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Stereospecific synthesis and structure–activity relationships of unsymmetrical 4,4-diphenylbut-3-enyl derivatives of nipecotic acid as GAT-1 inhibitors

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ABSTRACT

Two complementary stereospecific synthetic approaches for the preparation of unsymmetrical *ortho*-substituted *N*-(4,4-diphenylbut-3-enyl) derivatives of nipecotic acid are described. Determination of the activity of the prepared compounds at the GAT-1 transporter highlighted differing SAR requirements of the *E*- and *Z*-phenyl rings, and led to the discovery of a compound with comparable potency to tiagabine. Some attempts to replace nipecotic acid with alternative novel amino acids are also described.

The amino acid 4-aminobutyric acid (GABA) is recognised as the major inhibitory neurotransmitter in the brain.[1–3] Considerable direct and indirect evidence suggests that alterations of the GABAergic system may to be implicated in several psychiatric and neurological disorders, including anxiety, pain, Huntington's chorea, epilepsy and Parkinson's disease.[4–8] A number of strategies are available for increasing GABAergic tone including agonism of GABA receptors, inhibition of GABA catabolism and inhibition of GABA reuptake. The latter approach requires inhibitors of one or more of the GABA uptake transporters (GAT 1–3, BGT-1),[9] of which the GAT-1 transporter has been most extensively studied.

The therapeutic potential of the earliest GAT-1 inhibitors, such as nipecotic acid (**1**) and guvacine (**2**), was limited by their inability to penetrate the blood–brain barrier (BBB). Subsequently, it was demonstrated that modification of these structures with a lipophilic side chain led to highly potent GAT-1 inhibitors exemplified by SKF-89976A (**3**), SKF-100300A (**4**) and tiagabine (**5**) (Fig. 1), which are able to cross the blood-brain barrier.[10] The pharmacology of these compounds has been extensively studied.[11,12]

In particular, tiagabine has been shown to exhibit anticonvulsant activity in clinical trials and is currently approved as an add-on therapy for the treatment of partial seizures.

A body of preclinical evidence suggests that GAT-1 inhibitors have the potential to function as effective anxiolytic agents.[13] Furthermore, at the time this work was initiated proof of concept

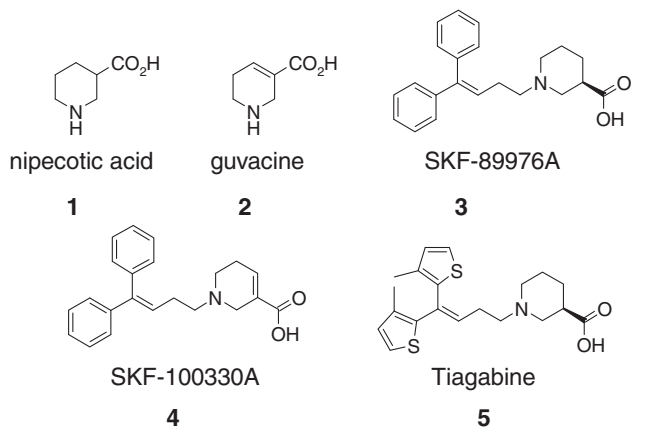

Figure 1. Some examples of potent GABA uptake inhibitors described in the literature.

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(POC) clinical trials undertaken by Cephalon had shown tiagabine held promise as a novel approach for the treatment of generalised anxiety disorder (GAD) and post traumatic stress disorder (PTSD).[14] As part of a research project aimed at the identification of novel GABA reuptake inhibitors for the treatment of anxiety disorders we were stimulated to revisit the SKF-89976A scaffold to perform a wider exploration of the structure–activity relationship (SAR) as remarkably little data are available for the aryl substitution of this system. Despite reports indicating the importance of an '*ortho*' substituent in more than one GAT-1 inhibitor series[15,16] and others proving the advantageousness of having unsymmetrical substitution patterns in the two aryl rings[17] only one example of an unsymmetrical, *ortho*-substituted derivative of SKF-89976A has been reported.[18] Herein, we describe the synthesis and the biological activity of a series of novel unsymmetrical *ortho*-substituted *N*-(4,4-diarylbut-3-enyl) derivatives of nipecotic acid (**6**) (Fig. 2). We also communicate a limited exploration of novel alternative amino acid head groups which were evaluated using the SKF-89976/tiagabine side chain (**7**) (Fig. 2).

