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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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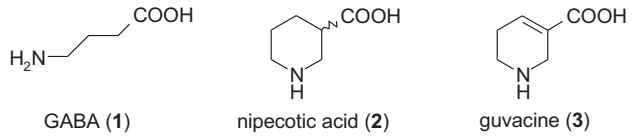


Fig. 1. GABA (1) and cyclic GABA uptake inhibitors.

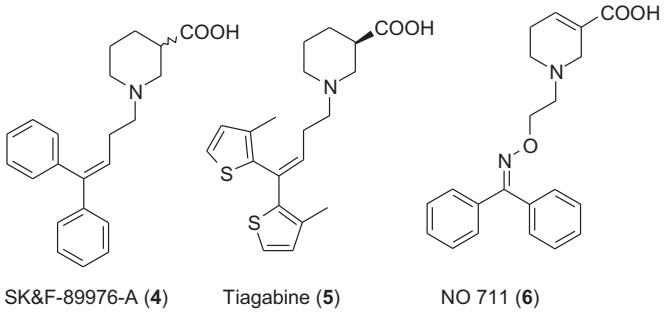


Fig. 2. Cyclic GABA uptake inhibitors of mGAT1.

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].

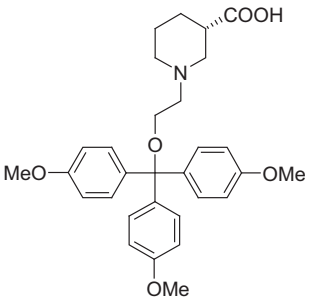
With potent and selective inhibitors of mGAT2, mGAT3, and mGAT4 still lacking, the therapeutic potential of these GABA transporters is not particularly clear so far. But anticonvulsant activity has been demonstrated for the moderate mGAT4 selective nipecotic acid derivative (*S*)-SNAP-5114 [28,30] (**7**, 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].

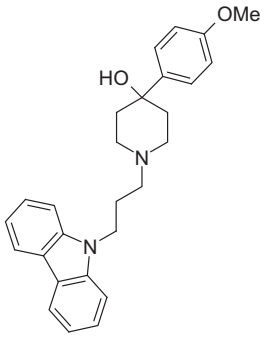
In this publication, we present the syntheses and biological evaluation of potential GABA uptake inhibitors based on imidazole derivatives. The drug development started with 1*H*-imidazol-4-yl-acetic acid (**20**, Fig. 3), a conformational restricted analogue of GABA. First evidence of partial agonistic 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

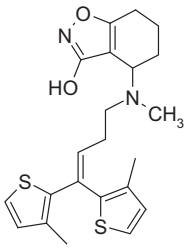
Table 1
GABA uptake inhibitors.



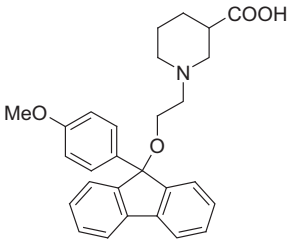
(*S*)-SNAP-5114 (7)



NNC 05-2045 (8)



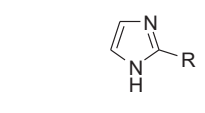
EF1502 (9)



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.



R =			R =			R =		
	COOH	**11**		COOH	**19**		CH_2COOH	**26**
	CH_2COOH	**12**		CH_2COOH	**20**		$(CH_2)_2COOH$	**27**
	$(CH_2)_2COOH$	**13**		$(CH_2)_2COOH$	**21**		$(CH_2)_3COOH$	**28**
	$(CH_2)_3COOH$	**14**		$(CH_2)_3COOH$	**22**		$(CH_2)_4COOH$	**29**
	$(CH_2)_4COOH$	**15**		$(CH_2)_4COOH$	**23**		$(CH_2)_5COOH$	**30**
	$CH{=}CHCOOH_{(E)}$	**16**		$CH{=}CHCOOH_{(E)}$	**24**		$(CH_2)_6COOH$	**31**
	$CH{=}CHCOOH_{(Z)}$	**17**		$(CH_2)_2CH{=}CHCOOH_{(E)}$	**25**		$(CH_2)_7COOH$	**32**
	$(CH_2)_2CH{=}CHCOOH_{(E)}$	**18**						

Fig. 3. Different monocyclic imidazole compounds.

with a carboxylic acid side chain as GABA uptake inhibitors, the structure of 1*H*-imidazol-4-ylacetic acid (**20**, Fig. 3) was systematically modified. Thus, by changing the length and the position of the side chain various analogues of **20** (**11**–**32**, Fig. 3) were synthesized as potential GABA uptake inhibitors and characterized with regard to their potency at and selectivity for the transporters. For the 2-substituted imidazole derivatives the side chain was extended from one C-atom (1*H*-imidazol-2-carboxylic acid (**11**)) up to five C-atoms (5-(1*H*-imidazol-2-yl)pentanoic acid (**15**)). In addition, the unsaturated compounds (2*E*)-3-(1*H*-imidazol-2-yl) acrylic acid (**16**), the *Z*-isomer (2*Z*)-3-(1*H*-imidazol-2-yl)acrylic acid (**17**) as well as (2*E*)-5-(1*H*-imidazol-2-yl)pent-2-enoic acid (**18**) were synthesized as structural variations of **13** and **15**, and evaluated for their biological activity to study the effects of the side chain geometry on the potency of these compounds. To complete the array of test compounds, carboxylic acid side chains of different length were also attached to the 4-position of the imidazole core structure which resulted in ω-imidazole-4-yl alkanoic and alkenoic acids **19**–**25**. Attaching carboxylic acid side chains at the ring nitrogen led to the ω-imidazole-1-yl alkanoic acids **26**–**32**.

2. Chemistry

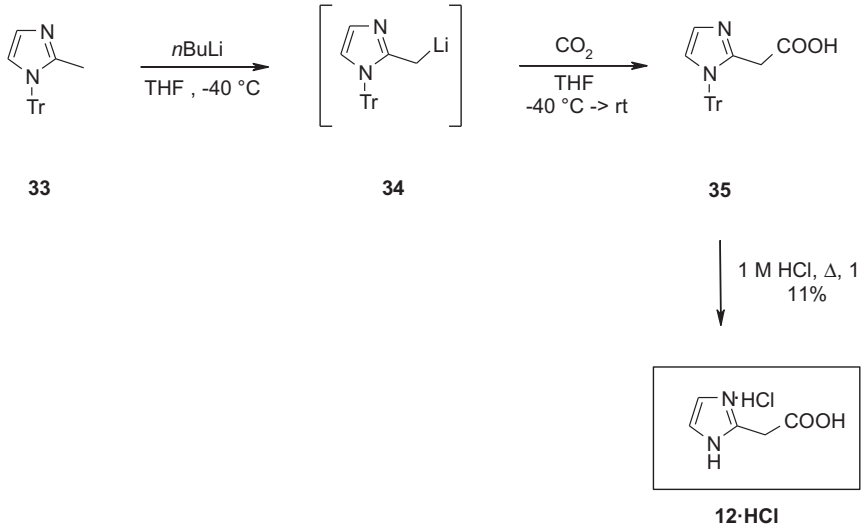
2.1. ω-Imidazole-2-yl alkanoic and alkenoic acids

1*H*-Imidazol-2-carboxylic acid (**11**) was prepared according to literature [36] and 1*H*-imidazol-2-yl propanoic acid **13** by hydrolysis of 3-(1*H*-imidazol-2-yl)propanoic acid ethyl ester [37] with NaOH in aqueous EtOH (yield 86%).

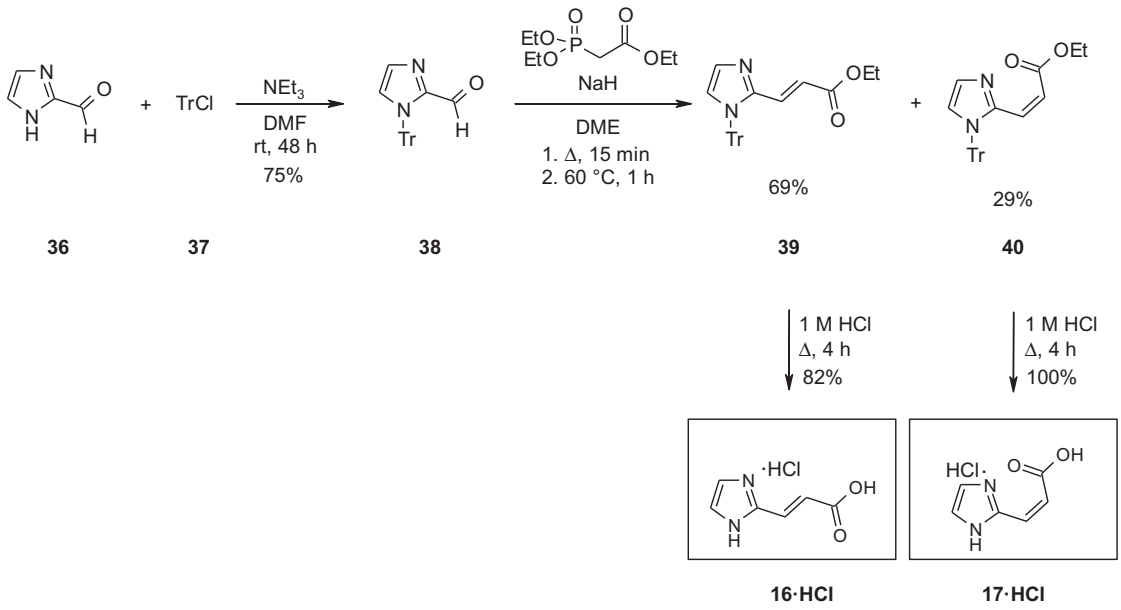
For the syntheses of the ω-imidazole-2-yl alkanoic acids **12** and **14**–**18**, the synthetic sequences outlined in Schemes 1–4 were followed.

The synthesis of the 2-(1*H*-imidazol-2-yl)acetic acid hydrochloride (**12**·HCl) was realized starting from 2-methyl-1-triphenylmethyl-1*H*-imidazole (**33**) which was treated with *n*BuLi in THF at −40 °C and subsequently with carbon dioxide to provide the acetic acid derivative **35**. Subsequent removal of the protecting group performed by refluxing **35** in 1 M HCl yielded 2-(1*H*-imidazol-2-yl)acetic acid hydrochloride (**12**·HCl), albeit in low yield (11%, Scheme 1).

For the synthesis of the stereoisomeric propenoic acid derivatives **16** and **17**, first 1*H*-imidazol-2-carbaldehyde (**36**) was



Scheme 1. Synthesis of **12**·HCl (Tr = Triphenylmethyl).



Scheme 2. Synthesis of **16**·HCl and **17**·HCl (Tr = Triphenylmethyl).

transformed into the N-triphenylmethyl protected derivative **38** by treatment with triphenylmethyl chloride in the presence of NEt_3 (yield 75%). When subjected to a Horner–Wadsworth–Emmons reaction analogous to a procedure published by Hunt [37], **38** yielded the isomeric mixture of the propenoic ethyl esters **39** [38] and **40** which were isolated in pure form. Subsequent deprotection and hydrolysis afforded the test compounds **16**·HCl and **17**·HCl in high yields (Scheme 2).

