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Original article

Development of imidazole alkanoic acids as mGAT3 selective GABA uptake inhibitors

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Dedicated with the best wishes to Prof. Fritz Eiden on occasion of his 85th birthday.

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ABSTRACT

A new series of potential GABA uptake inhibitors starting from of 1*H*-imidazol-4-ylacetic acid with the carboxylic acid side chain originating from different positions and varying in length have been synthesized and tested for the inhibitory potency at the four GABA uptake transporters mGAT1–4 stably expressed in HEK cells. Further two bicyclic compounds with a rigidified carboxylic acid side chain were included in this study. The results of the biological tests indicated that most ω-imidazole alkanoic and alkenoic acid derivatives exhibit the highest potencies as GABA uptake inhibitors at mGAT3.

1. Introduction

Since the initial isolation of γ-aminobutyric acid (GABA) in brain tissue in 1950 [1–3] and its identification as the major inhibitory neurotransmitter in the central nervous system (CNS) in the 1960s [4], about 40% of brain synapses were identified to be GABAergic. In the same decade, the existence of an active transport system deactivating the GABAergic signalling was discovered [5]. In 1986, Radian et al. purified the first GABA transporter showing dependence on Na^+ and Cl^- for transport [6]. By molecular cloning from different species, including mice, rats, and humans, the existence of four distinct subtypes of GABA transporters has been shown [7,8]. According to the nomenclature used for mice, the GABA transporter subtypes are denoted as mGAT1, mGAT2, mGAT3, and mGAT4. In contrast, GATs originating from other species including humans and rats are termed GAT-1, BGT-1, GAT-2, and GAT-3 [9–11], respectively.[1] The GABA-transporters differ in their regional distribution. In contrast to mGAT2 and mGAT3, which exist inside and outside the brain [12,13], the prevalent GABA transporters subtypes mGAT1 and mGAT4 are mainly located in the CNS. Thereby mGAT1 is widely distributed in the brain and predominantly found in the cerebral cortex, the cerebellum, the basal ganglia, and the hippocampus [12,13]. The distribution of mGAT4 is more restricted. Strong intensities are observed in certain brainstem nuclei, in the thalamus, in the hypothalamus, the retina, and olfactory bulb [12,14]. The four subtypes also differ with respect to their cellular and subcellular localization. Thus, mGAT1 is primarily found on presynaptic neurons in the synapse and to a minor extent on processes of astrocytes enveloping synapses. mGAT4 is predominantly expressed on distal processes of astrocytes in direct contact with GABAergic synapses [14,15]. mGAT2 is found to a large extend on astrocytes, but outside the synaptic cleft of GABA neurons [15,16]. mGAT3 has been found to be primarly located on leptomeninges [15,17]. In very low level, it seems to be also present throughout the brain on astrocytes and neurons at extrasynaptic sites. The different carrier proteins vary also in their affinity to GABA (**1**) [9]. mGAT1, mGAT3, and mGAT4 show high affinity, whereas mGAT2 possesses low-affinity to GABA (**1**), but also is able to carry betaine [7,12].

A number of CNS disorders such as epilepsy [18], Morbus Parkinson [19], and Chorea Huntington [20] are associated with a reduced neurotransmission in the GABAergic system and can potentially be alleviated by drugs that augment GABAergic neurotransmission [18,21,22]. Inhibition of the uptake by GABA transporters and the associated increase of GABA concentration in the synaptic cleft [23] has, for example, been realized with the cyclic

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[1] Since the biological activity of the compounds described in this paper was evaluated using murine transporters, the mice nomenclature will preferably be used in this publication. If test results obtained for GABA transporters of other species are quoted, the nomenclature of the respective species will be given in parenthesis.

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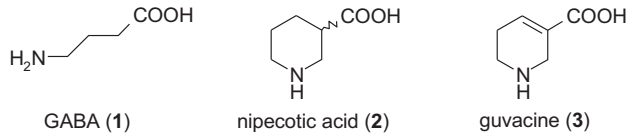


Fig. 1. GABA (1) and cyclic GABA uptake inhibitors.

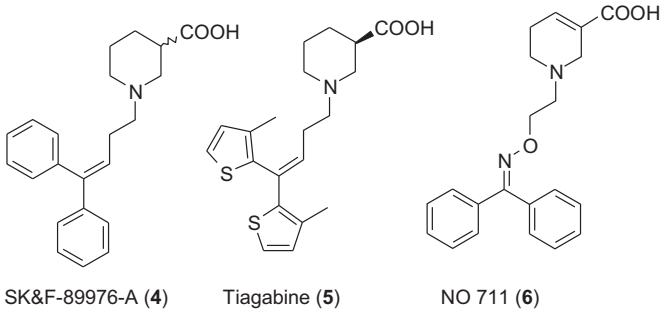


Fig. 2. Cyclic GABA uptake inhibitors of mGAT1.

compounds (*R*,*S*)-nipecotic acid (**2**, Fig. 1) and guvacine (**3**, Fig. 1) belonging to the first generation of GABA uptake inhibitors. Although both compounds show *in vitro* high GABA uptake inhibition without intrinsic activity at $GABA_A$-receptors, the pharmacological usefulness of these compounds was limited as they lack the capability to cross the blood–brain barrier [24].