The synthetic routes used for the preparation of compounds **6** are illustrated in Scheme 1.

Initially an established approach for the preparation of 4,4-diarylbut-3-enyl derivatives (Method A) was employed. In the first step, the cyclopropylphenylcarbinol intermediate **8** was prepared by reaction of commercially available cyclopropylphenylketones with an appropriate Grignard reagent, then treatment with hydrobromic acid in acetic acid afforded *E*/*Z* mixtures of unsymmetric 4-bromo-1,1-diaryl-1-butenes **9**. Nucleophilic substitution with ethyl (*R*)-nipecotate and subsequent saponification gave corresponding *E*/*Z* mixtures of products **11**. Unfortunately, we were unable to separate these mixtures, or the intermediates **9** and **10**, into pure *E* and *Z* isomers such that SAR analysis became impractical. Consequently efforts were made to develop alternative synthetic routes to allow stereospecific preparation of *E* and *Z* isomers. Two complementary stereospecific syntheses (Methods B and C, Scheme 1) were devised that allowed variation of the *E*- or *Z*-aryl ring at a late stage, facilitating SAR exploration. Method B exploits the known propensity of sodium bis(2-methoxyethoxy) aluminium hydride (Red-Al®) to reduce 3-butynol derivatives stereoselectively; the high stereoselectivity observed during this reduction is a consequence of chelation-assisted hydroalumination giving *Z*-vinylaluminiums which in turn can be trapped with electrophiles.[19,20] Hence, commercially available 3-butyn-1-ol was transformed under Sonogashira conditions into the corresponding 4-phenyl-3-butyn-1-ol **12**. Reduction of this alcohol with Red-Al® in THF followed by addition of iodine at -78 °C afforded isomerically pure (*Z*)-4-iodio-4-aryl-3-buten-1-ol **13**. Activation of the alcohol as its methanesulfonate ester and nucelophilic substitution with ethyl (*R*)-nipecotate afforded derivative **14** which was transformed into the corresponding 4,4-diaryl-butenyl derivative **15** through a Suzuki-type coupling with an appropriately substituted boronic acid. Alkaline saponification then provided the target products **6**.

Alternatively, Method C derives its stereoselectivity from the syn nature of the hydrostannylation of alkynes.[21] In this case the same starting material, 3-butyn-1-ol, was first converted into 3-butyn-1-yl methanesulfonate and then used to alkylate ethyl (*R*)-nipecotate. Alkyne **16** was arylated under Sonogashira

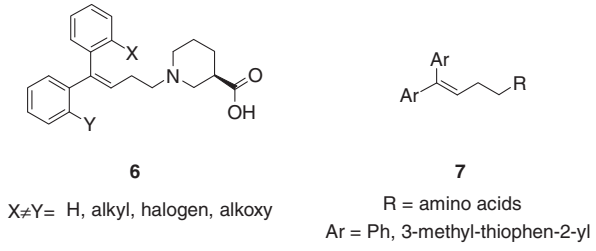
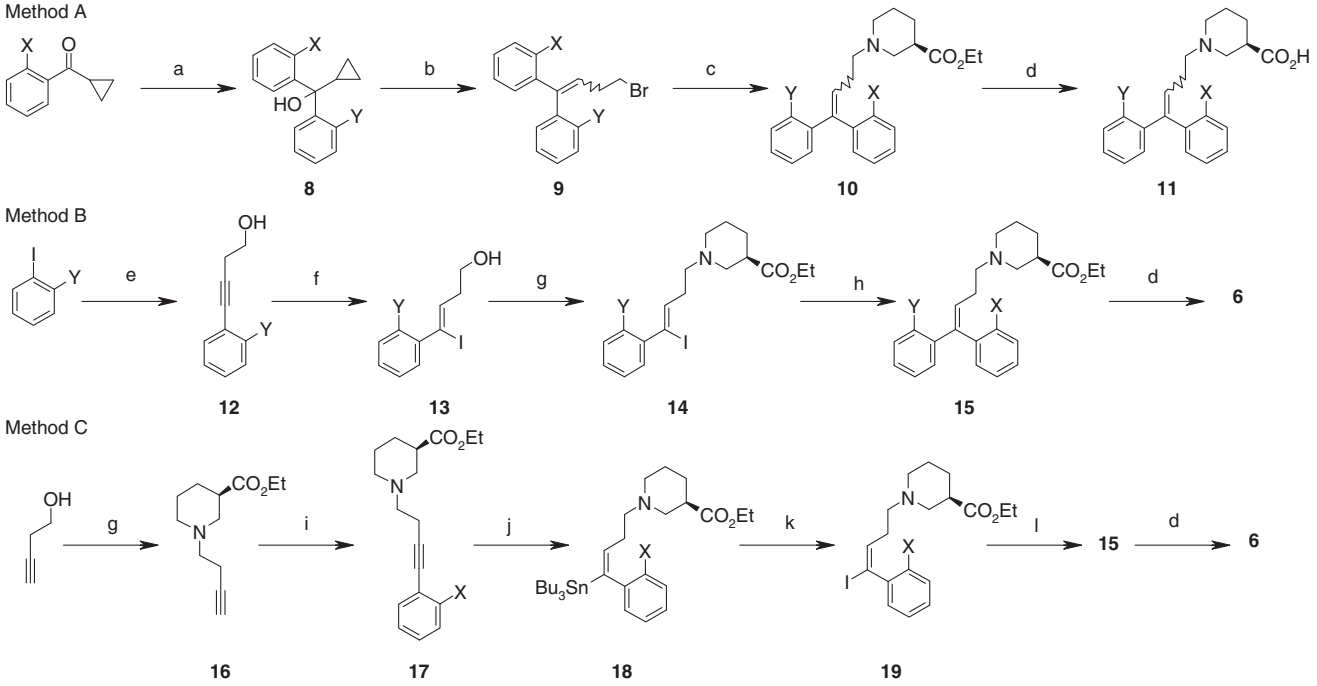