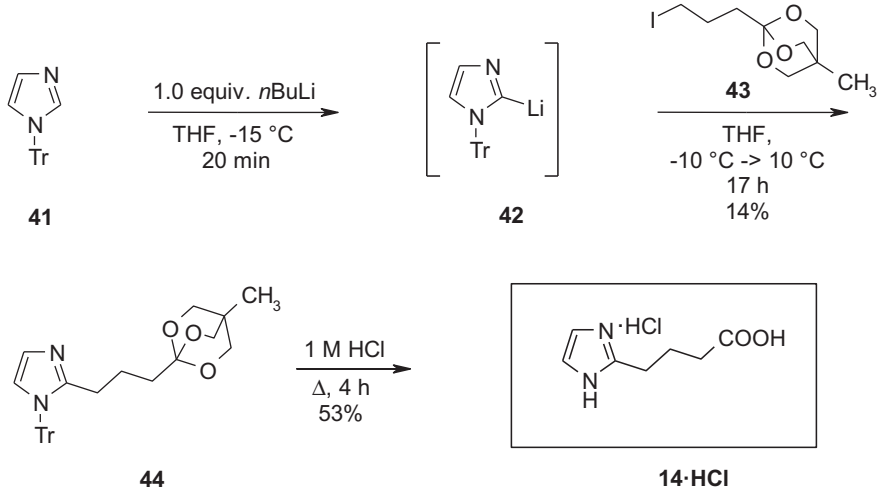
For the synthesis of 4-(1*H*-imidazol-2-yl)butanoic acid hydrochloride (**14**·HCl), 1-triphenylmethyl-1*H*-imidazole (**41**) [39] was treated with *n*BuLi to generate **42**, followed by 1-(3-iodopropyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**43**) in THF providing **44** in 14% yield, which was converted to the target compound **14**·HCl by heating in aqueous HCl (53%, Scheme 3).

The preparation of the test compounds **15** and **18** exhibiting a C-5 carboxylic acid side chain was realized starting from 3-(1-triphenylmethyl-1*H*-imidazol-2-yl)propanal (**45**) [37,40]. Upon subjection of **45** to a Horner–Wadsworth–Emmons reaction, the unsaturated ester **46** was obtained. Hydrolysis of **46** carried out by refluxing in 2 M HCl yielded the free, unsaturated amino acid **18**·HCl (70%) which upon catalytic hydrogenation over 5% Pd–C in MeOH provided the saturated 5-(1*H*-imidazol-2-yl)pentanoic acid hydrochloride **15**·HCl (74%).

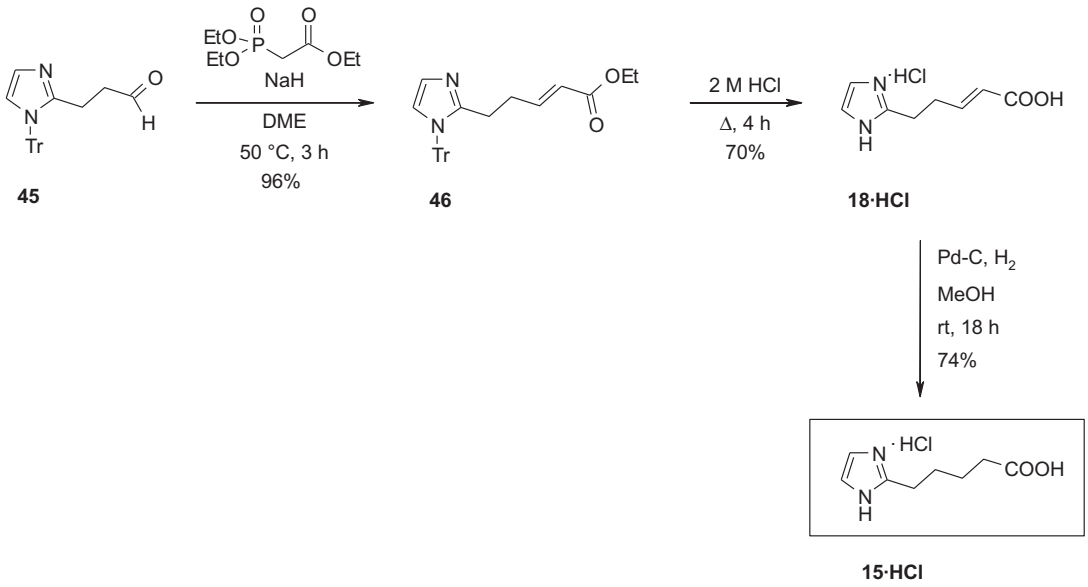
2.2. ω-Imidazole-4-yl alkanoic and alkenoic acids

In this series of compounds with the carboxylic acid side chain originating from the 4-position of the imidazole ring, 1*H*-imidazol-4-ylcarboxylic acid (**19**), 1*H*-imidazol-4-ylacetic acid (**20**), and (2*E*)-3-(1*H*-imidazol-4-yl)acrylic acid (**24**) were commercially available. 3-(1*H*-imidazol-4-yl)propanoic acid (**21**) [41] and 4-(1*H*-imidazol-4-yl)butanoic acid (**22**) [42–45] were synthesized according to literature procedures.

For the synthesis of 5-(1*H*-imidazol-4-yl)pentanoic acid hydrochloride (**23**·HCl), a strategy analogous to that employed in the preparation of 5-(1*H*-imidazol-2-yl)pentanoic acid hydrochloride (**15**·HCl) was used. Thus, 3-(1-triphenylmehtyl-1*H*-imidazol-4-yl)propanal (**47**) prepared from the N-protected 3-(1*H*-imidazole-4-yl)propanoic acid methyl ester according to a literature procedure [46] was transformed into (2*E*)-5-(1-triphenylmethyl-1*H*-imidazol-4-yl)pent-2-enoic acid



Scheme 3. Synthesis of **14**·HCl (Tr = Triphenylmethyl).



Scheme 4. Synthesis of **15**·HCl and **18**·HCl (Tr = Triphenylmethyl).

ethyl ester (**48**) via a Horner–Wadsworth–Emmons reaction. Subsequent deprotection of the imidazole moiety and hydrolysis of the ethyl ester group by heating **48** in a mixture of 2 M HCl and EtOH to reflux yielded the free amino acid **25**·HCl. Finally, the desired saturated amino acid **23**·HCl was obtained by catalytic hydrogenation over 5% Pd–C in MeOH in 72% yield (Scheme 5).

2.3. ω-Imidazole-1-yl alkanoic acids

Compounds **27** [47] and **28** [48] belonging to the third series of ω-imidazole alkanoic acids with the alkanoic acid side chain originating from the 1-position of the imidazole ring were prepared according to literature procedures. The syntheses of the remaining ω-imidazole-1-yl alkanoic acids, compounds **26** as well as **29**–**31** and **32**, were performed according or rather analogue to modified literature procedures [49–51].

3. Results and discussion

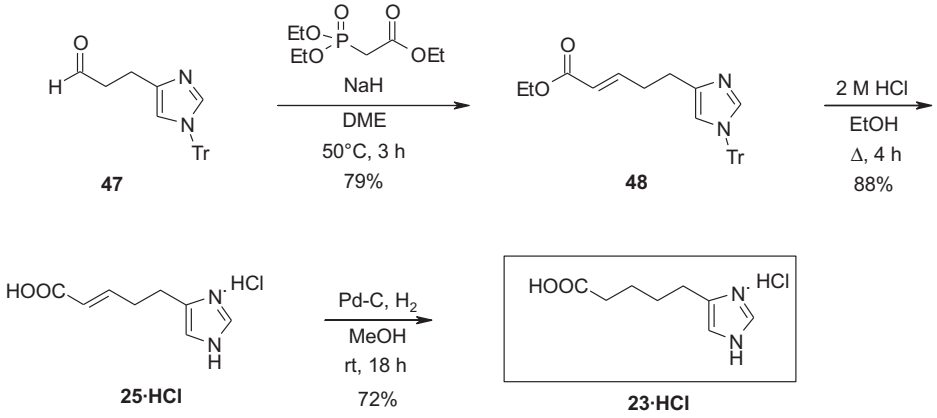
3.1. Biological evaluation

The compounds of all three series of different imidazole alkanoic and alkenoic acids were evaluated in [^{3}H]GABA uptake assays for their inhibitory potency at the four subtypes of GABA transporters, i.e. mGAT1, mGAT2, mGAT3, and mGAT4. These [^{3}H]GABA uptake assays were performed in a uniform manner using HEK cell lines, each stably expressing one of the four GABA transporter subtypes [30]. The potency of the tested compounds to inhibit [^{3}H]GABA uptake is given as pIC_{50} value. For test compounds of low potency, unable to reduce the specific [^{3}H]GABA uptake to a value below 50% at a specific concentration (100 μM, 1 mM, 10 mM), no pIC_{50} values were determined. Instead, for these compounds, the percentage of the remaining specific [^{3}H]GABA uptake at the specified concentration is given.

In addition to the three series of 1*H*-imidazole derivatives with different carboxylic acid side chains, a related series of homologous ω-aminoalkanoic acids with chain length ranging from two to seven carbons including GABA (**1**) was evaluated for their potencies at the different GABA transporters. The data obtained for these reference compounds are listed in Table 2, those for the imidazole derivatives in Tables 3, 5, and 7.

3.1.1. ω-Imidazole-2-yl alkanoic and alkenoic acids

For better legibility the potencies of ω-imidazole-2-yl alkanoic acids are, in addition, presented in a graphical manner as function of the chain length (Fig. 4). As can be seen from Fig. 4 the compounds are most potent at mGAT3 with 3-(1*H*-imidazol-2-yl) propanoic acid hydrochloride (**13**·HCl) exhibiting an alkyl chain



Scheme 5. Synthesis of **23**·HCl and 25·HCl (Tr = Triphenylmethyl).

Table 2
pIC_{50} values of ω-aminoalkanoic acids at mGAT1–mGAT4.

Compound	No.	mGAT1	mGAT2	mGAT3	mGAT4
Gylcine	**49**	59.8%[a]	2.65 ± 0.10	2.87, 2.85	2.51 ± 0.12
β-Alanine	**50**	2.59 ± 0.03	3.48 ± 0.10	4.66 ± 0.06	4.46 ± 0.13
GABA	**1**	5.14 ± 0.09	4.56 ± 0.06	5.09 ± 0.23	5.18 ± 0.13
δ-Aminovalerianic acid	**51**	4.13 ± 0.09	3.87 ± 0.02	3.72 ± 0.05	3.30 ± 0.04
ϵ-Aminohexanoic acid	**52**	80.8%	55.8%	70.9%	65.1%
ζ-Aminoheptanoic acid	**53**	107.0%	61.8%	96.4%	84.3%

If not stated otherwise the results are given as means of pIC_{50}-values ± SEM of three independent experiments each performed in triplicate, percentages represent specific binding remaining in presence of 1 mM.
[a] 10 mM inhibitor.

length of three C-atoms inhibiting mGAT3 best. According to Fig. 4 (and Table 3), **13**·HCl exhibits not only the highest affinity of the tested compounds to the mGAT3 transporter with a pIC_{50} value of 4.54, but also good subtype selectivity. Notably, the efficacy of compound **13**·HCl at mGAT3 compared to mGAT4 is 10, compared to mGAT2 18 and compared to mGAT1 even ≥34 times higher (Table 4). Both, elongation and reduction of the chain length cause lower inhibitory potencies (see Fig. 4). For example, 2-(1*H*-imidazol-2-yl)acetic acid hydrochloride (**12**·HCl) possesses lower inhibitory potency at mGAT3, but the potency remains still detectable as does the subtype selectivity. Further shortening leads to 1*H*-imidazol-2-carboxylic acid hydrochloride (**11**·HCl), which is then devoid of any significant inhibitory potency ($pIC_{50} \leq 3.0$). The obtained biological data demonstrate also the influence of the presence of a double bond on the potency of the compounds. The unsaturated, *E*-configured (2*E*)-3-(1*H*-imidazol-2-yl)acrylic acid hydrochloride (**16**·HCl) possesses the same number of C-atoms as 3-(1*H*-imidazol-2-yl)propanoic acid hydrochloride (**13**·HCl), but exhibits lower potency at mGAT3, though this is still significant (see Table 4). In contrast, the *Z*-isomer (2Z)-3-(1*H*-imidazol-2-yl)acrylic acid hydrochloride (**17**·HCl) is lacking any reasonable affinity at mGAT3, but also at any other GABA transporter subtype. When the

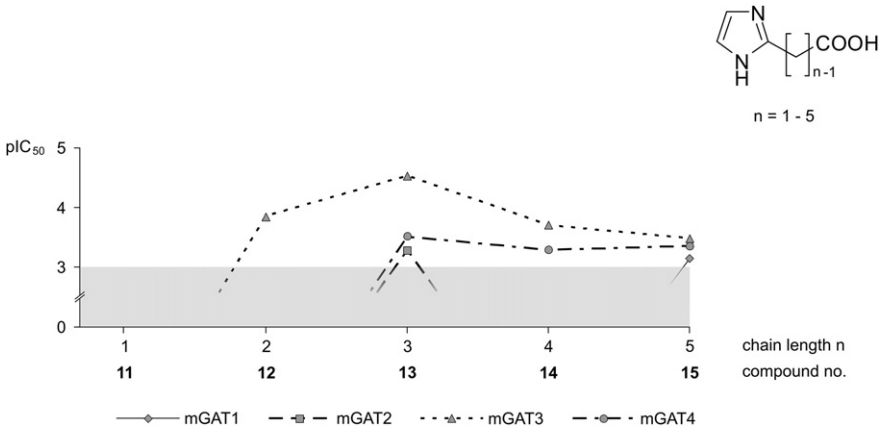


Fig. 4. Potencies of imidazole-2-yl-compounds. (Lines are drawn between the data points only for better legibility and do not represent mathematical relations.)

