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Synthesis and evaluation of highly potent $GABA_A$ receptor antagonists based on gabazine (SR-95531)

Favaad Iqbal [a], Ryan Ellwood [a], Martin Mortensen [b], Trevor G. Smart [b], James R. Baker [a,*]

[a] Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, United Kingdom
[b] Department of Neuroscience, Physiology & Pharmacology, University College London, Gower Street, London WC1E 6BT, United Kingdom

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ABSTRACT

A selection of highly potent analogues based on the gabazine structure is described. Their syntheses are carried out in just four steps, and their potencies for antagonism at the $GABA_A$ receptor were measured. All antagonists showed significantly higher potencies compared to the parent competitive antagonist, gabazine.

$GABA_A$ receptors are ligand-gated Cl^- channels belonging to the Cys-loop superfamily of ionotropic receptors.[1] These receptors are expressed in a variety of isoforms. They play an important role in inhibiting cell excitation in the central nervous system.[2] In addition to mediating the effects of endogenous γ-aminobutyric acid (GABA), $GABA_A$ receptors are also modulated by an array of compounds including neurosteroids and benzodiazepines.[3]

A number of small molecule antagonists are known for the $GABA_A$ receptor.[4–9] Heaulme et al. showed SR 95103 (**1**, Fig. 1) to be a selective antagonist at the GABA binding sites on $GABA_A$ receptors.[10] Subsequent structure–activity relationship studies demonstrated that a series of aminopyridazine derivatives acted as selective, competitive antagonists at $GABA_A$ receptors.[10,11] These reports showed that the potency of these antagonists was clearly linked to the presence of an aromatic ring at the 6-position of the pyridazine ring and that the carboxylic acid side-chain was essential for antagonism at the $GABA_A$ receptor agonist binding sites. Furthermore, they also noted that substituents attached to the aromatic ring influenced antagonist potency. The most potent $GABA_A$ antagonist reported was the *para*-methoxy analogue, gabazine, also known as SR-95531 (**2**, Fig. 1).[11] Ueno et al. demonstrated that gabazine partially inhibited direct activation of the receptor by the barbiturate pentobarbital and by the steroid alphaxolone, not by blocking their binding, but possibly by acting as an allosteric inhibitor of $GABA_A$ receptor channel opening.[7] Since its inception, gabazine has become a widespread tool in scientific research of the $GABA_A$ receptor.[12–15]

In our ongoing studies into the development of labelled analogues of gabazine as probes for the $GABA_A$ receptor, we discovered that a relatively minor addition to the gabazine skeleton significantly enhances its antagonist potency. Herein, we report on highly potent gabazine analogues that inhibit $GABA_A$ receptor function, describing their synthesis and pharmacological evaluation.

Our initial target was the benzyl analogue of gabazine (**7a**, Scheme 1), the testing of which would indicate whether substituents in this position are tolerated.

The preparation of novel arylpyridazine **4** outlined in Scheme 1 adopts a microwave Suzuki–Miyaura protocol for the coupling of 3-amino-6-chloropyridazine **3** and 4-hydroxybenzeneboronic acid affording **4** in 72% yield.[16–18] We installed the benzyl motif in **5a** via a Williamson ether synthesis with benzyl bromide, achieving the synthesis of **5a** in a reasonable 41% yield. The ability to achieve selective N(2)-alkylation of arylpyridazines stems from a combination of steric effects from the adjacent phenyl

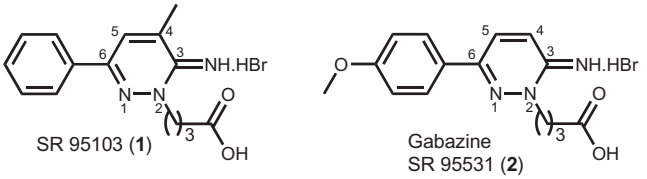

Figure 1. Structure of SR 95103 (**1**) and gabazine (**2**).

* Corresponding author. Tel.: +44 (0)20 7679 2653; fax: +44 (0)20 7679 7463.
E-mail address: j.r.baker@ucl.ac.uk (J.R. Baker).

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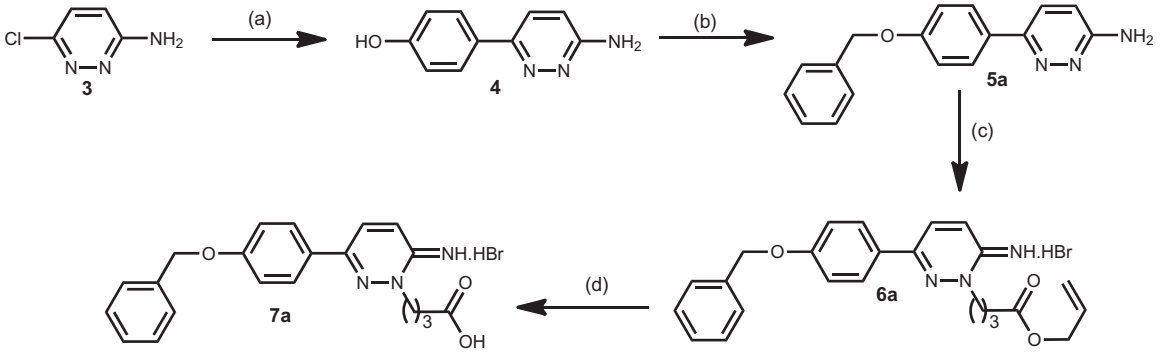
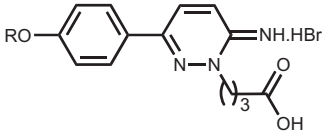


Scheme 1. Reagents and conditions: (a) 4-hydroxybenzeneboronic acid, bis(triphenylphosphine)palladium(II) dichloride, potassium carbonate, MeCN/H_2O, 120 °C, 72%; (b) sodium hydride, benzyl bromide, DMF, 0 °C, 41%; (c) allyl-4-bromobutyrate, DMF, 80 °C, 59%; (d) palladium(II) acetate, triethyl phosphite, dimedone, THF/H_2O, 56%.