Figure 2. General structures of diaryl butenyl derivatives explored.



Scheme 1. Synthetic routes to butenyl GAT-1 inhibitors. Reagents and conditions: (a) 2-Y-phenylmagnesium halide, THF, rt to reflux; (b) AcOH, HBr; 10 °C; (c) methyl (*R*)-nipecotate tartrate, DIPEA, DMF, 60 °C; (d) NaOH, EtOH, rt then HCl, EtOH; (e) CuI, $Pd(PPh_3)_4$, TEA, 3-butyn-1-ol; (f) Red-Al, THF, reflux then I_2, −78 °C; (g) MsCl, TEA, DCM then methyl (*R*)-nipecotate tartrate, K_2CO_3, KI, 2-butanone; (h) 2-X-phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, 110 °C, microwave irradiation; (i) 1-iodo-2-X-benzene, CuI, $Pd(PPh_3)_4$, TEA; (j) Bu_3SnH, $PdCl_2(PPh_3)_2$, THF; (k) I_2, DCM, rt; (l) 2-Y-phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, 110 °C, microwave irradiation.

conditions to give **17**, which was then subjected to a regio and stereospecific hydrostannylation to afford the *E*-vinylstannane **18**. The vinylstannane **18** itself is a suitable intermediate for the preparation of **15** via Stille coupling but in practise it was most convenient to transform the vinylstannane into vinyliodide **19** via stereospecific iodo-destannylation. The synthetic sequence was completed, as in Method B, with a Suzuki-type coupling and alkaline saponification.

The affinity of the compounds prepared for the hGAT-1 transporter was measured in an equilibrium binding assay[22] utilising [^{3}H]-tiagabine as radioligand.[23] Data for a selection of the compounds tested is shown in Table 1.