Table 3
pIC_{50} values of ω-imidazole-2-yl alkanoic and alkenoic acids at mGAT1–mGAT4.

Compound	No.	mGAT1	mGAT2	mGAT3	mGAT4
 R =					
COOH	**11**·HCl	62.4%	75.5%	70.2%	98.6%
CH_2COOH	**12**·HCl	75.3%	63.8%	3.86 ± 0.12	63.4%
$(CH_2)_2COOH$	**13**·HCl	62.8%	3.28 ± 0.19	**4.54** ± **0.15**	3.51 ± 0.03
$(CH_2)_3COOH$	**14**·HCl	62.3%	53.4%	3.71 ± 0.04	3.29 ± 0.10
$(CH_2)_4COOH$	**15**·HCl	3.14 ± 0.03	57.8%	3.49 ± 0.06	3.36 ± 0.03
$CH_{(E)}{=}CHCOOH$	**16**·HCl	73.3%	77.3%	3.71 ± 0.05	47.2%
$CH_{(Z)}{=}CHCOOH$	**17**·HCl	102%	92.2%	79.1%	93.6%
$(CH_2)_2CH_{(E)}{=}CHCOOH$	**18**·HCl	3.33, 3.49[a]	3.20 ± 0.13	53.5%	3.51 ± 0.18

If not stated otherwise the results are given as means of pIC_{50}-values ± SEM of three independent experiments each performed in triplicate, percentages represent specific binding remaining in presence of 1 mM inhibitor.
[a] As only two independent experiments were performed, the results of the GABA uptake are given instead of the means of pIC_{50}-values ± SEM.

Table 4
Subtype selectivity and pIC_{50} values (mean ± SEM, $n = 3$) at mGAT3.

Compound no.	pIC_{50}	Subtype selectivity		
		mGAT3:mGAT1	mGAT3:mGAT2	mGAT3:mGAT4
12·HCl	3.86 ± 0.12	≥7:1[a]	≥7:1[a]	≥7:1[a]
13·HCl	4.54 ± 0.15	≥34:1[a]	18:1	10:1
16·HCl	3.71 ± 0.05	≥7:1[a]	≥7:1[a]	≥7:1[a]

[a] For compounds reducing [^{3}H]GABA uptake to not less than 50% at 1 mM a $pIC_{50} \leq 3.0$ is assumed.

chain length reaches five C-atoms, the saturated as well as the unsaturated compounds, **15**·HCl and **18**·HCl, show neither good affinities nor subtype selectivities.

3.1.2. ω-Imidazole-4-yl alkanoic and alkenoic acids

As shown in Fig. 5, wherein the potencies of ω-imidazole-4-yl alkanoic acids are presented in a graphical manner as function of the chain length, also for this series of compounds the highest potencies are observed for mGAT3 with 1*H*-imidazol-4-ylacetic acid (**20**), 3-(1*H*-imidazol-4-yl)propanoic acid (**21**) and 4-(1*H*-imidazol-4-yl)butanoic acid hydrochloride (**22**·HCl) representing the most potent mGAT3 inhibitors. For these compounds the pIC_{50} values are declining with extension of the side chain from two to four carbons from 4.76 to 4.60, but only formally, as the observed differences lack statistical significance (see Table 5). Notably, as mentioned in the introduction, **20**, starting point of our study, shows, good mGAT3 selectivity compared to mGAT1 (35:1), but compared to mGAT2 as well as mGAT4 the selectivity is distinctly less pronounced (Table 6). The homologous compound with a side chain of three C-atoms, **21**, achieves more positive results, as the mGAT3 selectivity against mGAT1, mGAT2, and mGAT4 is higher in each case (see Table 6). But, this is not the case for the butanoic acid derivative **22**·HCl as compound **22** exhibits lower mGAT3 selectivity in relation to mGAT1, mGAT2, and mGAT4 as compared to the acetic acid derivative **20**. Even more, compound **22**·HCl lacks any selectivity for mGAT3 in relation to mGAT2, the differences between the pIC_{50} values for these transporters are missing statistical significance.

Remarkably, the unsaturated analogue of 3-(1*H*-imidazol-4-yl) propanoic acid (**21**), compound (2*E*)-3-(1*H*-imidazol-4-yl)acrylic acid (**24**), is, in contrast to **21**, devoid of any significant inhibitory potency ($pIC_{50} \leq 3.0$, see Table 5). 5-(1*H*-Imidazol-4-yl)pentanoic acid hydrochloride (**23**·HCl) and (2*E*)-5-(1*H*-imidazol-4-yl)pent-2-enoic acid hydrochloride (**25**·HCl) with further elongated side chain (five C-atoms) exhibit only low affinity to mGAT3 and miss subtype selectivity. The reduction of the chain length to one C-atom (1*H*-imidazol-4-ylcarboxylic acid (**19**)) is accompanied with an even more serious loss of potency, being now almost neglectable ($pIC_{50} \leq 3.0$, see Table 5).

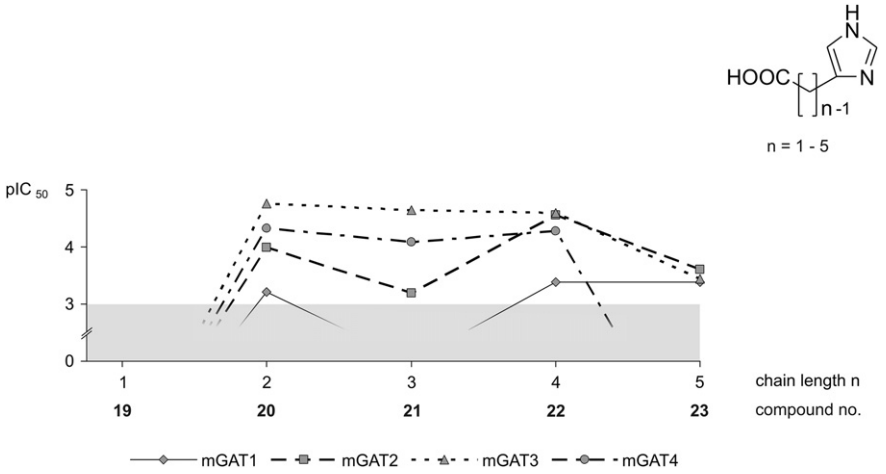
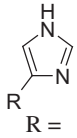


Fig. 5. Potencies of ω-imidazole-4-yl alkanoic acids. (Lines are drawn between the data points only for better legibility and do not represent mathematical relations.)

Table 5
pIC_{50} values of test compounds at mGAT1–mGAT4.

Compound	No.	mGAT1	mGAT2	mGAT3	mGAT4
 R =					
COOH	**19**	90.8%	91.1%	67.5%	85.5%
CH_2COOH	**20**	3.21 ± 0.12	3.99 ± 0.05	**4.76 ± 0.08**	4.33 ± 0.01
$(CH_2)_2COOH$	**21**	61.7%	3.19 ± 0.06	**4.64 ± 0.15**	4.09 ± 0.15
$(CH_2)_3COOH$	**22**·HCl	3.38 ± 0.14	4.56 ± 0.08	4.60 ± 0.17	4.28 ± 0.08
$(CH_2)_4COOH$	**23**·HCl	3.38 ± 0.07	3.61 ± 0.03	3.44 ± 0.09	64.7%
$CH{=}CHCOOH_{(E)}$	**24**	54.8%[a]	66.6%[a]	105%[a]	95.8%[a]
$(CH_2)_2CH{=}CHCOOH_{(E)}$	**25**·HCl	3.91 ± 01	3.42 ± 0.14	3.60 ± 0.05	3.68 ± 0.04

If not stated otherwise the results are given as means of pIC_{50}-values ± SEM of three independent experiments each performed in triplicate, percentages represent specific binding remaining in presence of 1 mM.
[a] 10 mM inhibitor.

Table 6
Subtype selectivity and pIC_{50} values (mean ± SEM, $n = 3$) at mGAT3.

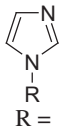
Compound no.	pIC_{50}	Subtype selectivity		
		mGAT3:mGAT1	mGAT3:mGAT2	mGAT3:mGAT4
20	4.76 ± 0.08	35:1	6:1	3:1
21	4.64 ± 0.15	≥44:1[a]	29:1	4:1
22·HCl	4.60 ± 0.17	16:1	1:1	2:1

[a] For compounds reducing [^{3}H]GABA uptake to not less than 50% at 1 mM a $pIC_{50} \leq 3.0$ is assumed.

3.1.3. ω-Imidazole-1-yl alkanoic acids

Table 7 presents the pIC_{50} values of ω-imidazol-1-yl alkanoic acids which are, in addition, displayed in Fig. 6 in a graphical manner as function of the chain length of the alkanoic acid residue. As 1*H*-imidazole-1-carboxylic acid is unstable, the series of compounds studied starts with 1*H*-imidazol-1-ylacetic acid (**26**) having a chain length of two C-atoms. According to Fig. 6, ω-imidazol-1-yl alkanoic acids having a chain length of two and three C-atoms, respectively, exhibit higher affinity to the mGAT3 transporter than to other subtypes, especially to mGAT1. 3-(1*H*-Imidazol-1-yl)propanoic acid (**27**) (three C-atoms) displays high potency at mGAT3 as well as high mGAT3 selectivity compared to mGAT1 (≥48:1) and good mGAT3 selectivity compared to mGAT2 (19:1) (Table 8). Only the mGAT3:mGAT4 selectivity is moderate (7:1). 1*H*-Imidazol-1-ylacetic acid (26) possessing one C-atom less than 3-(1*H*-imidazol-1-yl)propanoic acid (27) shows slightly lower potency at mGAT3, but still high mGAT3 subtype selectivity compared to mGAT1 (≥23:1) and mGAT4 (14:1) (Table 8). By reaching an alkyl chain length of four, the mGAT3 selectivity compared to the other three transporters is reduced. Thus, 4-(1*H*-imidazol-1-yl)butanoic acid (28) having one C-atom more than 27, shows slightly lower potency at mGAT3 (pIC_{50}: 4.40 ± 0.07) with distinctly diminished subtype selectivity as compared to the latter compound (see Table 8). Further elongation of the chain length (compounds 29–32) causes reduced mGAT3-affinity in combination with very low or even vanished subtype selectivity (Fig. 7 and 8).

Table 7
pIC_{50} values of test compounds at mGAT1–mGAT4.

