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Original article

Synthesis, biological evaluation and structure–activity relationship of new GABA uptake inhibitors, derivatives of 4-aminobutanamides

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ABSTRACT

Six series of 2-substituted 4-aminobutanamide derivatives were synthesized and evaluated for their ability to inhibit GABA transport proteins mGAT1–4 stably expressed in HEK-293 cell lines. The pIC_{50} values determined were in the range 4.23–5.23. Two compounds (**15b** and **15c**) were selected for further *in vitro* studies. These compounds were also subjected to preliminary behavioral studies to evaluate their anticonvulsant, antidepressant-like, and antinociceptive activities in mice. Their influence on motor coordination was also assessed. We report that, among a spectrum of *in vivo* activities, both **15b** and **15c** displayed significant activity against pentylenetetrazole (PTZ)-induced seizures.

1. Introduction

4-Aminobutyric acid (GABA) transporters (GATs) have been extensively investigated as prospective tools for studies on their key role in the dysfunction of GABAergic neurotransmission. To date, four different plasma-membrane transport proteins that mediate the uptake of synaptic GABA into neurons and glial cells have been identified and characterized. Following the nomenclature used by the Human Genome Organization (HUGO), these transporters are termed GAT1 (SLC6a1), BGT1 (SLC6a12), GAT2 (SLC6a13) and GAT3 (SLC6a11) which correspond to mouse mGAT1, mGAT2, mGAT3 and mGAT4, respectively [1–6]. Because the characterization of the test compounds was performed using mouse GABA transporters, the mouse nomenclature is used in the present paper.

The four transporter subtypes differ in their localization, affinity for GABA and pharmacological function. mGAT1 and mGAT4 are almost exclusively located in the central nervous system (CNS). Moreover, mGAT1 is primarily localized in presynaptic neurons and to a minor extent in synaptically apposed astrocytes [7,8], whereas mGAT4 is predominantly localized on distal astrocytes which are in direct contact with GABAergic neurons [9]. Thus, mGAT1 and mGAT4 can affect many brain functions including muscle relaxation, cognition, and memory [10,11]. By contrast, mGAT2 and mGAT3 are predominantly expressed in the liver and at lower levels in kidneys, whereas in the brain their significant concentrations are restricted to the leptomeninges (mGAT3 and mGAT2) and to cerebral blood vessels (mGAT3), indicating that these transporters are unlikely to play an important role in the inactivation of GABA [12,13].

Following the identification of GAT inhibitors it was demonstrated that they enhance the GABA tone, and therefore hold promise the treatment of several diseases in which GABA function is reduced, including epilepsy, migraine, neuropathic pain, Huntington's chorea, Parkinson's disease [14–18]. However, to explore both the physiological function of GATs and their individual structure, further compounds that selectively target and modulate GATs are needed.

Tiagabine (Fig. 1), a derivative of nipecotic acid, has been developed and marketed as an add-on treatment for partial epilepsy, and this drug is also in clinical trials for new indications

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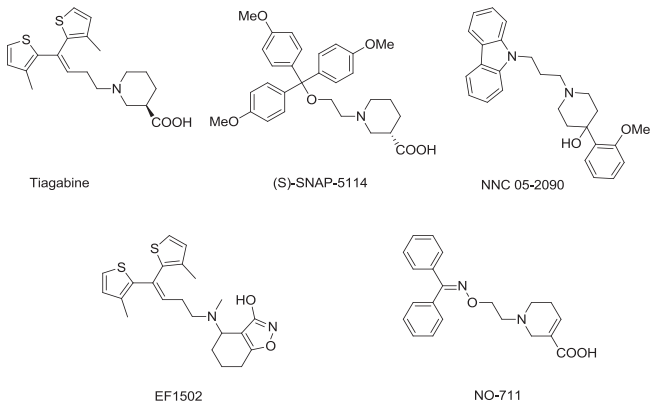

Fig. 1. Structure of lipophilic GABA uptake inhibitors of mGAT1–mGAT4.

including diabetic neuropathy and migraine. Tiagabine is a highly selective blocker of the GAT1 transporter, and this may limit its activity to regions of the CNS in which GAT1 plays a significant role (the cortex, cerebellum, and hippocampus) [19]. This drug strongly inhibits GABA uptake in synaptosomal preparations from rat brain, as well as in cultured neurons and glial cells *in vitro* [20]. Tiagabine generates a dose-dependent increase in extracellular GABA levels in rat brain *in vivo*, and specifically suppresses various chemically-induced seizures evoked by pentylenetetrazole (PTZ) and DMCM (6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate), as well as kindled seizures, whereas it only weakly influences maximal electroshock seizures [19]. Tiagabine demonstrates antinociceptive effects at moderate doses (7.2 and 24.3 μmol/kg) against thermally evoked pain in the mouse hot-plate test [21]. Furthermore, it has an antiallodynic activity and anxiolytic-like properties in rodent models [21–23]. Unfortunately, the utility of tiagabine is limited in part because of adverse effects, such as asthenia, diarrhea, dizziness and tremor [24,25].