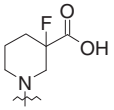
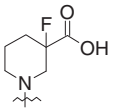
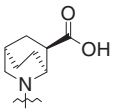
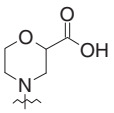
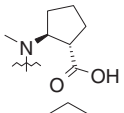
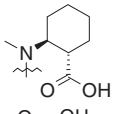
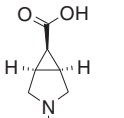
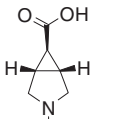
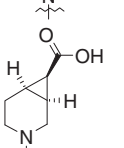
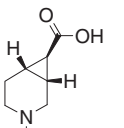
An analysis of the monosubstituted derivatives reveals distinct SAR differences between the *E*- and *Z*-aryl rings. All *ortho*-substituents examined on the *E*-phenyl ring led to a loss of activity in comparison to the parent SKF-89976A (**6a**–**6k**, Table 1). Activity appears to drop with the size of the substituent and with electron withdrawing groups indicating that both steric and electronic factors are important. In contrast, many of the same *ortho*-substituents on the *Z*-aryl ring led to increased GAT-1 inhibitory activity (**6l–6n**, **6p–6q**, **6s**–**6t**, **6v**). Bis-*ortho* substitution of the *Z*-aryl ring was therefore investigated (**6u**, **6w**) but the presence of a second *ortho*-substituent does not lead to further gains in affinity. A methyl substituent appears to be optimal although ethyl, chlorine and bromine also give appreciable increases in affinity. Indeed, the benefits of an *ortho*-methyl substituent have previously been noted by Knutsen[15] who reported the symmetrical di-methyl derivative **6ag**, however, our results clearly show that the second methyl group, on the *E*-aryl group is actually detrimental and lowers affinity compared to the unsymmetrical monosubstituted derivative (**6p** vs **6ag**). Likewise the majority of the unsymmetrical disubstituted derivatives prepared ($X \neq H \neq Y$) showed lower activity than the corresponding monosubstituted analogues ($Y = H$; $X \neq H$) reflecting the negative influence of substitution on the *E*-ring (e.g., see **6ad–6ag** vs **6p**). Curiously, however, disubstituted derivatives with an *ortho*-fluorine substituent on the *E*-ring ($Y = F$; $X \neq H$) had comparable or slightly higher affinities that the corresponding monosubstituted analogues ($Y = H$; $X \neq H$), despite the

Table 1
In vitro affinity (pK_i[a]) of compounds **6**

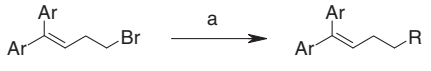
Compound	X	Y	pK_i (hGAT-1)
Tiagabine (**5**)	—	—	7.77
SKF-89976A (**3**)	H	H	7.14
6a	H	F	6.43
6b	H	Cl	6.78
6c	H	Br	6.70
6d	H	CF_3	5.72
6e	H	Me	7.03
6f	H	Et	6.99
6g	H	Ph	6.17
6h	H	OMe	6.43
6i	H	OCF_3	6.19
6j	H	2,6-di-F	6.16
6k	H	2-Me,4-F	6.42
6l	F	H	7.16
6m	Cl	H	7.39
6n	Br	H	7.54
6o	CF_3	H	7.03
6p	Me	H	7.69
6q	Et	H	7.53
6r	Ph	H	5.97
6s	OMe	H	7.25
6t	OCF_3	H	7.23
6u	2,6-di-F	H	6.48
6v	2-Me,4-F	H	7.47
6w	2,6-di-Me	H	7.11
6x	F	Me	7.14
6y	F	Et	7.05
6z	F	F	7.33
6aa	F	Cl	6.80
6ab	F	CF_3	6.45
6ac	Me	F	7.83
6ad	Me	Cl	7.21
6ae	Me	CF_3	6.42
6af	Me	OMe	7.15
6ag	Me	Me	7.38
6ah	Et	F	7.42
6ai	Cl	F	7.74
6aj	Br	F	7.66
6ak	OCF_3	F	7.28
6al	OMe	F	7.27
6am	2-Me,4-F	F	7.72
6an	CF_3	F	7.41

[a] [^{3}H]-Tiagabine radioligand binding assay to determine affinity at human recombinant GAT-1 transporters transiently expressed in HEK293 membranes using BacMam technology. Each determination lies within 0.3 log units of the mean.

Table 2
In vitro affinity(pK_i[a]) of compounds **7**

Compound	Ar	R	pK_i (hGAT-1)
Tiagabine (**5**)			7.77
SKF-89976A (**3**)			7.14
7a			6.86
7b			6.08
7c			5.56
7d			<5
7e			<5
7f			<5
7g			<5
7h			<5
7i			<5
7j			<5

[a] See Table 1.



Scheme 2. Synthetic route to afford compounds **7**. Reagents and conditions: (a) (i) amino acid ester, DIPEA, DMF, 65 °C; (ii) NaOH, EtOH, rt then HCl in Et_2O.

fact that in isolation a fluorine substituent on the *E*-ring led to a net fall in affinity (**6a**).