Compound	No.	mGAT1	mGAT2	mGAT3	mGAT4
 R =					
CH_2COOH	**26**	73.3%	3.70 ± 0.05	4.37 ± 0.10	3.21 ± 0.11
$(CH_2)_2COOH$	**27**	72.0%	3.30 ± 0.15	4.59 ± 0.02	3.72 ± 0.09
$(CH_2)_3COOH$	**28**	3.93 ± 0.11	4.12 ± 0.14	4.40 ± 0.07	4.42 ± 0.02
$(CH_2)_4COOH$	**29**·HCl	3.94 ± 0.04	3.65 ± 0.11	3.79 ± 0.03	3.65 ± 0.10
$(CH_2)_5COOH$	**30**·HCl	2.99 ± 0.10	3.79 ± 0.07	3.63 ± 0.04	3.63 ± 0.07
$(CH_2)_6COOH$	**31**·HCl	3.17 ± 0.10	56.6%	3.32, 3.11[a]	71.1%
$(CH_2)_7COOH$	**32**·HCl	68.3%	79.3%	56.2%	58.6%

If not stated otherwise the results are given as means of pIC_{50}-values ± SEM of three independent experiments each performed in triplicate, percentages represent specific binding remaining in presence of 1 mM inhibitor.

[a] As only two independent experiments were performed, the results of the GABA uptake are given instead of the means of pIC_{50}-values ± SEM.

Table 8
Subtype selectivity and pIC_{50} (mean ± SEM, $n = 3$) at mGAT3.

Compound no.	pIC_{50}	Subtype selectivity		
		mGAT3:mGAT1	mGAT3:mGAT2	mGAT3:mGAT4
26	4.37 ± 0.10	≥23:1[a]	5:1	14:1
27	4.59 ± 0.02	≥48:1[a]	19:1	7:1
28	4.40 ± 0.07	3:1	2:1	1:1

[a] For compounds reducing [^{3}H]GABA uptake to not less than 50% at 1 mM a $pIC_{50} \leq 3.0$ is assumed.

3.2. Synthesis and biological evaluation of bicyclic systems

Most of the imidazole derivatives described above are predominantly active as mGAT3 inhibitors. Among them, 1*H*-imidazol-4-ylacetic acid (**20**) and its homologue 3-(1*H*-imidazol-4-yl) propanoic acid (**21**) exhibit the highest potencies at mGAT3, identical within the limits of error. But **21** clearly surpasses the imidazole derivative **20** with a shorter side chain with respect to its subtype selectivity in favour of mGAT3 compared to mGAT1 and mGAT2. For this reason the conformationally restrained 4,5,6,7-tetrahydro-1*H*-benzimidazol-4-carboxylic acid (**54**) and 4,5,6,7-tetrahydro-1*H*-benzimidazol-5-carboxylic acid (**55**) were included in this study as cyclic analogues of **20** and of **21**, with the aim to improve subtype selectivity.

3.2.1. Synthesis of bicyclic systems

The synthesis of the ethyl ester derivative of the bicyclic 4,5,6,7-tetrahydro-1*H*-benzimidazol-4-carboxylic acid (**54**) (see Fig. 7) was performed according to methods described in literature [52]. The subsequent hydrolysis of the ester function carried out in 1 M HCl at 100 °C (3 h) afforded the amino acid **54**·HCl in 90% yield.

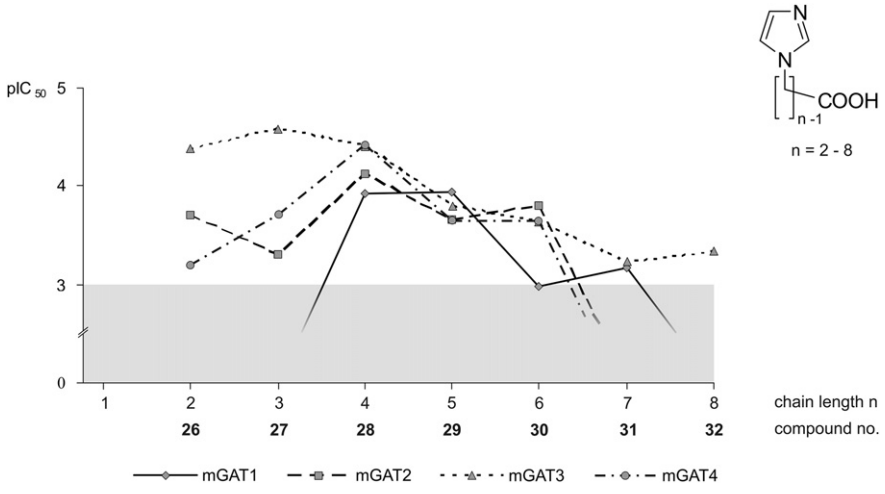


Fig. 6. Potencies of imidazole-1-yl derivatives. (Lines are drawn between the data points only for better legibility and do not represent mathematical relations.)

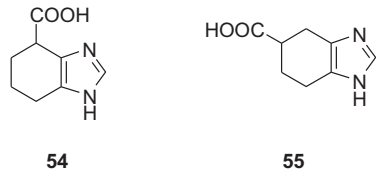


Fig. 7. Bicyclic compounds.

The synthesis of 4,5,6,7-tetrahydro-1*H*-benzimidazol-5-carboxylic acid (**55**) was performed in analogy to the preparation of **54**. The required starting material ethyl 3-bromo-4-oxocyclohexanecarboxylate (**56**) was prepared as described by Berger et al. [53]. Reaction of **56** with formamide at 150 °C for 1.5 h yielded the ethyl 4,5,6,7-tetrahydro-1*H*-benzimidazol-5-carboxylate (**59**). As attempts to purify **59** by CC remained unsuccessful, the reaction product containing compound **59** was treated with sodium hydride and di-*tert*-butyl dicarbonate to substitute the NH moiety of the imidazole ring which was considered to be the origin of the encountered problem. The resulting, 1:1 mixture of the two N-substituted regioisomers **57** and **58**, could then, indeed, be easily purified by CC. Deprotection was accomplished by treating the obtained 1:1 mixture of **57** and **58** using trifluoroacetic acid in ethanol at room temperature providing pure ethyl 4,5,6,7-tetrahydro-1*H*-benzimidazol-5-carboxylate (**59**) in high yield (94%). The target compound 4,5,6,7-tetrahydro-1*H*-benzimidazole-5-carboxylic acid hydrochloride (**55**·HCl) was finally obtained in 90% yield, after heating **59** in aqueous HCl to 100 °C to hydrolyse the ester function (Scheme 6).

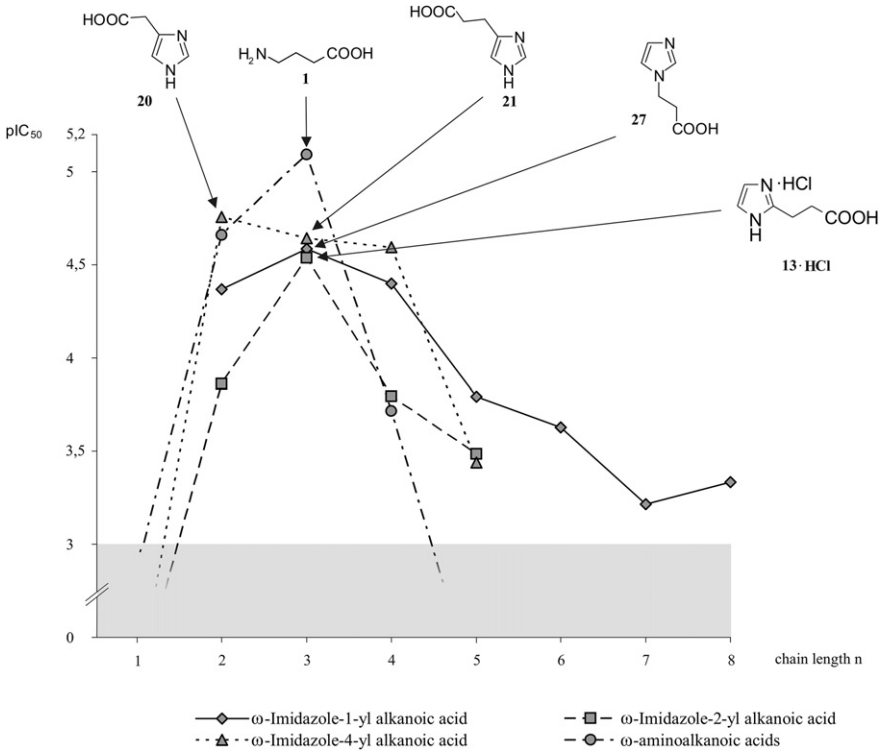
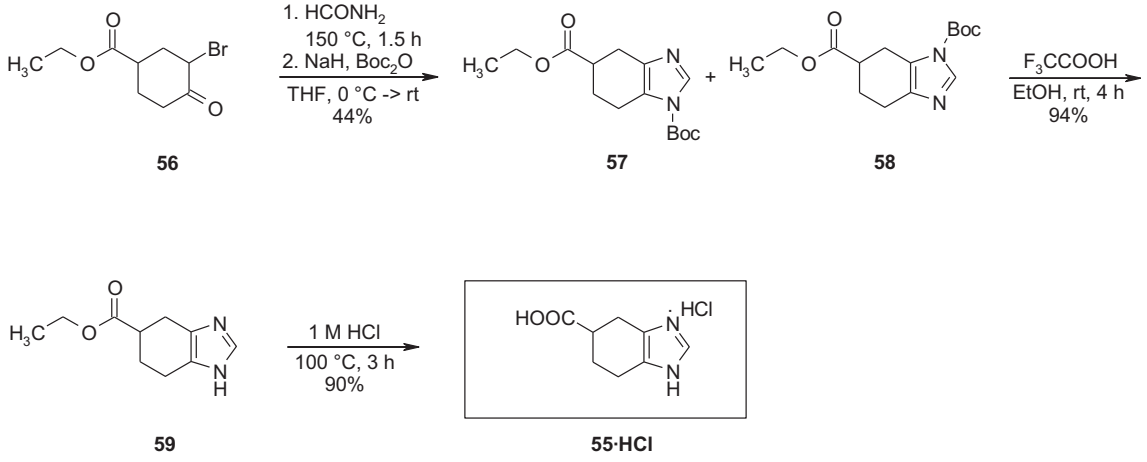


Fig. 8. Summary of the potencies of tested imidazole compounds at mGAT3. (Lines are drawn between the data points only for better legibility and do not represent mathematical relations.)

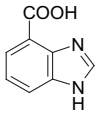
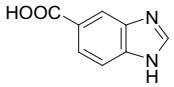
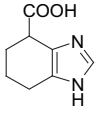
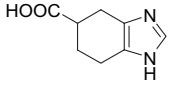


Scheme 6. Synthesis of **55**·HCl.

3.2.2. Biological evaluation of bicyclic systems

In addition to **54**·HCl and **55**·HCl, two commercially available substances, 1*H*-benzimidazol-4-carboxylic acid hydrochloride (**60**·HCl) and 1*H*-benzimidazol-5-carboxylic acid (**61**) were evaluated for their inhibitory potency at mGAT1, mGAT2, mGAT3, and mGAT4. The results obtained in the biological test are illustrated in Table 9.

Table 9
pIC_{50} values of ω-aminoalkanoic acids at mGAT1–mGAT4.

Compound	No.	mGAT1	mGAT2	mGAT3	mGAT4
	**60**·HCl	95.9%[a]	94.2%[a]	78.2%[a]	68.2%[a]
	**61**	108%[a]	74.2%[a]	103%[a]	67.8%[a]
	**54**·HCl	59.0%	60.8%	97.6%	81.6%
	**55**·HCl	85.0%	65.4%	81.5%	82.4%

If not stated otherwise the results are given as means of pIC_{50}-values ± SEM of three independent experiments each performed in triplicate, percentages represent specific binding remaining in presence of 1 mM.
[a] 100 μM inhibitor.

As shown in Table 9, the bicyclic compounds 4,5,6,7-tetrahydro-1*H*-benzimidazol-4-carboxylic acid hydrochloride (**54**·HCl) and 4,5,6,7-tetrahydro-1*H*-benzimidazol-5-carboxylic acid hydrochloride (**55**·HCl) are devoid of any reasonable potency at any of the four GABA transport proteins. This applies to the fully unsaturated bicyclic systems as well, the inhibitory potency of 1*H*-benzimidazol-4-carboxylic acid hydrochloride (**60**·HCl) and 1*H*-benzimidazol-5-carboxylic acid (**61**) is very low or even negligible.

