, J = 8.8 Hz, 4H), 7.24–7.31 (m, 6H).

Step e. (±)-1-[2-[Bis(4-methylphenyl)(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (5). An aqueous solution of LiOH (2.38 mL, 1 M, 2.38 mmol) was added to a solution of the ester obtained in step d (0.59 g, 1.1 mmol) in ethanol (20 mL), and the reaction mixture was stirred for 24 h at room temperature. Upon completion of the reaction it was neutralized carefully with a solution of NaH_2PO_4 and concentrated under reduced pressure. After extraction with methylene chloride, the solution was dried over sodium sulfate and filtered. Removal of the solvent under reduced pressure gave (±)-1-[2-[bis(4-methylphenyl)(4-methoxyphenyl)methoxy]-ethyl]-3-piperidinecarboxylic acid as a solid. Yield: 0.42 g, 75%. Mp: 85–90 °C. ^{1}H NMR ($CDCl_3$): 1.58–3.35 (m, 13H), 2.29 (s, 6H), 3.78 (s, 3H), 6.78 (d, J = 8.8 Hz, 2H), 7.08 (d, J = 8.8 Hz, 4H), 7.24–7.31 (m, 6H).

(±)-1-[2-[Tris-(4-fluorophenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (6). Step a. Tris(4-trifluorophenyl)methanol. To magnesium turnings (115 mg, 4.8 mmol) in a flame-dried flask under nitrogen were added THF (6 mL) and 1-bromo-4-fluorobenzene (0.75 mL, 6.9 mmol), and the solution was refluxed for 20 min. The reaction mixture was cooled to 0 °C, and 4,4′-difluorobenzophenone (1 g, 4.6 mmol) in THF was added in a dropwise fashion. The solution was refluxed for 2 h and, upon completion, was quenched with a saturated solution of ammonium chloride at 0 °C. It was concentrated, extracted with ethyl acetate (30 mL), washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the crude product by flash chromatography (18:1 hexane–ethyl acetate) produced tris(4-trifluorophenyl)methanol. Yield: 0.64 g, 45%. ^{1}H NMR ($CDCl_3$): 2.78 (s, 1H), 6.78 (d, J = 8.6 Hz, 2H), 6.93 (m, 4H), 7.08 (d, J = 8.6 H, 2H), 7.19 (m, 4H).

Step b. (±)-1-[2-[Tris(4-fluorophenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid Ethyl Ester. To solution of tris(4-trifluorophenyl)methanol (0.64 g, 2 mmol) in benzene (10 mL), was added 34 μL of concentrated sulfuric acid, and the solution was heated at 65 °C for 5 min. After addition of bromoethanol (0.22 mL, 3.1 mmol), the reaction mixture was stirred at room temperature for 20 min. It was partitioned between benzene (30 mL) and water (15 mL), dried over calcium chloride, filtered, concentrated, and used as such for the subsequent step. Yield: 0.1 g, 7.7%.

A mixture of ethyl nipecotate (0.04 mL, 0.28 mmol) in 1,4-dioxane (15 mL), the bromo compound noted above (0.1 g, 0.28 mmol), potassium carbonate (0.11 g, 8.6 mmol), and sodium iodide (30 mg) was refluxed overnight. After concentration of the reaction mixture, it was extracted with ethyl acetate (30 mL), washed with water (15 mL), dried over sodium sulfate, and filtered, and the solvent was removed under reduced pressure. Purification of the crude product by flash chromatography (3:1 hexane–ethyl acetate) produced (±)-1-[2-[tris-(4-fluorophenyl)methoxy]ethyl]-3-piperidinecarboxylic acid ethyl ester as a syrup. Yield: 0.1 g, 83%. ^{1}H NMR ($CDCl_3$): 1.21 (t, J = 7.8 Hz, 3H), 1.45–2.1 (m, 5H), 2.2 (t, J = 10.6 Hz, 1H), 2.49–2.56 (m, 1H), 2.64 (t, J = 6.1 Hz, 2H), 2.70–2.71 (m, 1H), 2.9–3.02 (m, 1H), 3.2 (t, J = 6.1 Hz, 2H), 4.09 (q, J = 7.1 Hz, 2H), 6.78 (d, J = 8.6 Hz, 2H), 6.93 (m, 4H), 7.08 (d, J = 8.6 Hz, 2H), 7.19 (m, 4H).