This obstacle was overcome by introducing characteristic lipophilic side chains to the parent compounds like nipecotic acid and guvacine leading to several GABA uptake inhibitors such as SK&F-89976-A [25] (**4**, Fig. 2), Tiagabine [26] (**5**, Fig. 2), or NO 711 [27] (**6**, Fig. 2) which, apart from being systemically active, were found to be selective and, as compared to the parent compounds, distinctly more potent inhibitors of mGAT1 [28]. This research culminated in the development of Tiagabine (**5**) which is in clinical use for the therapy of epilepsy since 1997 classifying mGAT1 an approved drug target for the therapy of this disease [29].

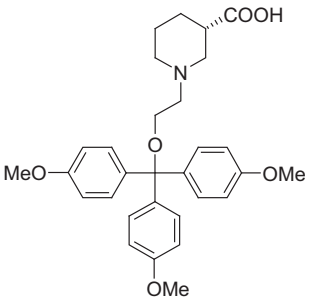
With potent and selective inhibitors of mGAT2, mGAT3, and mGAT4 still lacking, the therapeutic potential of these GABA transporters is not particularly clear so far. But anticonvulsant activity has been demonstrated for the moderate mGAT4 selective nipecotic acid derivative (*S*)-SNAP-5114 [28,30] (**7**, Table 1), the poorly mGAT2 selective N-substituted 4-hydroxy-4-aryl-piperidine derivative NNC 05-2045 [31] (**8**, Table 1) and the mGAT1/mGAT2 selective compound EF1502 [32] (**9**, Table 1).

Up to date, only few selective inhibitors for mGAT3 are published. The majority of compounds inhibiting this transporter are also inhibitors of mGAT4 indicating that the binding sites of the transporters mGAT3 and mGAT4 posses similar structural characteristics [7]. SNAP-5294 (**10**, Table 1) developed by Murali Dhar et al. [28] shows the best inhibitory potency at mGAT3 in combination with a reasonable selectivity for this transporter, which mainly originates from the lipophilic side chain present in the molecule [28].

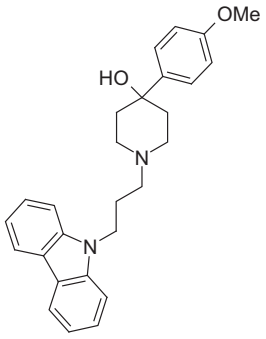
In this publication, we present the syntheses and biological evaluation of potential GABA uptake inhibitors based on imidazole derivatives. The drug development started with 1*H*-imidazol-4-yl-acetic acid (**20**, Fig. 3), a conformational restricted analogue of GABA. First evidence of partial agonistic activity at $GABA_A$-receptors as well as at $GABA_C$-receptors of the histamine metabolite 1*H*-imidazol-4-ylacetic acid (**20**) was provided by Tunnicliff in 1998 [33]. Recently, Madsen et al. [34] reported on the influence of various substituted 1*H*-imidazol-4-ylacetic acid derivatives on the activity of $GABA_A$-receptors and $GABA_C$-receptors. In parallel, we had found, when screening for new GABA uptake inhibitors applying a test system based on stably expressed mGAT1–mGAT4 in HEK cells [35], that, apart from effects on GABA receptors, 1*H*-imidazol-4-ylacetic acid (**20**) inhibits also the GABA uptake. Besides a moderate inhibitory effect at mGAT1 (pIC_{50} value of 3.208 ± 0.121) the imidazole compound **20** exhibits a particularly high inhibitory potency at mGAT3 (pIC_{50} value of 4.756 ± 0.080). With a subtype selectivity of >10:1 for mGAT3 compared to mGAT1, we considered 1*H*-imidazol-4-ylacetic acid (**20**) a promising starting point for the development of mGAT3 selective GABA uptake inhibitors.

In order to determine the relationship between the structure and the activity as well as the selectivity of imidazole derivatives

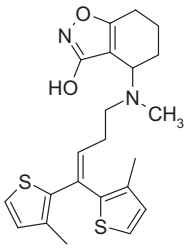
Table 1
GABA uptake inhibitors.



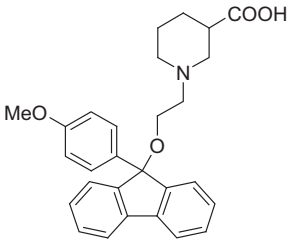
(*S*)-SNAP-5114 (7)



NNC 05-2045 (8)



EF1502 (9)



SNAP-5294 (10)

Compound	Uptake-inhibition IC_{50} (μM)[a]			
	mGAT1	mGAT2	mGAT3	mGAT4
7 [30]	85 ± 14	35 ± 13	22 ± 4	3.0 ± 0.3
8 [31]	19 ± 2	1.4 ± 0.3	41 ± 11	15 ± 4
9 [32]	4	22	>300	>300
10 [28]	132 ± 50 (hGAT-1)	27 ± 10%[b] (hBGT-1)	51 ± 4 (rGAT-2)	142 ± 21 (hGAT-3)

[a] The IC_{50} values originate from literature. The references are given in parenthesis after the corresponding compound.
[b] Percent inhibition at 100 μM.