4. Conclusion

In summary, new series of GABA uptake inhibitors based on the structure of 1*H*-imidazol-4-ylacetic acid (**20**) modified by changing the length and the position of the side chain have been synthesized and tested on stably expressed mGAT1–mGAT4 in HEK cells. Most of the synthesized ω-imidazole alkanoic and alkenoic acid derivatives inhibited GABA uptake with the highest potency at mGAT3.

Though the ω-imidazole alkanoic and alkenoic acid derivatives studied do not reach the affinity of GABA (**1**), compounds exhibiting good mGAT3 inhibitory potency in combination with good subtype selectivity, in contrast to GABA (**1**), were found. Of all synthesized substances, 3-(1*H*-imidazol-2-yl)propanoic acid hydrochloride (**13**·HCl), 1*H*-imidazol-4-ylacetic acid (**20**), 3-(1*H*-imidazol-4-yl) propanoic acid (**21**), and 3-(1*H*-imidazole-1-yl)propanoic acid (**27**) exhibited the best inhibitory potency at mGAT3 associated with a preference for this transporter (see Fig. 8).

Within a given series of ω-imidazole alkanoic acids elongation or reduction of the length of the side chain resulted in less potent or selective compounds. Furthermore, integration of the alkyl side chain into a bicyclic ring system led to ineffective compounds.

Although the starting compound 1*H*-imidazol-4-ylacetic acid (**20**) (pIC_{50} mGAT1: 3.21 ± 0.12; pIC_{50} mGAT2: 3.99 ± 0.05; pIC_{50} mGAT3: 4.76 ± 0.08; pIC_{50} mGAT4: 4.33 ± 0.01) as well as the chain-extended analogue 3-(1*H*-imidazol-4-yl)propanoic acid (**21**) (pIC_{50} mGAT1: 61.7% 1 mM; pIC_{50} mGAT2: 3.19 ± 0.06; pIC_{50} mGAT3: 4.64 ± 0.15; pIC_{50} mGAT4: 4.09 ± 0.15) showed great inhibitory potency at mGAT3 in combination with a better mGAT3 selectivity, especially compared to mGAT1 and, for compound **21**, also compared to mGAT2 than the amino acid GABA (**1**), the preference compared to mGAT4 still remains low (see Table 10). 3-(1*H*-imidazol-2-yl)propanoic acid (**13**) (mGAT1: 62.8% 1 mM; pIC_{50} mGAT2: 3.28 ± 0.19; pIC_{50} mGAT3: 4.54 ± 0.15; pIC_{50} mGAT4: 3.51 ± 0.03) and 3-(1*H*-imidazol-1-yl)propanoic acid (**27**) (mGAT1: 72.0% 1 mM; pIC_{50} mGAT2: 3.30 ± 0.15; pIC_{50} mGAT3: 4.59 ± 0.02; pIC_{50} mGAT4: 3.72 ± 0.09) possess within the limits of error identical or slightly lower inhibitory potency than 1*H*-imidazol-4-ylacetic acid (**20**). However, while the mGAT3 selectivity of compounds **13**·HCl and **27** compared to mGAT1 is similar to that of the ω-imidazole-2-yl alkanoic acids **20** and **21**, the mGAT3:mGAT2 preference is, compared to the regio isomer **21**, reduced, but still considerably better than that of the starting compound **20**. Especially worth-mentioning is the improved mGAT3:mGAT4 selectivity, which is 7:1 and 10:1, respectively, and thus better than that of compounds **20** and **21**.

Up to date, only few selective inhibitors of mGAT3 are published. In this study two compounds, 3-(1*H*-imidazol-2-yl)propanoic acid·hydrochloride (**13**·HCl) and 3-(1*H*-imidazol-1-yl)propanoic acid (**27**), were characterized, which might represent, due to their good inhibitory potencies at mGAT3 in combination with an, even for mGAT4, improved subtype selectivity, good starting points for the development of GABA uptake inhibitors of mGAT3 with high potency and subtype selectivity.

Table 10
Subtype selectivity and pIC_{50} values (mean ± SEM, $n = 3$) at mGAT3.

Compound no.	pIC_{50}	Subtype selectivity		
		mGAT3:mGAT1	mGAT3:mGAT2	mGAT3:mGAT4
1	5.14 ± 0.09	1:1	3:1	1:1
13·HCl	4.54 ± 0.15	≥34:1[a]	18:1	10:1
20	4.76 ± 0.08	35:1	6:1	3:1
21	4.64 ± 0.15	≥44:1[a]	29:1	4:1
27	4.59 ± 0.02	≥48:1[a]	19:1	7:1

[a] For compounds reducing [^{3}H]GABA uptake to not less than 50% at 1 mM a $pIC_{50} \leq 3.0$ is assumed.

5. Experimental

5.1. Materials and methods

Solvents were p.a. quality and freshly distilled before use. Purchased reagents were used without further purification. TLC plates were made from silica gel 60 F_{254} on aluminium sheet (Merck). Column chromatography (CC) was carried out using Merck silica gel 60 (mesh 0.040–0.063 mm) as stationary phase. As strong basic ion exchange resin Amberlite IRA 410, 20–50 mesh (Fa. Fluka) and as strong acidic ion exchange resin Amberlite IR 120 were used. Melting points: m.p. (uncorrected) were determined with a Büchi 512 Melting Point apparatus. NMR spectroscopy: ^{1}H NMR spectra were recorded at rt with a JNMR-GX (JEOL, 400 or 500 MHz) using TMS as internal standard and integrated with the NMR software Nuts (2D Version 5.097, Acorn NMR, 1995). IR spectroscopy: FT-IR Spectrometer 410 (Jasco); samples were measured as KBr-pellets. Mass spectrometry (MS): Mass Spectrometer 5989 A with 59980 B

particle beam LC/MS interface (Hewlett Packard) or Applied Biosystems LC-MS/MS-Mass Spectrometer API 2000; analysis was carried out using chemical ionization (CH_5^+) or electron impact ionization. High resolution mass spectrometry (HRMS): JEOL MS-Station JMS-700, FAB (Xenon, 6 KV, MBA, reference PEG), LTQ FT (Thermo Finnigan). Elementary analysis: Elementaranalysator Rapid (Heraeus).

5.1.1. General procedure 1 (GP1)

Pd–C (10%, 40.0 mg) was added to the alkenoic acid (1.0 equiv) solved in MeOH. After hydrogenation for 18 h at rt and room pressure, the catalyst was removed and the filtrate was evaporated and dried with $MgSO_4$. Purification was realized using a strong basic ion exchange resin.

5.1.2. General procedure 2 (GP2)

The imidazole derivative (1.0 equiv) was dissolved in HCl (1 M). After 4 h reflux, the reaction solution was washed three times with CH_2Cl_2. The combined organic layers were extracted with H_2O. Then, the combined aqueous layers were concentrated.

5.1.3. General procedure 3 (GP3)

At 0 °C triethylphosphonoacetate (1.3 equiv) was treated with NaH (1.0 equiv) suspended in DME. The reaction solution was warmed up to rt and subsequently stirred for the given time. The imidazole derivative (1.0 equiv) solved in DME was added. The reaction solution was warmed up to the given temperature and stirring was continued for the given time. Having evaporated the solvent the residue was purified by CC (EtOAc/*n*-Pentane = 1/1).

5.1.4. General procedure 4 (GP4)

Imidazole (2.0 equiv) in acetone was treated with K_2CO_3 (6.0 equiv), NaI (40 mg, 0.27 mmol) and the respective bromoalkanoic acid methyl ester (1.0 equiv) or bromoalkanenitrile (1.0 equiv). After the given time at rt, the reaction mixture was filtered, evaporated, and purified by CC.

5.1.5. General procedure 5 (GP5)

The carboxylic acid ester was dissolved in NaOH (1 M) and stirred for the given time at rt before a pH-value of 6 with HCl (0.5 M) was adjusted and the solution was concentrated.

5.1.6. General procedure 6 (GP6)

Imidazole (2.0 equiv) in THF was treated with NaH (2.0 equiv). After 3 h at rt, the respective bromoalkanoic acid ester (1.0 equiv) was added and stirring was continued for the given time. Then, the reaction solution was filtered, evaporated, and purified by CC.

5.1.7. General procedure 7 (GP7)

The carboxylic acid ethyl ester (1.0 equiv) was dissolved in HCl (1 M or 2 M) and warmed up to 100 °C for 3 h followed by concentration.

*5.1.8. 2-(1H-Imidazol-2-yl)acetic acid hydrochloride (**12**·HCl)*

*n*BuLi (1.6 M in hexane, 15.8 mmol, 9.80 mL) was added to a solution of 2-methyl-1-triphenylmethyl-1*H*-imidazole (33) (1.62 mg, 15.0 mmol) suspended in THF (25 mL) at −40 °C. After the reaction mixture was stirred for 1 h at rt, it was cooled again to −40 °C and solid carbon dioxide (5 g) was added. After the mixture was stirred for 1 h, H_2O (1 mL) was added and it was warmed up to rt. The solvent was evaporated and the residue was dissolved in HCl (0.5 M, 0.4 mol, 200 mL) and refluxed for 1 h. After cooling to rt, the reaction mixture was washed three times with Et_2O and concentrated. The residue was purified using a strong basic ion exchange resin.

Colourless crystals; Yield: 260 mg (11%); mp 95 °C. IR (KBr) ν cm^{-1}: 3386, 3147, 2988, 1727, 1620, 1352, 1290, 1218, 613; 500 MHz ^1H NMR (CD_3OD): δ = 4.19 (s, 2H, CH_2), 7.25 (s, 2H, $CH_{[5H\text{-}Imid.]}$, $CH_{[4H\text{-}Imid.]}$); 126 MHz ^{13}C NMR (CD_3OD): δ = 32.1 (CH_2), 120.4 ($CH_{[5C\text{-}Imid.]}$, $CH_{[4C\text{-}Imid.]}$), 142.6 ($C_{[2C\text{-}Imid.]}$), 169.1 (COO); MS (EI, 70 eV): *m*/*z* (%) = 126 (5) [M^+], 82 (100); MS (CI, CH_5^+): *m*/*z* (%) = 127 (13) [M^++1], 83 (100); HMRS (FAB, NBA) calcd. for $C_5H_7N_2O_2$: 127.0508; found 127.0483.

*5.1.9. 3-(1H-Imidazol-2-yl)propanoic acid hydrochloride(**13**·HCl)*

To 3-(1*H*-imidazol-2-yl)propanoic acid ethyl ester [37] (68 mg, 0.40 mmol) in EtOH (0.5 mL) NaOH (2 M, 1.5 mmol, 0.75 mL) was added. The mixture was stirred for 48 h at rt before a pH-value of 1–2 with HCl (0.5 M) was adjusted. After concentration, the crude product was purified using a strong basic ion exchange resin.

Colourless crystals; Yield: 61 mg (86%); mp 221 °C. IR (KBr) ν cm^{-1}: 3085, 2968, 2727, 1725, 1619, 1507, 1429, 1212, 1188; 500 MHz ^1H NMR (CD_3OD): δ = 3.21 (m, 2H, CH_2COO), 3.30 (m, 2H, CH_2CH_2COO), 7.41 (s, 2H, $CH_{[4H\text{-}Imid.]}$, $CH_{[5H\text{-}Imid.]}$); 126 MHz ^{13}C NMR (CD_3OD): δ = 23.6 (CH_2CH_2COO), 31.0 (CH_2CH_2COO), 119.0 ($CH_{[4C\text{-}Imid.]}$, $CH_{[5C\text{-}Imid.]}$), 147.7 ($C_{[2C\text{-}Imid.]}$), 173.7 (COO); MS (EI, 70 eV): *m*/*z* (%) = 140 (16) [M^+], 95 (100); MS (CI, CH_5^+): *m*/*z* (%) = 141 (100) [M^++1]; HMRS (EI) calcd. for $C_6H_8N_2O_2$: 140.0586; found 140.0583.