Table 1
Structure–activity relationships of gabazine (**2**) and its analogues (**7a**–**c**)



Entry	Compound	IC_{50} (nM)	Relative potency
i	**2** (R = CH_3)	349	1
ii	**7a** (R = $C_6H_5CH_2$)	11	32
iii	**7b** (R = *m*-$MeOC_6H_4CH_2$)	7	50
iv	**7c** (R = *m*-$NO_2C_6H_4CH_2$)	3	116

IC_{50} values are mean (n = 5–8). Relative potency is determined as an IC_{50} ratio with respect to gabazine (= 1).

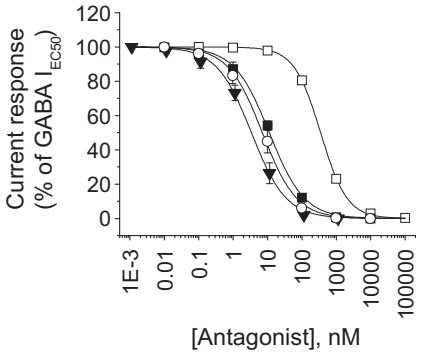


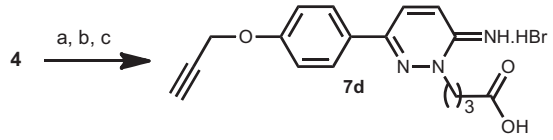
Figure 2. Concentration inhibition curves for gabazine **2** (open squares), **7a** (filled squares), **7b** (open circles) and **7c** (filled triangles) on recombinant $\alpha1\beta2\gamma2S$ $GABA_A$ receptors activated by EC_{50} GABA (10 μM).

group and resonance stabilisation.[11] N(2)-alkylation of arylpyridazines can be achieved through reaction with an appropriate bromoester; ethyl-4-bromobutyrate is commonly used. However, the harsh deprotection conditions that would be required were of concern.[11] We chose to react **5a** with the corresponding allyl ester, affording the protected antagonist **6a** in 59% yield. Allyl ester **6a** was easily deprotected using a relatively benign Pd-mediated method in 56% yield, thus affording the desired analogue **7a** in just four steps.

We examined the potency of this compound, using patch clamp electrophysiology with recombinant $\alpha1\beta2\gamma2S$ $GABA_A$ receptors transiently expressed in HEK293 cells. By constructing concentration inhibition curves for the response to EC_{50} GABA, the lower IC_{50} for **7a** (IC_{50} = 11 nM, Table 1, entry ii) indicated a 32-fold increase in antagonist potency compared to gabazine (IC_{50} = 349 nM, entry i). We also synthesised the *meta*-methoxy analogue **7b** in order to assess the feasibility of attaching tags to this position. The potency of this compound was increased further (IC_{50} = 7 nM, entry iii). Interestingly, installing an electron-withdrawing nitro-group in **7c** even further enhanced antagonist potency (IC_{50} = 3 nM, entry iv).

Our findings indicate that there is significant scope to modify the gabazine skeleton on the alkoxy substituent and considerably enhance antagonist potency at the $GABA_A$ receptor (Fig. 2).

In our final analogue **7d** (Scheme 2), we installed a propargyloxy group in place of the methoxy-group on **2**, thereby creating an alternative diverse point of attachment for prospective labelling groups. By employing a similar synthetic strategy as previous, we were able to isolate **7d**, which again showed an increased potency (IC_{50} = 40 nM; n = 6) compared to gabazine.



Scheme 2. Reagents and conditions: (a) propargyl bromide, sodium hydride, DMF, 0 °C, 71%; (b) allyl-4-bromobutyrate, DMF, 80 °C, 88%; (c) tetrakis(triphenylphosphine)palladium(0), morpholine, 86%.

In summary, we have developed a novel series of $GABA_A$ receptor antagonists. We have demonstrated that by the simple addition of a benzyl group, the antagonist potency of arylpyridazine analogues of GABA can be greatly increased. This is exemplified by **7a**, where we observed a 30-fold increase in potency compared to gabazine. We also found the inclusion of an electron-donating methoxy-group or an electron-withdrawing nitro-group on the *meta*-position of the benzyl ring (**7b** and **7c**, respectively) were not only tolerated but also enabled further increases in potency. Finally, we demonstrated that the presence of an additional benzyl group is not the only means of eliciting increments in antagonist potency. In the case of **7d**, a simple propargyl group will clearly suffice. The versatility of the alkyne and benzyl groups provides useful tools for further structural exploration of the core antagonist structure.

Acknowledgements

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Supplementary data

The electrophysiological methods and cell transfection methods have all been previously described.[19] Supplementary data (experimental procedures and characterisation of compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.067.

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