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Original article

Synthesis of *N*-substituted acyclic β-amino acids and their investigation as GABA uptake inhibitors

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ABSTRACT

In this publication, we describe the synthesis of new inhibitors for the GABA transporter subtypes GAT1 and especially GAT3. We started with 3-aminopropanoic acid possessing a distinct preference for GAT3 in comparison to GAT1 and furthermore its homolog 3-aminobutanoic acid. A series of respective *N*-substituted amino acids was synthesized by selective *N*-monoalkylation of these parent structures with 6 different arylalkyl alcohols via a Mitsunobu-type reaction. The resulting compounds were investigated for their inhibitory potency GABA transporter subtypes. Among all tested compounds the 4,4-diphenylbut-3-enyl substituted 3-aminobutanoic acid (*rac*)-**6b** showed highest potency with a pIC_{50} value of 5.34 at GAT1. Unfortunately, the expected GAT3 potency for 2-[tris(4-methoxyphenyl)methoxy] ethyl substituted derivatives was not as high as observed for the respective nipecotic acid derivatives.

1. Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS). In the last four decades, several neuro-pathological and psychiatric diseases have been connected directly or indirectly with the GABAergic neurotransmission such as e.g. dyskinesia, epilepsy, anxiety states, depression, and several other behavioral disorders [1]. Interesting pharmacological targets in search for new drugs in this field are therefore the receptors, transporters, and metabolic enzymes that are involved in the GABAergic neurotransmission. Thus, a promising approach to the successful treatment of cerebral disorder is represented by drugs affecting GABA-uptake. GABA-uptake from the synaptic cleft into the glial cells and presynaptic neurons takes place by transmembrane proteins, the so called GABA transporters. Of the GABA transporters (GATs) for different subtypes are known [2–9]. These are termed mGAT1, mGAT2, mGAT3, and mGAT4 when originating from mice but are named GAT-1, GAT-2, GAT-3, and BGT1, respectively, for all other species, e.g. rats and humans whereby the species is indicated by a respective prefix (e.g. hGAT-1) (for a survey of different nomenclatures of GATs see Dalby et al. [10]). An additional nomenclature has been proposed by the Human Genome Organization (HUGO). Referring to the latter the GAT homologs are denoted as GAT1 (encoding gene: SLC6A1), BGT1 (SLC6A12), GAT2 (SLC6A13) and GAT3 (SLC6A11). For the sake of simplicity, the HUGO nomenclature is used in terms of a species independent nomenclature in the following, although it is – if taken accurately – restricted to human.

Substantial differences have been found with respect to the distribution of these four subtypes in the organism. GAT1 and GAT3 are located almost exclusively in the central nervous system, where GAT1 has been shown to be predominantly responsible for the transport of GABA into neurons and GAT3 into glial cells. BGT1 and GAT2 have repeatedly been reported to be widely distributed throughout the brain, too, though in low concentrations and in peripheral organs like the liver and the kidneys. However, according to recent studies from Danbolt et al. BGT1 and GAT2 are not detectable in brain parenchyma. In the brain, both proteins show distinct expressions in the leptomeninges and GAT2, in addition, in a subpopulation of brain blood vessels only [11,12]. Thus, only GAT1 and GAT3 but not BGT1 and GAT2 can be considered to play a role in neurotransmitter inactivation [13]. Accordingly, compounds addressing GAT1 and GAT3 with high potency and selectivity are of special interest when it comes to the development of CNS drugs

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enhancing GABA neurotransmission. This is especially true for potent and selective GAT3 inhibitors as those are lacking so far.