*5.1.10. 4-(1H-Imidazol-2-yl)butyric acid hydrochloride (**14**·HCl)*

1-Triphenylmethyl-2-[3-(4-methyl-2,6,7-trioxabicyclo[2.2.2] octan-1-yl)propyl]-1*H*-imidazole (**44**) (150 mg, 0.312 mmol) was stirred in methanolic H_2SO_4 (1.1 mol, 0.30 mL) for 20 min at rt. After removing the solvent in vacuum, the residue was dissolved in HCl (2 M, 6 mmol, 3 mL) and refluxed for 4 h. The aqueous phase was washed three times with CH_2Cl_2 followed by concentration. Purification was achieved with a strong basic ion exchange resin.

Colourless oil; Yield: 31.3 mg (52.6%); IR (KBr) ν cm^{-1}: 3418, 3106, 2983, 1715, 1621, 1416, 1198; 500 MHz ^1H NMR (CD_3OD): δ = 2.07 (m, 2H, CH_2CH_2COO), 2.41 (m, 2H, CH_2COO), 3.05 (m, 2H, $CH_2CH_2CH_2COO$), 7.43 (s, 2H, $CH_{[4H\text{-}Imid.]}$, $CH_{[4H\text{-}Imid.]}$); 100 MHz ^{13}C NMR (CD_3OD): δ = 23.7 (CH_2CH_2COOH), 26.0 ($CH_2CH_2CH_2COOH$), 33.5 (CH_2COOH), 120.1 ($CH_{[5C\text{-}Imid.]}$, $CH_{[4C\text{-}Imid.]}$), 142.5 ($C_{[2C\text{-}Imid.]}$), 175.8 (COOH); MS (EI, 70 eV): *m*/*z* (%) = 154 (4) [M^+], 95 (100); MS (CI, CH_5^+): *m*/*z* (%) = 155 (100) [M^++1], 137 (16); HMRS (EI) calcd. for $C_7H_{10}N_2O_2$: 154.0742; found 154.0741. Anal. Calcd. for $C_7H_{10}N_2O_2$·HCl (190.63)+ 0.25H_2O (195.13) (%): C 43.09, H 5.94, N 14.35. Found: C 43.46, H 6.36, N 14.77.

*5.1.11. 5-(1H-Imidazol-2-yl)pentanoic acid hydrochloride (**15**·HCl)*

According to **GP1**: (2*E*)-5-(1*H*-imidazol-2-yl)pent-2-enoic acid hydrochloride (**18**·HCl) (79 mg, 0.39 mmol), MeOH (2 mL).

Colourless crystals; Yield: 65.4 mg (73.7%); mp 173 °C. IR (KBr) ν cm^{-1}: 3434, 2920, 1700, 1616, 1404, 1209; 400 MHz ^1H NMR (D_2O): δ = 1.55–1.63 (m, 2H, $CH_2CH_2CH_2$), 1.73–1.81 (m, 2H, $CH_2CH_2CH_2$), 2.38 (t, *J* = 6.9 Hz, 2H, CH_2COO), 3.00 (t, *J* = 7.4 Hz, 2H, CCH_2CH_2), 7.30 (s, 2H, $CH_{[5H\text{-}Imid.]}$, $CH_{[4H\text{-}Imid.]}$); 100 MHz ^{13}C NMR (D_2O): δ = 23.5 (CH_2), 24.9 (CH_2), 26.7 (CH_2), 33.3 (CH_2COO), 118.4 ($CH_{[5C\text{-}Imid.]}$, $CH_{[4C\text{-}Imid.]}$), 148.0 ($C_{[2C\text{-}Imid.]}$), 178.5 (COO); MS (EI, 70 eV): *m*/*z* (%) = 168 (7) [M^+], 95 (100); MS (CI, CH_5^+): *m*/*z* (%) = 169 (100) [M^++1], 151 (10). HMRS (EI) calcd. for $C_8H_{12}N_2O_2$: 168.0899; found 168.0899. Anal Calcd. for $C_8H_{12}N_2O_2$·HCl (204.66) + 0.2H_2O (208.26) (%): C 46.14, H 6.49, N 13.45. Found C 46.39, H 6.28, N 13.16.

*5.1.12. (2E)-3-(1H-Imidazol-2-yl)acrylic acid hydrochloride (**16**·HCl)*

According to **GP2**: (2*E*)-3-(1-Triphenylmethyl-1*H*-imidazol-2-yl)acrylic acid ethyl ester (**39**) (408 mg, 1.00 mmol), HCl (1 M, 10 mmol, 10 mL).

Colourless crystals; Yield: 175 mg (100%); mp 163 °C. IR (KBr) ν cm^{-1}: 2884, 2753, 1714, 1603, 1444, 1375, 1337, 1248, 1192, 1141, 1117; 500 MHz ^{1}H NMR (CD_3OD): δ = 6.88 (d, *J* = 16.2 Hz, 1H, CHCOO), 7.51 (d, *J* = 16.2 Hz, 1H, *CH*CHCOO), 6.87 (d, *J* = 1.2 Hz, 1 H, $CH_{[5H\text{-}Imid.]}$), 7.69 (d, *J* = 1.2 Hz, 2 H, $CH_{[4H\text{-}Imid.]}$); 126 MHz ^{13}C NMR (CD_3OD): δ = 122.5 ($CH_{[4C\text{-}Imid.]}$, $CH_{[4C\text{-}Imid.]}$), 124.8 (CH), 130.0 (CH), 140.8 ($C_{[2C\text{-}Imid.]}$), 167.2 (COO); MS (EI, 70 eV): *m*/*z* (%) = 138 (42) [M^+], 94 (100); MS (CI, CH_5^+): *m*/*z* (%) = 139 (100) [M^++1], 121 (15); HMRS (EI) calcd. for $C_5H_6N_2O_2$: 138.0421; found 138.0429.

5.1.13. (2Z)-3-(1H-imidazol-2-yl)acrylic acid hydrochloride (*17*·HCl)

According to **GP2**: (2*Z*)-3-(1-Triphenylmethyl-1*H*-imidazol-2-yl)acrylic acid ethyl ester (**40**) (408 mg, 1.00 mmol), HCl (1 M, 10 mmol, 10 mL).

Colourless crystals; Yield: 143 mg (82%); mp 182–185 °C. IR (KBr) ν cm^{-1}: 2884, 2753, 1714, 1603, 1444, 1375, 1337, 1248, 1192, 1141, 1117; 500 MHz ^{1}H NMR (CD_3OD): δ = 6.55 (d, *J* = 12.0 Hz, 1H, CHCOO), 7.04 (d, *J* = 12.0 Hz, 1H, *CH*CHCOO), 7.70 (s, 2H, $CH_{[4H\text{-}Imid.]}$, $CH_{[5H\text{-}Imid.]}$); 126 MHz ^{13}C NMR (CD_3OD): δ = 122.2 ($CH_{[4C\text{-}Imid.]}$, $CH_{[5C\text{-}Imid.]}$), 123.8 (CH), 128.7 (CH), 140.8 ($C_{[2C\text{-}Imid.]}$), 169.3 (COO); MS (EI, 70 eV): *m*/*z* (%) = 138 (45) [M^+], 94 (100); MS (CI, CH_5^+): *m*/*z* (%) = 139 (100) [M^++1], 121 (16); HMRS (ESI) calcd. for $C_6H_7N_2O_2$: 139.0508; found 139.0503.

5.1.14. (2E)-5-(1H-Imidazol-2-yl)pent-2-enoic acid hydrochloride (*18*·HCl)

According to **GP2**: (2*E*)-5-(1-Triphenylmethyl-1*H*-imidazol-2-yl)pent-2-enoic acid ethyl ester (**46**) (294 mg, 0.670 mmol), HCl (1 M, 3 mmol, 3 mL).

Colourless crystals; Yield: 95 mg (70%); mp 170 °C. IR (KBr) ν cm^{-1}: 3431, 2928, 2835, 2365, 1719, 1606, 1580, 1507, 1461, 1295, 1250; 500 MHz ^{1}H NMR (D_2O): δ = 2.60 (m, 2H, CH_2CH), 3.07 (t, *J* = 7.3 Hz, 2H, CH_2CH_2), 5.74 (dt, *J* = 15.7 Hz, 1.4 Hz, 1H, CHCOO), 6.82 (dt, *J* = 15.7 Hz, 7.0 Hz, 1H, CH_2CH), 7.19 (s, 2H, $CH_{[5H\text{-}Imid.]}$, $CH_{[4H\text{-}Imid.]}$); 126 MHz ^{13}C NMR (D_2O): δ = 24.0 (CH_2CH_2CH), 29.2 (CH_2CH_2CH), 118.7 ($CH_{[5C\text{-}Imid.]}$, $CH_{[4C\text{-}Imid.]}$), 123.0 (*C*HCOO), 146.7 ($CH_{[2C\text{-}Imid.]}$), 147.3 (CH_2CH), 170.4 (COO). MS (EI, 70 eV): *m*/*z* (%) = 166 (5) [M^+], 121 (100), 81 (86); MS (CI, CH_5^+): *m*/*z* (%) = 167 (100) [M^++1], 149 (13); Anal. Calcd. for $C_8H_{10}N_2O_2$·HCl (202.64) + 0.25H_2O (207.14) (%): C 46.39, H 5.60, N 13.52. Found C 46.13, H 5.30, N 13.33.

5.1.15. 5-(1H-Imidazol-4-yl)pentanoic acid hydrochloride (*23*·HCl)

According to **GP1**: (2*E*)-5-(1*H*-imidazol-4-yl)pent-2-enoic acid hydrochloride (**25**·HCl) (101 mg, 0.50 mmol), MeOH (3 mL).

Colourless crystals; Yield: 75.4 mg (74%); mp 173 °C. IR (KBr) ν cm^{-1}: 3462, 3096, 3024, 2865, 2362, 1699, 1624, 1476, 1338, 1269, 1228, 1203; 500 MHz ^{1}H NMR (CD_3OD): δ = 1.65–1.73 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.35 (t, *J* = 6.9 Hz, 2H, CH_2COO), 2.75 (t, *J* = 7.4 Hz, 2H, CCH_2CH_2), 7.32 (s, 1H, $CH_{[5H\text{-}Imid.]}$), 8.79 (s, 1H, $CH_{[2H\text{-}Imid.]}$); 100 MHz ^{13}C NMR (CD_3OD): δ = 24.4 (CCH_2), 24.5 (CH_2), 28.3 (CH_2), 33.6 (CH_2COO), 116.1 ($CH_{[5C\text{-}Imid.]}$), 134.0 ($CH_{[2C\text{-}Imid.]}$), 134.8 ($C_{[4C\text{-}Imid.]}$), 176.5 (COO); MS (EI, 70 eV): *m*/*z* (%) = 168 (2) [M^+], 151 (3), 95 (100); MS (CI, CH_5^+): *m*/*z* (%) = 169 (100) [M^++1], 151 (16); HMRS (EI) calcd. for $C_8H_{12}N_2O_2$: 168.0899; found 168.0872.

5.1.16. (2E)-5-(1H-Imidazol-4-yl)pent-2-enoic acid hydrochloride (*25*·HCl)

(2*E*)-5-(1-Triphenylmethyl-1*H*-imidazol-4-yl)pent-2-enoic acid ethyl ester (**48**): [60] According to **GP3**: Triethylphosphonoacetate (0.29 g, 1.3 mmol, 0.26 mL), NaH (26 mg, 1.0 mmol), DME (2 mL), 1 h, 3-(1-triphenylmethyl-1*H*-imidazol-2-yl)propanal (**47**) (367 mg, 1.00 mmol) [46], DME (1 mL), 50 °C, 3 h (2E)-5-(1H-Imidazol-4-yl) pent-2-enoic acid hydrochloride (**25**·HCl): According to **GP2**: (2*E*)-5-(1-Triphenylmethyl-1*H*-imidazol-4-yl)pent-2-enoic acid ethyl ester (**48**) (171 mg, 0.350 mmol) solved in HCl (2 M, 4 mmol, 2 mL) and, differing to **GP2**, EtOH (0.5 mL).