Step c. (±)-1-[2-[Tris(4-fluorophenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (6). An aqueous solution of LiOH (1.14 mL, 1 M, 1.14 mmol) was added to a solution of ester obtained in step c (0.25 g, 0.57 mmol) in ethanol (20 mL), and the reaction mixture was stirred for 24 h at room temperature. Upon completion of the reaction it was neutralized carefully with a solution of NaH_2PO_4 and concentrated under reduced pressure. After extraction with methylene chloride, the solution was dried over sodium sulfate and filtered. Removal of the solvent under reduced pressure gave the title compound. Yield: 0.14 g, 54%. Mp: 118–120 °C. ^{1}H NMR ($CDCl_3$): 1.22–3.35 (m, 13H), 6.78 (d, J = 8.6 Hz, 2H), 6.93 (m, 4H), 7.08 (d, J = 8.6 Hz, 2H), 7.19 (m, 4H).

(±)-1-[2-[Bis[3-(trifluoromethyl)phenyl](4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (7). Yield: 0.14 g, 68%. Mp: 70–72 °C. ^{1}H NMR ($CDCl_3$): 1.22–3.35 (m, 13H), 3.78 (s, 3H), 6.87 (d, J = 8.8 Hz, 2H), 7.20 (d, J = 8.8 Hz, 2H), 7.40–7.46 (m, 4H), 7.60 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H).

(±)-1-[2-[Bis(3-fluorophenyl)(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (8). Yield: 0.24 g, 64%. Mp: 110–115 °C. ^{1}H NMR ($CDCl_3$): 1.6–3.35 (m, 13H), 3.78 (s, 3H), 6.88 (d, J = 8.8 Hz, 2H), 7.01–7.07 (m, 6H), 7.19 (d, J = 8.8 Hz, 2H), 7.22–7.36 (m, 2H).

(±)-1-[2-[Bis(4-chlorophenyl)(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (9). Yield: 0.26 g, 61%. Mp: 115–120 °C. ^{1}H NMR ($CDCl_3$): 1.6–3.35 (m, 13H), 3.78 (s, 3H), 6.82 (d, J = 8.9 Hz, 2H), 7.22 (t, J = 8.9 Hz, 2H), 7.24 (d, J = 8.6 Hz, 4H), 7.37 (d, J = 8.6 Hz, 4H).

(±)-1-[2-[Bis(4-fluorophenyl)(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (10). Yield: 0.26 g, 61%. Mp: 90–95 °C. ^{1}H NMR ($CDCl_3$): 1.6–3.35 (m, 13H), 3.78 (s, 3H), 6.83 (d, J = 8.8 Hz, 2H), 6.96 (t, J = 8.6 Hz, 4H), 7.25 (d, J = 8.1 Hz, 2H), 7.36 (dd, J = 8.8, 8.98 Hz, 4H).

(±)-1-[2-[[9-(4-Methoxyphenyl)fluorenyl]methoxy]ethyl]-3-piperidinecarboxylic Acid (14). Yield: 0.36 g, 65%. Mp: 100–102 °C. ^{1}H NMR ($CDCl_3$): 1.55–3.35 (m, 13H), 3.78 (s, 3H), 6.74 (d, J = 8.9 Hz, 2H), 7.24–7.37 (m, 8H), 7.65 (d, J = 7.37 Hz, 2H).

(±)-1-[2-[(9-Phenylfluorenyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (15). Yield; 0.15 g, 68%. Mp: 115–120 °C. ^{1}H NMR ($CDCl_3$): 1.54–3.35 (m, 13H), 7.17–7.38 (m, 11H), 7.67 (d, J = 7.4 Hz, 2H).

(±)-1-[2-[(9-Phenylxanthyl)methoxy]ethyl]-3-piperidinecarboxylic Acid (16). Yield: 0.69 g, 88%. Mp: 105–108 °C. ^{1}H NMR ($CDCl_3$): 1.42–3.35 (m, 13H), 6.99–7.05 (m, 2H), 7.11–7.29 (m, 9H), 7.35–7.38 (m, 2H).

Method C: (±)-1-[2-[(Triphenyl)thioxomethoxy]ethyl]-3-piperidinecarboxylic Acid (12). Step a. (±)-1-[2-[(Triphenyl)thiomethoxy]ethyl]-3-piperidinecarboxylic Acid Ethyl Ester. To pentane-washed sodium hydride (60% dispersion, 0.145 mg, 3.6 mmol) in THF (10 mL) at 0 °C was added triphenylmethyl mercaptan (1 g, 3.6 mmol) over a period of 5 min, and the reaction mixture was stirred at room temperature for 10 min. The reaction mixture was cooled to 0 °C and treated with 1,2-dibromoethane (0.31 mL, 3.6 mmol). After being stirred at room temperature for 20 min, the reaction mixture was concentrated under reduced pressure and partitioned between ethyl acetate (20 mL) and water (10 mL). The ethyl acetate layer was dried over sodium sulfate, filtered, concentrated, and used as such for the subsequent step. Yield: 1 g, 72%.