Nipecotic acid (**1**), which can be considered as a rigidified β-alanine, has been found to be an inhibitor of GABA uptake in neuronal and astroglial cell cultures [14]. But due to its low lipophilicity, nipecotic acid (**1**) lacks the capability to cross the blood–brain barrier in sufficient amount [15]. This obstacle was overcome by attaching a lipophilic side chain to the nipecotic acid nitrogen leading to GABA uptake inhibitors such as SK&F-89976-A [(*rac*)-**2**] [16,17], tiagabine [(*R*)-**3**] [18,19] and (*S*)-SNAP-5114 [(*S*)-**4**] [20]. SK&F-89976-A [(*rac*)-**2**] displays a 4,4-diphenylbut-3-enyl side chain whereas in case of tiagabine [(*R*)-**3**] a 4,4-bis(3-methyl thiophene-1-yl)but-3-enyl moiety is present, both of which not only enhance the potency of these compounds but also substantially improve the subtype selectivity in favor of GAT1 inhibition as compared to the basic compounds (*rac*)-**1** and (*R*)-**1**, respectively. For (*S*)-SNAP-5114 [(S)-**4**] this subtype selectivity is reversed, this compound possessing the highest inhibitory potency at GAT3 which has largely to be ascribed to the presence of the 2-[tris(4-methoxyphenyl)methoxy]ethyl residue. A similar effect, the rever sal of subtype selectivity from GAT1 to GAT3, has been observed for several other amino acids acting as GAT inhibitors when 4,4-diarylbutenyl moieties as in (*rac*)-**2** or (*R*)-**3** were replaced by the sterically more demanding *N*-substituent of (S)-SNAP-5114 [(*S*)-4] these residues were applied [21]. Interestingly, for GAT1 and GAT3 inhibitors like **3** and **4** and related compounds the change in subtype selectivity is commonly paralleled by a switch in biological enantioselectivity, the highest potency and subtype selectivity for GAT1 inhibitors residing in the (*R*)- and for GAT3 inhibitors in the (*S*)-enantiomer (or the corresponding homochiral compound) (Fig. 1).

β-Alanine (3-aminopropanoic acid, **5a**) is long known to inhibit GABA uptake into glial cells [22] and in the meantime it has been intensively studied on the four GABA transporter subtypes [23]. In the test system that has been established in our group and is based on HEK293 cells stably expressing the individual murine GABA transporters, β-alanine (**5a**) was found to possess a reasonable potency at and subtype selectivity for GAT3 ($pIC_{50} = 4.66 \pm 0.06$) as compared to GAT1 (GAT1: $pIC_{50} = 2.55 \pm 0.03$). Also the methyl substituted derivative 3-aminobutanoic acid [(*rac*)-**6a**] displays good inhibitory potency at GAT3 ($pIC_{50} = 4.08 \pm 0.03$) but this time also at GAT1 ($pIC_{50} = 4.30 \pm 0.10$) thus being, in contrast to β-alanine (**5a**), almost equally potent at both transporters. With the inhibitory potencies at GAT1 [for (*rac*)-**6a**] and at GAT3 [for **5a** and (*rac*)-**6a**] being in the range of (*rac*)-nipecotic acid [(*rac*)-**1a**] we considered these compounds suitable starting points for the development of new GABA uptake inhibitors for these GABA transporters, especially GAT3. Thereby it was hoped the subtype selectivity inherent to the parent compounds **5a** and (*rac*)-**6a** might also positively contribute to that of appropriately *N*-substituted compounds (Fig. 2).

In this context, it should be mentioned that attachment of lipophilic residues – known to enhance inhibitory potency at GAT1 and GAT3 – to the β-amino acids **5a** and (*rac*)-**6a** were expected to provide data for the establishment of structure activity relationships for the respective acyclic amino acid derivatives, yet unknown so far.