Another GAT1 inhibitor which is effective in neuropathic pain is NO711 (Fig. 1). In the chronic constriction injury (CCI) model in mice NO711 induced late-onset and long-lasting analgesic effect. These results indicate that mGAT1 may be involved in the occurrence and development of neuropathic pain [26].

mGAT4 inhibitors are represented by moderately potent (*S*)-SNAP-5114, the most selective mGAT4 inhibitor up to now (Fig. 1) [27,28]. Recently, it was found that (*S*)-SNAP-5114 exerted antinociceptive effects by promoting the activation of $GABA_A$ and $GABA_B$ receptors in the spinal cord. Its analgesic efficacy has been confirmed in the tail flick test (a model of acute thermal nociception), in the late phase of the formalin test (a model of persistent pain) and in CCI neuropathic pain model. Studies conducted by *Kataoka* et al. [29] demonstrated that mGAT4 inhibitors might be useful in treatment of various painful conditions. However, the physiological role of mGAT4 in the CNS is not completely understood [27–29].

Our earlier studies focused on the search for novel biologically active compounds and demonstrated that several 2-substituted 4-hydroxybutanamides with affinity for GATs display not only anticonvulsant effects in some rodent models of seizures [30,31] but also antinociceptive properties in thermally and chemically induced acute and tonic pain models [30,32,33].

We present herein the synthesis and biological evaluation of six series of new 4-aminobutanamide derivatives.

The strategy in the development of these new compounds was motivated by our previous structural characterization and biological evaluation of 2-substituted derivatives of 4-hydroxybutanamides [31,34,35] and of compounds bearing a phthalimide group at the 4-position of the butanamides [36]. Following our previous investigations which showed that 4-(1,3-dioxoisoindolin-2-yl)butanamide derivatives with the 2-(4-benzhydryl)-piperazin-1-yl residue exhibited only weak GAT inhibition [36], we sought to obtain butanamide derivatives with a primary amine group at the 4-position. Thus, drug development commenced from GABA, which shows low selectivity for GATs [37]. Preliminary SAR studies indicated that the benzyl substituent on the amide group and the aromatic and lipophilic substituent at the 2-position of the 4-hydroxybutanamide moiety are crucial for their activity. In addition, the presence of a tertiary amino group, for example *N*-methyl-4,4-diphenylbut-3-en-1-amine (*N*-methyl-*N*-DPB), in analogues of GABA uptake inhibitors is necessary for effective interaction with the GABA uptake system [35,38]. To mimic the biaryl moieties of known GAT inhibitors a different *N*-bulky and lipophilic biaryl group was introduced at the 2-position of 4-aminobutanamide (Fig. 2).

2. Chemistry

To prepare the target compounds **15**–**20**(**a**–**e**), GABA was used as a starting material. The reaction routes to all the target compounds are outlined in Scheme 1.

First, the amine group of GABA was protected by phthalimide. Phthalimide-protected amino acid **1** was easily obtained from the parent amino acid and phthalic anhydride [39]. In the case of substituted α-halo amides **3a**–**e**, 4-(1,3-dioxoisoindolin-2-yl)butanoic acid (**1**) was brominated using *N*-bromosuccinimide. This ionic bromination procedure proved to be tolerant of phthalimido group and was superior to the standard Hell-Vollard-Zielinski procedure [40]. The molecule obtained, 2-bromo-4-(1,3-dioxoisoindolin-2-yl) butanoic acid (**2**), was purified by method described by Kolasa et *al.* [41]. Further treatment with thionyl chloride generated the acid chloride which then was reacted with various substituted derivatives of benzylamine at room temperature in dry THF for 12 h, giving molecules **3a**–**e**. The resulting amides (**3a**–**e**) were a substrate for the *N*-alkylation of earlier obtained amines (**4**–**8**).

The amines necessary for the reactions, namely 4-diphenylmethylene piperidine (**4**) and 4-benzhydryl piperidine (**5**), were prepared according to a published procedure [42]. *N*-Methyl-4,4-diphenylbut-3-en-1-amine (**6**) and 4,4-diphenylbut-3-en-1-amine (**7**) were prepared according to a published procedure using 1,1′-(4-bromobut-1-ene-1,1-diyl)dibenzene as a starting

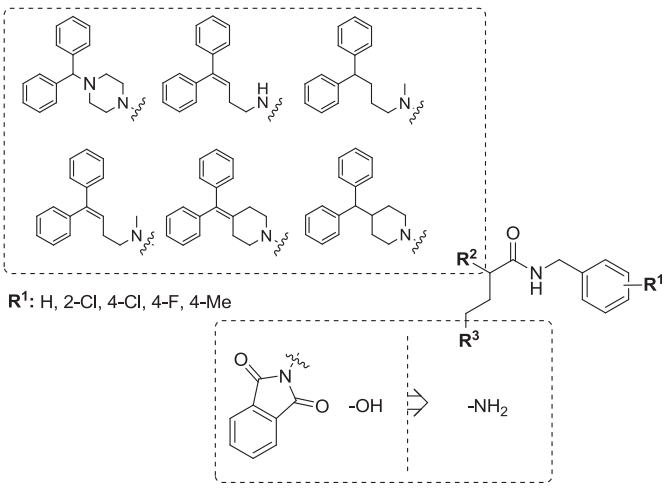

Fig. 2. Schematic structure of designed 2-substituted 4-aminobutanamides.