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Synthesis of 4-substituted nipecotic acid derivatives and their evaluation as potential GABA uptake inhibitors

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Dedicated to Prof. Dr. Gunther Seitz with warmest wishes on the occasion of his 80th birthday

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ABSTRACT

In this study, we disclose the design and synthesis of novel 4-susbtituted nipecotic acid derivatives as inhibitors of the GABA transporter mGAT1. Based on molecular modeling studies the compounds are assumed to adopt a binding pose similar to that of the potent mGAT1 inhibitor nipecotic acid. As substitution in 4-position should not cause an energetically unfavorable orientation of nipecotic acid as it is the case for *N*-substituted derivatives this is expected to lead to highly potent binders. For the synthesis of novel 4-substituted nipecotic acid derivatives a linear synthetic strategy was employed. As a key step, palladium catalyzed cross coupling reactions were used to attach the required biaryl moieties to the ω-position of the alkenyl- or alkynyl spacers of varying length in the 4-position of the nipecotic acid scaffold. The resulting amino acids were characterized with respect to their binding affinities and inhibitory potencies at mGAT1. Though the biological activities found were generally insignificant to poor, two compounds, one of which possesses a reasonable binding affinity for mGAT1, *rac*-**57**, the other a notable inhibitory potency at mGAT4, *rac*-**84**, both displaying a slight subtype selectivity for the individual transporters, could be identified.

1. Introduction

γ-Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the mammalian central nervous system (CNS). Pathologic GABAergic neurotransmission is known to be affiliated with a number of neurological disorders such as epilepsy,[1,2] Alzheimer's disease,[3] depression,[4] and neuropathic pain.[5] One approach to amplify the inhibitory neurotransmission within the CNS is to increase the GABA level in the synaptic cleft by inhibition of membrane bound GABA Transporters (GATs). Four different GABA transporters have been identified, which are termed GAT1, GAT2, GAT3 and BGT-1, according to a nomenclature suggested by HUGO.[6] When cloned from mouse cells, these transport proteins are denoted as mGAT1 (≙GAT1), mGAT2 (≙BGT-1), mGAT3 (≙GAT2) and mGAT4 (≙GAT3) respectively.[7] In the central nervous system mGAT1 and mGAT4 are the most prevalent GABA transporters, with mGAT1 being particularly expressed on presynaptic neurons[8,9] and mGAT4 majorly located on glia cells, adjacent to GABAergic neurons.[10] The abundance of mGAT2 and mGAT3 in the brain is very limited which largely precludes their relevance for the termination GABAergic neurotransmission in the CNS.[9,11]

Many of the so far synthesized GABA uptake inhibitors are based on nipecotic acid (**2**), a cyclic amino acid that shows in vitro activity as inhibitor of [^{3}H]GABA uptake and that can be considered as a GABA (**1**) analog.[12] A common approach to obtain potent mGAT1 inhibitors is the attachment of lipophilic substituents to the amino group of nipecotic acid (**2**). These residues consist of a side chain bearing different aromatic moieties at the ω-position which result in an increased inhibitory potency and subtype selectivity for GAT1 of these compounds compared to the parent amino acid **2**. GABA uptake inhibitors, like SK&F-89976A (**3**) and tiagabine [(*R*)-**4**] represent two prominent examples for this class of bioactive compounds of which tiagabine is the only clinically accepted drug in antiepileptic therapy (Fig. 1).[13]

The determination of the first crystal structure of a bacterial homolog of the transporters of the SLC6 family, the Leucin transporter (LeuT)[14] in 2005, opened the possibility for first structure based analysis of ligand binding. Two investigations analyzed the binding of GABA and small inhibitors to homology models of GAT-1 using docking calculations.[15,16] A later study analyzed the binding of tiagabine in GAT-1 using docking and molecular dynamics calculations.[17]

From this it could be concluded that two different binding poses exist, which are preferably adopted depending on the structure of the inhibitor. (*R*)-nipecotic acid [(*R*)-**2**] was reported to preferably adopt a binding pose with the piperidine nitrogen facing to the intracellular side, whereby the carboxylic moiety forms the same interactions with the protein observed for the native ligand GABA

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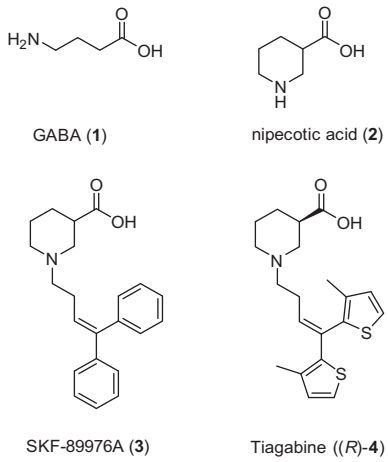

Figure 1. GABA uptake inhibitors.

(Fig. 2A).[18] Interestingly, a large inhibitor like tiagabine (**4**) adopts an inverse binding pose, in which the interactions of the carboxylic moiety with the protein are the same as for (*R*)-nipecotic acid [(*R*)-**2**], but wherein the amino nitrogen points towards the extracellular side and the lipophilic residue on the nitrogen extends towards the extracellular region of the transporter (Fig. 2B). The high binding affinity of tiagabine towards hGAT1 is thus believed to result from π–aliphatic and π–π-interactions of the lipophilic side chain in the extracellular vestibule.