To the bromo compound obtained above (1.0 g, 3.6 mmol) were sequentially added 1,4-dioxane (25 mL), sodium carbonate (1.15 g, 10.8 mmol), sodium iodide (0.543 g, 3.6 mmol), and ethyl nipecotate (0.62 mL, 3.96 mmol), and the contents were refluxed for 18 h. The reaction mixture was concentrated and partitioned between ethyl acetate (50 mL) and water (20 mL). The ethyl acetate layer was dried over sodium sulfate, filtered,

concentrated, and purified by flash chromatography (4:1 hexane–ethyl acetate) to yield (±)-1-[2-[(triphenyl)thiomethoxy]-ethyl]-3-piperidinecarboxylic acid as a syrup. Yield: 1.1 g, 69%. ^{1}H NMR ($CDCl_3$): 1.21 (t, J = 7.1 Hz, 3H), 1.45–1.81 (m, 5H), 2.0 (t, J = 10.4 Hz, 1H), 2.25–2.6 (m, 6H), 2.75–2.85 (m, 1H), 4.09 (q, J = 7.1 Hz, 2H), 7.18–7.49 (m, 15H).

Step b. (±)-1-[2-[(Triphenyl)thiomethoxy]ethyl]-3-piperidinecarboxylic Acid (12). An aqueous solution of LiOH (0.655 mL, 1 M, 6.55 mmol) was added to a solution of the ester obtained in step a (0.18 g, 0.33 mmol) in ethanol (7 mL), and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture then neutralized carefully with a solution of NaH_2PO_4 and concentrated under reduced pressure. After extraction with methylene chloride (20 mL), the solution was dried over sodium sulfate and filtered. Removal of the solvent under reduced pressure gave (±)-1-[2-[(triphenyl)thiomethoxy]ethyl]-3-piperidinecarboxylic acid. Yield: 0.338 g, 60%. Mp: 58–60 °C. ^{1}H NMR (CD_3OD): 1.48–2.85 (m, 13H), 7.18–7.49 (m, 15H).

Method D: (±)-1-[2-[Tris(4-methoxyphenyl)thiomethoxy]ethyl]-3-piperidinecarboxylic Acid (13). Step a. (±)-1-(2-Bromoethyl)-3-piperidinecarboxylic Acid Ethyl Ester. Triphenylphosphine (1.95 g, 7.46 mmol) in dichloromethane (20 mL) was added dropwise to a well-stirred solution of (±)-1-(2-hydroxyethyl)-3-(piperidinecarboxylic acid ethyl ester obtained in method A, step a (1.5 g, 7.46 mmol) and carbon tetrabromide (1.72 g, 11.1 mmol) in dichloromethane (10 mL) at room temperature. After 1 h the reaction mixture was treated with water (10 mL), extracted with dichloromethane (30 mL), washed with brine (15 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purification by flash chromatography (7:2 hexane–ethyl acetate) gave (±)-1-(2-bromoethyl)-3-piperidinecarboxylic acid ethyl ester as a syrup. Yield: 1.1 g, 56%. ^{1}H NMR ($CDCl_3$): 1.25 (t, J = 7.8 Hz, 3H), 1.35–2.07 (m, 5H), 2.21 (t, J = 10.6 Hz, 1H), 2.45–2.60 (m, 1H), 2.72 (s, 1H), 2.77 (t, J = 6.1 Hz, 2H), 2.94–2.99 (m, 1H), 3.43 (t, J = 6 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H).

Step b. (±)-1-(2-Mercaptoethyl)-3-piperidinecarboxylic Acid Ethyl Ester. To (±)-1-(2-bromoethyl)-3-piperidinecarboxylic acid ethyl ester (0.61 g, 2.3 mmol) in DMF (3 mL) was added sodium thiophosphate dodecahydrate (1.83 g, 4.6 mmol) in 10 mL of water. The mixture was stirred for 5 h, after which the pH was lowered to 4.0 with 3.5% citric acid. The mixture was stirred overnight at room temperature. It was extracted with dichloromethane (3 × 15 mL), washed with brine (15 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purification of the crude product by flash chromatography (5:2 hexane–ethyl acetate) gave (±)-1-(2-mercaptoethyl)-3-piperidinecarboxylic acid ethyl ester. Yield: 0.12 g, 25%. ^{1}H NMR ($CDCl_3$): 1.24 (t, J = 7.8 Hz, 3H), 1.42–2.18 (m, 7H), 2.27 (t, J = 10.6 Hz, 1H), 2.54–2.68 (m, 4H), 2.70–2.73 (m, 1H), 2.91–2.95 (m, 1H), 4.12 (q, J = 7.1 Hz, 2H).