Though **5a** and (*rac*)-**6a** display also reasonable potencies at BGT1 and GAT2 no attention should be paid to the development of inhibitors of these GABA transporter subtypes as these transporters due to their low abundance in the brain are of no significant relevance for the development of CNS active compounds [12]. For our study the 4,4-diphenylbut-3-enyl (**b**), the 2-[2-(10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)ethoxy]ethyl (**c**), the 2-[2-(5*H*-dibenzo[*b*,*f*]azepin-5-yl)ethoxy]ethyl (**d**) and the 2-[2-(10*H*-phenothiazin-10-yl)ethoxy]ethyl (**e**) moiety were selected to be employed as *N*-substituents which according to the results published by Andersen et al. [24] improve the potency at GAT1 as well as the subtype selectivity for this transporter. With regard to the development of more potent GAT3 inhibitors from **5a** and (*rac*)-**6a** the 2-[tris(4-methoxyphenyl)methoxy]ethyl (**f**) and the (*E*)-2-[tris(4-methoxy phenyl)]but-2-en-1-yl (**g**) residue should be used as *N*-substituents, which are known to enhance potency and subtype selectivity in favor of this transporter as demonstrated by (*S*)-SNAP-5114 [(*S*)-**4**] and the recently introduced DDPM-1457 [25], two of the most potent inhibitors of this subtype of GABA transporters.

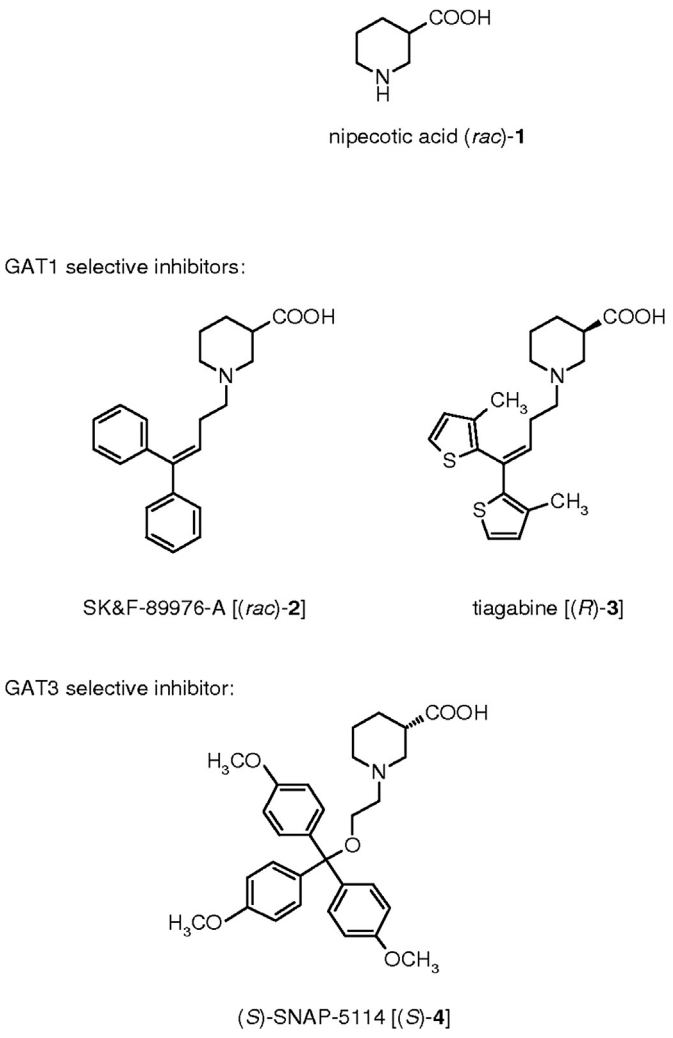

Fig. 1. Structures of GABA uptake inhibitors.

2. Chemistry

First attempts to synthesize the desired compounds were undertaken by reacting methyl 3-aminopropanoate hydrochloride (**9**) with the respective alkyl halides but resulted in yields that were not fully satisfying (Scheme 1). Thus, the exemplarily performed reaction of methyl 3-aminopropanoate (**7**) with the bromides **7b** and **7f** (K_2CO_3, KI, CH_3CN, 45 °C, 24 h) led to the corresponding *N*-alkylated ester derivatives **10b** and **10f** in moderate yields of 52% and 49% only.

Therefore, we decided to perform the synthesis of all other *N*-substituted amino acids by a variant of the Mitsunobu reaction