In this context we assumed that nipecotic acid derivatives, which bear a lipophilic side chain with ω-biaryl moieties attached to the 4-position could be interesting as potential GABA uptake inhibitors. We supposed that compounds of this kind would exert a similar binding pose with regard to the amino acid scaffold as the unsubstituted nipecotic acid **2**, and as suchswould benefit from the binding energy of the nipecotic acid as well as from positive interactions of the lipophilic residue, which is oriented towards the extracellular side partly occupying the so called vestibule or S2 above the central binding site.

Thus, we synthesized a variety of 4-substituted nipecotic acid derivatives, the structure of which is depicted by the general formula in Figure 3, to investigate them for their biological activities at mGAT1.

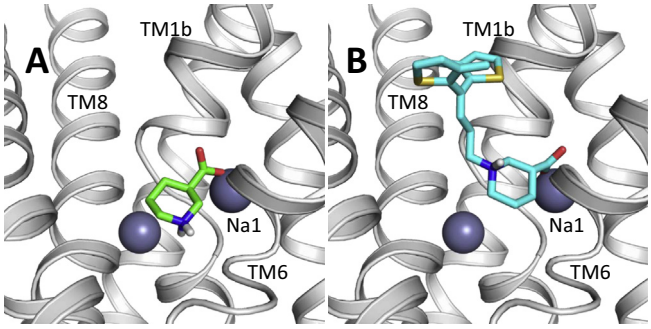

Figure 2. Side view of the hGAT1 homology model perpendicular to the membrane z-axis showing the central part of the transporter. The extracellular side is on the top and the intracellular side is at the bottom of the pictures. (A) Nipecotic acid (green carbon atoms) docked into the homology model of hGAT1, the nitrogen atom pointing to the intracellular side of the protein, (B) Tiagabine (cyan carbon atoms) docked into the homology model of hGAT1, the nitrogen atom pointing to the extracellular side of hGAT1.

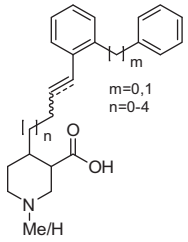

Figure 3. General structure of target compounds.

2. Results and discussion

2.1. Chemistry

For the synthesis of the target compounds with the general structures like *rac*-**7**, *rac*-**8**, *rac*-**11** and *rac*-**12**, 4-substituted nipecotic acid derivatives with a terminal alkyne function of the general structure *rac*-**5** or *rac*-**9**, bearing an *N*-Boc or *N*-Methyl group should be employed. The respective *cis*-diastereomers (not depicted) could be employed accordingly. These substrates, *rac*-**5** and *rac*-**9**, were accessible by an organocopper-(I)-mediated conjugate addition of Grignard reagents to heterocyclicα,β -unsaturated *N*-methyl- or *N*-Boc guvacine derivatives, as recently published by us.[19] For the introduction of aryl moieties at the terminal position of the 4-substituent of the nipecotic acid residue, we planned to transform the alkyne functionality in *rac*-**5** and *rac*-**9** into (*E*)-vinylboronic acid ester moieties (→ *rac*-**6** and *rac*-**10**). The resulting nipecotic acid esters, *rac*-**6** and *rac*-**10**, should then be subjected to 'Suzuki–Miyaura' reactions. Subsequent ester hydrolysis, and in case of the use of *rac*-**6** also the removal of the Boc-protecting group should lead to the 4-substituted nipecotic acid derivatives *rac*-**7** and *rac*-**11** with a ω-arylalkenyl-moiety. Likewise, the alkynes *rac*-**5** and *rac*-**9** appeared to be also well suited for the construction of nipecotic acid derivatives *rac*-**8** and *rac*-**12** with ω-aryl alkyne moieties by Sonogashira reactions and subsequent hydrolysis of the ester function and where necessary removal of the Boc protecting group (Scheme 1).

Additionally, some unprotected nipecotic acid derivatives with short side chains in 4-position devoid of aromatic residues should be synthesized and evaluated for their biological activity at the GABA transporters. That way, we intended to gain insight into how the individual parts of the 4-substituent of the nipecotic acid, the spacer and the aryl moiety, contribute to the biological activity of the entire molecule.

2.1.1. Preparation of the vinyl boronates

Herein we now report the synthesis and biological evaluation of the above mentioned compounds.

For the construction of the target compounds like *rac*-**7**, *rac*-**11**, *rac*-**8** and *rac*-**12** exhibiting aryl moieties attached to the 4-position of nipecotic acid via alkenyl or alkynyl chains of different length (Scheme 1) the respective pure 4-substituted nipecotic acid derivatives with (*E*)-1-vinylboronic acid ester moiety (*rac*-**6**, *rac*-**10**) or 4-ω-alkynyl substituted derivatives (*rac*-**5**, *rac*-**9**) were required. Depending on whether in the final compounds the amino group of the nipecotic acid moiety should carry an *N*-methyl group or be unsubstituted, the synthesis commenced from either the *N*-methyl derivatives *rac*-**13**, *rac*-**14** (Scheme 2) or the *N*-Boc protected compounds *rac*-**19**–*rac*-**22**, which were available to us as pure but racemic diastereomers from previous studies.[19]