Colourless crystals; Yield: 63 mg (88%); mp 170 °C. IR (KBr) ν cm^{-1}: 3430, 3090, 3007, 2825, 2604, 2362, 1694, 1646, 1617, 1476, 1416, 1294, 1228, 1181; 500 MHz ^{1}H NMR (CD_3OD): δ = 2.61 (m, 2H, CH_2CH), 2.92 (t, *J* = 7.2 Hz, 2H, CCH_2CH_2), 5.86 (dt, *J* = 15.6/1.3 Hz, 1H, *CH*COO), 6.93 (dt, *J* = 15.6/8.6 Hz, 1H, CH_2CH), 6.35 (s, 1H, $CH_{[5H\text{-}Imid.]}$), 8.80 (s, 1H, $CH_{[2H\text{-}Imid.]}$); 100 MHz ^{13}C NMR (CD_3OD): δ = 23.3 (CCH_2CH_2), 31.0 (CH_2CH), 116.5 ($CH_{[5C\text{-}Imid.]}$), 123.6 (*C*HCOO), 133.7 ($CH_{[2C\text{-}Imid.]}$), 134.2 ($C_{[4C\text{-}Imid.]}$), 147.1 (CH_2CH), 168.9 (COO); MS (EI, 70 eV): *m*/*z* (%) = 166 (7) [M^+], 121 (46), 81 (100); MS (CI, CH_5^+): *m*/*z* (%) = 167 (100) [M^++1], 149 (21); HMRS (EI) calcd. for $C_8H_{10}N_2O_2$: 166.0742; found 166.0770.

5.1.17. 1H-Imidazol-1-ylacetic acid hydrochloride (*26*·HCl)

1*H*-Imidazol-1-ylacetic acid methyl ester.

Synthesis was performed according to a modified literature procedure [50].

According to **GP4**: Imidazole (1.36 g, 20.0 mmol), acetone (15 mL), K_2CO_3 (8.29 g, 60.0 mmol), NaI (40 mg, 0.27 mmol), 2-bromoacetic acid methyl ester (1.53 g, 10.0 mmol), 7 h, CC (CH_2Cl_2/C_2H_5OH = 9/1).

Colourless crystals; Yield: 1.08 g (77%); mp 55 °C. IR (KBr) ν cm^{-1}: 3386, 3112, 2985, 2956, 1748, 1626, 1511, 1294, 1221; 500 MHz ^{1}H NMR ($CDCl_3$): δ = 3.72 (s, 3H, $COOCH_3$), 4.65 (s, 2H, CH_2COO), 6.89 (s, 1H, $CH_{[5H\text{-}Imid.]}$), 7.03 (s, 1H, $CH_{[4H\text{-}Imid.]}$), 7.43 (s, 1H, $CH_{[2H\text{-}Imid.]}$); 126 MHz ^{13}C NMR (CD_3OD): δ = 53.1 (CH_3), 122.1 ($CH_{[5C\text{-}Imid.]}$), 128.6 ($CH_{[4C\text{-}Imid.]}$), 139.5 ($CH_{[2C\text{-}Imid.]}$), 170.1 (COO); MS (EI, 70 eV): *m*/*z* (%) = 140 (18) [M^+], 81 (100); MS (CI, CH_5^+): *m*/*z* (%) = 141 (100) [M^++1]; HMRS (EI) calcd. for $C_6H_8N_2O_2$: 140.0586; found 140.0596.

NMR, IR and melting point (lit.: 55–56 °C) are according to literature values [54].

1*H*-Imidazol-1-ylacetic acid hydrochloride (**26**·HCl).

Synthesis was performed analogue to a modified literature procedure [49].

According to **GP5**: 1*H*-Imidazol-1-ylacetic acid methyl ester (100 mg, 0.700 mmol), NaOH (1 M, 1 mmol, 1 mL).

Colourless crystals; Yield: 114 mg (100%); mp 216 °C (lit.: 193–195 °C [55]); IR (KBr) ν cm^{-1}: 3129, 3067, 2951, 2846, 2360, 2340, 1732, 1581, 1404, 1226; 500 MHz ^{1}H NMR (CD_3OD): δ = 5.18 (s, 2H, CH_2COOH), 7.60 (s, 1H, $CH_{[5H\text{-}Imid.]}$), 7.65 (s, 1H, $CH_{[4H\text{-}Imid.]}$), 9.00 (s, 1H, $CH_{[2H\text{-}Imid.]}$); 126 MHz ^{13}C NMR (D_2O): δ = 50.6 (CH_2), 120.7 ($CH_{[5C\text{-}Imid.]}$), 124.8 ($CH_{[4C\text{-}Imid.]}$), 137.9 ($CH_{[2C\text{-}Imid.]}$), 169.2 (COOH); MS (EI, 70 eV): *m*/*z* (%) = 126 (80) [M^+], 81 (100); MS (CI, CH_5^+): *m*/*z* (%) = 127 (100) [M^++1].

NMR, IR are according to literature values [55].

5.1.18. 5-(1H-Imidazol-1-yl)pentanoic acid (*29*)

5-(1*H*-Imidazol-1-yl)pentanoic acid ethyl ester.

Synthesis was performed according to a modified literature procedure [49].

According to **GP6**: Imidazole (1.36 g, 20.0 mmol), THF (20 mL), NaH (510 mg, 20.0 mmol), 5-bromovalerianic acid ethyl ester (2.00 g, 10.0 mmol, 1.58 mL), 16 h, CC (CH_2Cl_2/C_2H_5OH = 95/5).

Colourless oil; Yield: 542 mg (30%); IR (KBr) ν cm^{-1}: 3400, 3112, 2940, 2363, 1729, 1509, 1452, 1374, 1230, 1188; 500 MHz ^{1}H NMR (CD_3OD): δ = 1.22 (t, *J* = 7.2 Hz, 3H, $COOCH_2CH_3$), 1.53–1.60 (m, 2H, CH_2), 1.78–1.84 (m, 2H, CH_2), 2.34 (t, *J* = 7.4 Hz, 2H, CH_2COO), 4.04 (t, *J* = 7.1 Hz, 2H, NCH_2), 4.10 (q, *J* = 7.2 Hz, 2H, $COOCH_2$), 6.96 (s, 1H, $CH_{[5H\text{-}Imid.]}$), 7.13 (s, 1H, $CH_{[4H\text{-}Imid.]}$), 7.65 (s, 1H, $CH_{[2H\text{-}Imid.]}$); 126 MHz ^{13}C NMR (CD_3OD): δ = 14.5 ($COOCH_2CH_3$), 22.9 (CH_2), 31.5

(CH_2), 34.4 (CH_2COO), 47.6 (NCH_2), 61.5 ($COOCH_2$), 120.5 (CH[5C-Imid.]), 129.1 (CH[4C-Imid.]), 138.4 (CH[2C-mid.]), 174.9 (COO); MS (EI, 70 eV): m/z (%) = 196 (13) [M^+], 152 (35), 96 (100); MS (CI, CH_5^+): m/z (%) = 197 (100) [M^++1]; HMRS (EI) calcd. for $C_{10}H_{16}N_2O_2$: 196.1212; found 196.1207.

5-(1*H*-Imidazol-1-yl)pentanoic acid (**29**)

Synthesis was performed analogue to a modified literature procedure [49].

According to **GP5**: 5-(1*H*-Imidazol-1-yl)pentanoic acid ethyl ester (197 mg, 1.00 mmol), NaOH (1 M, 1.25 mmol, 1.25 mL). Differing to **GP5**, EtOH (1 mL) was added. 48 h. After concentration, the crude product was purified using a strong acidic ion exchange resin.

Colourless crystals; Yield: 163 mg (97%); mp 164 °C. IR (KBr) ν cm^{-1}: 3442, 3128, 2952, 2359, 1716, 1516, 1381, 1326, 1283, 1241, 1202, 1098; 500 MHz ^{1}H NMR (CD_3OD): δ = 1.54–1.60 (m, 2H, CH_2), 1.81–1.96 (m, 2H, CH_2), 2.29 (t, J = 7.3 Hz, 2H, CH_2COO), 4.07 (t, J = 7.1 Hz, 2H, NCH_2), 7.03 (s, 1H, CH[5H-Imid.]), 7.20 (s, 1H, CH[4H-Imid.]), 7.82 (s, 1H, CH[2H-Imid.]); 126 MHz ^{13}C NMR (CD_3OD): δ = 23.3 (CH_2), 31.5 (CH_2), 35.1 (CH_2COO), 48.0 (NCH_2), 120.9 (CH[5C-Imid.]), 128.0 (CH[4C-Imid.]), 138.1 (CH[2C-Imid.]), 178.1 (COOH); MS (EI, 70 eV): m/z (%) = 168 (39) [M^+], 96 (42), 82 (100); MS (CI, CH_5^+): m/z (%) = 169 (100) [M^++1]; HMRS (EI) calcd. for $C_8H_{12}N_2O_2$: 168.0899; found 168.0889.

NMR, IR and melting point (lit.: 165–166.5 °C) are according to literature values [56].

*5.1.19. 6-(1H-Imidazol-1-yl)hexanoic acid hydrochloride (**30**·HCl)*

Synthesis was performed according to a modified literature procedure [49].

6-(1*H*-Imidazol-1-yl)hexanoic acid methyl ester: According to **GP6**: Imidazole (1.36 g, 20.0 mmol), THF (20 mL), NaH (510 mg, 20.0 mmol), 6-bromohexanoic acid ethyl ester (2.09 g, 10.0 mmol), 72 h, CC (CH_2Cl_2/C_2H_5OH = 9/1); 6-(1*H*-Imidazol-1-yl)hexanoic acid hydrochloride: According to **GP5**: 6-(1*H*-Imidazol-1-yl)hexanoic acid methyl ester (117 mg, 0.600 mmol), NaOH (1 M, 0.75 mmol, 0.75 mL). Differing to **GP5**, MeOH (0.5 mL) was added. 24 h.

Colourless crystals; Yield: 124 mg (95%); mp 126 °C (lit.: 139–140 °C[56]); IR (KBr) ν cm^{-1}: 3128, 3061, 2943, 2860, 2360, 2342, 1715, 1574, 1541, 1403, 1306, 1291, 1236, 1180; 500 MHz ^{1}H NMR (CD_3OD): δ = 1.32–1.40 (m, 2H, CH_2), 1.63–1.69 (m, 2H, CH_2), 1.88–1.95 (m, 2H, CH_2), 2.32 (t, J = 7.3 Hz, 2H, CH_2COO), 4.27 (t, J = 7.1 Hz, 2H, NCH_2), 7.57 (s, 1H, CH[5H-Imid.]), 7.67 (s, 1H, CH[4H-Imid.]), 8.97 (s, 1H, CH[2H-Imid.]); 126 MHz ^{13}C NMR (D_2O): δ = 23.8 (CH_2), 25.0 (CH_2), 29.1 (CH_2), 33.6 (CH_2COO), 49.3 (NCH_2), 119.9 (CH[5C-Imid.]), 122.0 (CH[4C-Imid.]), 134.5 (CH[2C-Imid.]), 178.9 (COO); MS (EI, 70 eV): m/z (%) = 182 (17) [M^+], 181 (60), 82 (100); MS (CI, CH_5^+): m/z (%) = 183 (100) [M^++1]; HMRS (EI) calcd. for $C_9H_{14}N_2O_2$: 182.1055; found 182.1065.

NMR and IR are according to literature values [56].

5.1.20. 7-(1H-Imidazol-1-yl)heptanoic acid hydrochloride (31·HCl)

7-(1*H*-Imidazol-1-yl)heptanenitrile.

Synthesis was performed analogue to a modified literature procedure [49].

According to **GP4**: Imidazole (1.36 g, 20.0 mmol), acetone (15 mL), K_2CO_3 (8.29 g, 60.0 mmol), NaI (40 mg, 0.27 mmol), 7-bromoheptanenitrile (1.92 g, 10.0 mmol, 1.51 mL), 16 h, CC (CH_2Cl_2/C_2H_5OH = 95/5).