Step c. (±)-1-[2-[Tris(4-methoxyphenyl)thiomethoxy]-ethyl]-3-piperidinecarboxylic Acid Ethyl Ester. To (±)-1-(2-mercaptoethyl)-3-piperidinecarboxylic acid ethyl ester obtained in step b (0.12 g, 0.55 mmol) in dry THF (10 mL, were added sequentially triethylamine (0.18 mL, 13 mmol), DMAP (30 mg), and trimethoxytrityl chloride (0.27 g, 0.7 mmol), and the reaction mixture was allowed to stir at room temperature overnight. It was then concentrated, extracted with dichloromethane (20 mL), washed with water (15 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purification of the crude product by flash chromatography (3:1 hexane–ethyl acetate) gave (±)-1-[2-[tris-(4-methoxyphenyl)thiomethoxy]ethyl]-3-piperidinecarboxylic acid ethyl ester as a syrup. Yield: 0.18 g, 57%. ^{1}H NMR ($CDCl_3$): 1.24 (t, J = 7.8 Hz, 3H), 1.42–2.29 (m, 5H), 2.31 (s, 4H), 2.32–2.8 (m, 4H), 3.78 (s, 9H), 4.08 (q, J = 7.1 Hz, 2H), 6.78 (d, J = 8.8 Hz, 6H), 7.27 (d, J = 8.8 Hz, 6H).

Step d. (±)-1-[2-[Tris(4-methoxyphenyl)thiomethoxy]-ethyl]-3-piperidinecarboxylic Acid (13). An aqueous solution of LiOH (0.65 mL, 1 M, 0.65 mmol) was added to a solution of the ester obtained in step c (0.18 g, 0.3 mmol) in ethanol (20 mL), and the reaction mixture was stirred for 24 h at room temperature. Upon completion of the reaction it was neutralized carefully with a solution of NaH_2PO_4 and concentrated under reduced pressure. After extraction with methylene chloride (20 mL), the solution was dried over sodium sulfate and filtered. Removal of the solvent under reduced pressure gave (±)-1-[2-[tris(4-methoxyphenyl)thiomethoxy]ethyl]-3-piperidinecarboxylic acid. Yield: 0.08 g, 47%. Mp: 85–90 °C. ^{1}H NMR ($CDCl_3$): 1.6–2.9 (m, 13 H), 3.78 (s, 9H), 6.78 (d, J = 8.8 Hz, 6 H), 7.27 (d, J = 8.8 Hz, 6H).

Cell Lines. In the present study we employed rat GAT-2 (rGAT-2)[5] and the human homologs of GAT-1 (hGAT-1, which we have recloned),[20] GAT-3 (hGAT-3),[9] and BGT-1 (hBGT-1).[10] Stable cell lines for each of these clones were generated in LM(tk$^-$) cells using the calcium phosphate method and selection in G-418, as described previously.[21] Cells were grown under standard conditions (37 °C, 5% CO_2) in Dulbecco's modified Eagles's medium (GIBCO, Grand Island, NY).

Transport Assay. GABA transport was measured as described previously,[5] with the following modifications. Cells grown in 24-well plates (well diameter 18 mm) were washed three times with HEPES-buffered saline (HBS, in mM: NaCl, 150; HEPEs, 20; $CaCl_2$, 1; glucose, 10; KCl, 5; $MgCl_2$, 1; pH 7.4) and allowed to equilibrate on a 37 °C slide warmer. After 10 min the medium was removed, and unlabeled drugs in HBS were added (450 μL/well). Transport was initiated by adding 50 μL per well of a concentrated solution of [^{3}H]GABA in HBS (final concentration = 50 nM). Nonspecific uptake was defined in parallel wells with 1 unlabeled GABA and was subtracted from total uptake (no competitor) to yield specific uptake; all data represent specific uptake. Plates were incubated at 37 °C for 10 min and then washed rapidly three times with ice-cold HBS, using a 24-position plate washer (Brandel, Gaithersburg, MA; Model PW-12). Cells were solubilized with 0.05% sodium deoxycholate/0.1 N NaOH (0.25 mL/well), an aliquot neutralized with 1 N HCl, and radioactivity was determined by scintillation counting. Protein was quantified in an aliquot of the solubilized cells using a BIO-RAD protein assay kit, according to the manufacturer's directions.

Lipophilic inhibitors were dissolved in DMSO. The final concentration of DMSO in the transport assay was ≤2%, and control experiments demonstrated that this concentration had no significant effect on transport.

Data Analysis. Competition curves were conducted in duplicate, using 10 concentrations of unlabeled drug. IC_{50} values (concentrations resulting in 50% inhibition of uptake) were derived using software from Graphpad. For low-affinity compounds it was not possible to generate full curves; for such compounds, the percent inhibition at 100 μM is presented. All data (Tables 1 and 2) represent means ± SEM.

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