Evidently the SARs on the two aryl groups are not simply additive but can synergise in the right combination. The optimal substitution pattern from our studies was found to be *ortho*-methyl on the *Z*-ring and *ortho*-fluoro on the *E*-ring giving a 4,4-diphenylbut-3-enyl derivative (**6ac**) of comparable affinity to tiagabine. Compound **6ac** was further profiled in vitro where it was found to have low CYP450 inhibition potential (IC_{50} >10 μM at 1A2, 2C9, 2C19 and 3A4 isoforms) and high selectivity versus the other GABA transporters (pIC_{50} <4 at BGT-1, GAT-2 and GAT-3).

As part of our exploration we also briefly investigated replacement of the nipecotic acid headgroup. Many amino acids and amino acid isosteres have previously been evaluated for their ability to inhibit GABA uptake,[24] with best results being achieved with conformationally restricted cyclic amino acids, most notably nipecotic acid. We therefore limited our efforts to a small number of nipecotic acid derivatives and novel cyclic β- and γ-amino acids. The products **7** (Table 2) were prepared uneventfully as outlined in Scheme 2; all compounds are racemic or *meso* and stereochemistry, where shown, is used to indicate the relative configurations of the substituents.

The requisite amino acid ester starting materials were purchased or prepared according to literature procedures then alkylated with the appropriate 4-bromo-1,1-diaryl-1-butenes **20** or **21**[15] and saponified to give products **7**.

Disappointingly, all of the structures investigated were considerably less active than the reference compounds **3** and **5**, with GAT-1 binding only being measurable for the modified nipecotic acid derivatives **7a–c** (Table 2). Our results support the strict spatial requirements proposed for GAT-1 activity[16] and furthermore, the 10-fold drop in potency observed upon introduction of an α-fluorine to nipecotic acid indicate the importance of maintaining suitable pK_a/pK_b of the amino acid functionality.

In conclusion, two complementary stereospecific syntheses of unsymmetric *N*-(4,4-diaryl-3-butenyl)nipecotic derivatives were developed. Access to isomerically pure products allowed insights into the SAR on the aryl rings that otherwise would not have been possible. Indeed, it was established that the previously reported beneficial effect of an *ortho*-substituent on the phenyl rings[15] is limited to *ortho* substitution of the *Z*-phenyl ring. In contrast *ortho* substitution of the *E*-phenyl ring is detrimental to activity, except in the specific case of *ortho*-fluoro substitution of the *E*-ring in combination with *ortho* substitution of the *Z*-ring; in this instance a synergistic effect appears to be operating such that GAT-1 affinities are further enhanced. Accordingly, the optimal substitution pattern was found to be *ortho*-methyl on the *Z*-ring and *ortho*-fluoro on the *E*-ring giving a 4,4-diphenylbut-3-enyl derivative (**6ac**) of comparable affinity to tiagabine.

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22. [^{3}H]-Tiagabine displacement experiments were performed at equilibrium by the addition of HEK293 BacMam membranes expressing hGAT-1 (30–45 μg of protein per well) to 96 deep-well plates containing test compounds (1% final DMSO), [^{3}H]-tiagabine (Amersham Custom Synthesis TRQ10938,~1 nM final concentration) and 100 μM SKF-89976-A for non specific binding in a total volume of 400 μl/well of assay buffer (50 mM Tris–HCl, 1000 mM NaCl, 5 mM KCl, pH 7.7). After 2 h incubation at room temperature the reaction was stopped by rapid filtration through GF/B unifilter 96 plates (Perkin–Elmer 6005177), using an Omnifilter-96 harvester (Packard). Filters were washed (6 × 1 ml) with ice-cold 0.9% saline and counted for radioactivity using a TopCount liquid scintillation counter.
Binding data were analysed using an iterative non-linear least square curve fitting programme (GraphPad Prism™). Affinities (pK_i values) were calculated from the IC_{50} values using the Cheng–Prusoff equation.
23. [^{3}H]-Tiagabine selectively and potently binds to the GAT-1 transporter but is not itself a substrate of GAT-1; see Braestrup, C.; Nielsen, E. B.; Sonnewald, U.; Knutsen, L. J. S.; Andersen, K. E.; Jansen, J. A.; Frederiksen, K.; Andersen, P. H.; Mortensen, A.; Suzdak, P. D. *J. Neurochem.* **1990**, *54*, 639.
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