Colourless oil; Yield: 926 mg (52%); IR (KBr) ν cm^{-1}: 3397, 3113, 2245, 1639, 1511, 1462, 1231, 1109, 1081; 500 MHz ^{1}H NMR ($CDCl_3$): δ = 1.30–1.35 (m, 2H, CH_2), 1.44–1.50 (m, 2H, CH_2), 1.61–1.67 (m, 2H, CH_2), 1.77–1.82 (m, 2H, CH_2), 2.33 (t, J = 7.0 Hz, 2H, CH_2CN), 3.94 (t, J = 7.0 Hz, 2H, NCH_2), 6.89 (s, 1H, CH[5H-Imid.]), 7.02 (s, 1H, CH[4H-Imid.]), 7.45 (s, 1H, CH[2H-Imid.]); 126 MHz ^{13}C NMR ($CDCl_3$): δ = 17.0 (CH_2), 25.1 (CH_2), 25.5 (CH_2), 28.1 (CH_2), 30.5 (CH_2CN), 46.7 (NCH_2), 118.7 (CN), 119.5 (CH[5C-Imid.]), 129.5 (CH[4C-Imid.]), 137.0 (CH[2C-Imid.]); MS (EI, 70 eV): m/z (%) = 177 (16) [M^+], 137 (21), 82 (100); MS (CI, CH_5^+): m/z (%) = 178 (100) [M^++1]; HMRS (EI) calcd. for $C_{10}H_{15}N_3$: 177.1266; found 177.1271.

NMR and IR are according to literature values [56].

7-(1*H*-Imidazol-1-yl)heptanoic acid hydrochloride (**31**·HCl).

Synthesis was performed analogue to a modified literature procedure [51].

To 7-(1*H*-imidazol-1-yl)heptanenitrile (354 mg, 2.00 mmol) in EtOH (3 mL) was added KOH (10 M, 10 mmol, 1.0 mL). Then, the reaction mixture was warmed up to 85 °C for 5 h. Having evaporated the solvent, the residue was purified using a strong basic ion exchange.

Colourless crystals; Yield: 190 mg (41%); mp 132 °C. IR (KBr) ν cm^{-1}: 3130, 3062, 2939, 2854, 1718, 1572, 1541, 1402, 1286, 1226, 1179, 1082; 500 MHz ^{1}H NMR (CD_3OD): δ = 1.34–1.44 (m, 4H, CH_2), 1.57–1.64 (m, 2H, CH_2), 1.88–1.94 (m, 2H, CH_2), 2.29 (t, J = 7.3 Hz, 2H, CH_2COO), 4.26 (t, J = 7.3 Hz, 2H, NCH_2), 7.57 (s, 1H, CH[5H-Imid.]), 7.67 (s, 1H, CH[4H-Imid.]), 8.96 (s, 1H, CH[2H-Imid.]); 126 MHz ^{13}C NMR (CD_3OD): δ = 24.2 (CH_2), 25.4 (CH_2), 27.9 (CH_2), 29.4 (CH_2), 33.1 (CH_2COO), 49.0 (NCH_2), 119.6 (CH[5C-Imid.]), 121.6 (CH[4C-Imid.]), 145.0 (CH[2C-Imid.]), 175.9 (COOH); MS (EI, 70 eV): m/z (%) = 196 (10) [M^+], 195 (72), 82 (100); MS (CI, CH_5^+): m/z (%) = 197 (100) [M^++1]; HMRS (EI) calcd. for $C_{10}H_{17}N_2O_2$: 197.1290; found 197.1284.

Melting point (lit.: 135–136.5 °C) is according to literature value [57].

*5.1.21. 8-(1H-Imidazol-1-yl)octanoic acid hydrochloride (**32**·HCl)*

8-(1*H*-Imidazol-1-yl)octanoic acid methyl ester.

Synthesis was performed analogue to a modified literature procedure [49].

According to **GP4**: Imidazole (1.36 g, 20.0 mmol), acetone (15 mL), K_2CO_3 (8.29 g, 60.0 mmol), NaI (40 mg, 0.27 mmol), 8-bromooctanoic acid methyl ester (2.37 g, 10.0 mmol) 16 h, CC (CH_2Cl_2/C_2H_5OH = 95/5).

Colourless oil; Yield: 1.72 g (77%); IR (KBr) ν cm^{-1}: 3409, 3111, 2934, 2858, 1734, 1509, 1438, 1362, 1230, 1199, 1173, 1080; 500 MHz ^{1}H NMR ($CDCl_3$): δ = 1.22–1.32 (m, 6H, CH_2), 1.55–1.61 (m, 2H, CH_2), 1.71–1.77 (m, 2H, CH_2), 2.27 (t, J = 7.3 Hz, 2H, CH_2), 3.64 (s, 3H, $COOCH_3$), 3.89 (t, J = 7.3 Hz, 2H, NCH_2), 6.87 (s, 1H, CH[5H-Imid.]), 7.02 (s, 1H, CH[4H-Imid.]), 7.42 (s, 1H, CH[2H-Imid.]); 126 MHz ^{13}C NMR ($CDCl_3$): δ = 24.7 (CH_2), 26.3 (CH_2), 28.7 (CH_2), 28.8 (CH_2), 31.0 (CH_2), 33.9 (CH_2COO), 47.0 (NCH_2), 51.4 ($COOCH_3$), 118.7 (CH[5C-Imid.]), 129.4 (CH[4C-Imid.]), 137.0 (CH[2C-Imid.]), 174.1 (COO); MS (EI, 70 eV): m/z (%) = 224 (18) [M^+], 223 (92), 82 (100); MS (CI, CH_5^+): m/z (%) = 225 (100) [M^++1]; HMRS (EI) calcd. for $C_{12}H_{20}N_2O_2$: 224.1525; found 224.1550.

NMR and IR are according to literature values [56].

8-(1*H*-Imidazol-1-yl)octanoic acid hydrochloride (**32**·HCl).

Synthesis was performed analogue to a modified literature procedure [49].

According to **GP5**: 8-(1*H*-Imidazol-1-yl)octanoic acid methyl ester (224 mg, 1.00 mmol), NaOH (1 M, 1.5 mmol, 1.5 mL), 24 h.

Colourless crystals; Yield: 241 mg (98%); mp 152 °C. IR (KBr) ν cm^{-1}: 3127, 2939, 2854, 2360, 1713, 1573, 1542, 1403, 1362, 1295, 1220, 1177, 1082; 500 MHz ^{1}H NMR (CD_3OD): δ = 1.35–1.39 (m, 6H, CH_2), 1.57–1.61 (m, 2H, CH_2), 1.89–1.92 (m, 2H, CH_2), 2.29 (t, J = 7.3 Hz, 2H, CH_2COO), 4.22 (t, J = 7.3 Hz, 2H, NCH_2), 7.54 (s, 1H, CH[5H-Imid.]), 7.64 (s, 1H, CH[4H-Imid.]), 8.95 (s, 1H, CH[2H-Imid.]); 126 MHz ^{13}C NMR ($CDCl_3$): δ = 24.5 (CH_2), 25.7 (CH_2), 28.3 (CH_2), 28.5 (CH_2), 29.7 (CH_2), 33.4 (CH_2COO), 49.2 (NCH_2), 119.8 (CH[5C-Imid.]), 122.0 (CH[4C-Imid.]), 135.0 (CH[2C-Imid.]), 176.2 (COO); MS (EI, 70 eV): m/z (%) = 209 (30) [M^+], 195 (13), 82 (100); HMRS (EI) calcd. for $C_{11}H_{18}N_2O_2$: 209.1290; found 209.1294.

NMR, IR and melting point (lit.: 153–154 °C) are according to literature values [49,56,57].

*5.1.22. 1-Triphenylmethyl-1H-imidazol-2-carbaldehyde (**38**)*

1*H*-Imidazol-2-carbaldehyde (**36**) (1.98 g, 20.0 mmol) in DMF (20 mL) was treated with NEt_3 (4.10 g, 40.0 mmol, 5.62 mL). The mixture was stirred for 15 min at rt before triphenylmethyl chloride (**37**) (6.26 g, 22.0 mmol), suspended in DMF (10 mL), was added. After stirring for 48 h the solution was diluted with EtOAc (20 mL/mmol) and washed with brine, sat. Na_2CO_3-solution and H_2O. The organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by CC (*n*-Pentane/EtOAc = 6/4).

Colourless crystals; Yield: 5.59 g (81%); mp 190 °C. IR (KBr) ν cm^{-1}: 3397, 3065, 2845, 1708, 1491, 1442, 1396, 1356, 1242, 1169; 500 MHz ^1H NMR (CD_3OD): δ = 7.01 (s, 1H, $CH_{[5H\text{-}Imid.]}$), 7.09–7.11 (m, 6H, CH_{ar}), 7.24–7.36 (m, 10H, CH_{ar}, $CH_{[4H\text{-}Imid.]}$), 9.21 (s, 1H, CHO); 126 MHz ^{13}C NMR ($CDCl_3$): δ = 76.9 ($C(C_6H_5)_3$), 126.9 (CH_{ar}), 128.1 (CH_{ar}), 129.6 ($CH_{[4C\text{-}Imid.]}$, $CH_{[5C\text{-}Imid.]}$), 142.1 ($C_{[2C\text{-}Imid.]}$), 145.6 (C_q), 178.6 (CHO); MS (CI, CH_5^+): *m/z* (%) = 338 (1) [M^++1], 243 (100), 167 (109); MS (FAB, NBA): *m/z* (%) = 339.2 (5) [M^++1], 243.2 (100), 165.1 (11); HMRS (ESI) calcd. for $C_{23}H_{18}N_2ONa_1$: 361.1317; found 361.1313.

NMR and melting point (lit.: 189–190 °C) are according to literature values [58].

*5.1.23. (2E)-3-(1-Triphenylmethyl-1H-imidazol-2-yl)acrylic acid ethyl ester (**39**) and (2Z)-3-(1-triphenylmethyl-1H-imidazol-2-yl) acrylic acid ethyl ester (**40**)*

At 0 °C triethylphosphonoacetate (3.0 g, 13.0 mmol, 2.68 mL) was added to NaH (278 mg, 11.0 mmol) suspended in DME (10 mL). The reaction solution was warmed up to rt and subsequently stirred for 1 h. 1-Triphenylmethyl-1*H*-imidazol-2-carbaldehyde (**38**) (3.38 g, 10.0 mmol) solved in DME (14 mL) was added. After 15 min at reflux conditions the reaction solution was cooled down to 60 °C and stirring was continued for 2 h. Having extracted the solution with CH_2Cl_2, purification was realized by CC (EtOAc/*n*-Pentane = 1/1).

39: Colourless crystals; Yield: 2.80 mg (69%); mp 198 °C. IR (KBr) ν cm^{-1}: 2991 1721, 1606, 1490, 1445, 1424, 1227, 1203, 1128; 400 MHz ^1H NMR ($CDCl_3$): δ = 1.13 (t, *J* = 7.1 Hz, 3H, CH_3), 4.00 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 6.53 (d, *J* = 15.2 Hz, 1H, *CH*COO), 6.70 (d, *J* = 15.2 Hz, 1H, *CH*CHCOO), 6.87 (d, *J* = 1.2 Hz, 1H, $CH_{[5H\text{-}Imid.]}$), 7.10–7.14 (m, 7H, CH_{ar}, $CH_{[4H\text{-}Imid.]}$), 7.31–7.35 (m, 9H, CH_{ar}); 100 MHz ^{13}C NMR ($CDCl_3$): δ = 14.1 (CH_2CH_3), 60.0 (CH_2CH_3), 75.1 ($C(C_6H_5)_3$), 119.3 (CH), 124.2 ($CH_{[5C-Imid.]}$), 128.1 (CH_{ar}), 128.2 (CH_{ar}, $CH_{[4C\text{-}Imid.]}$), 129.8 (CH_{ar}), 132.5 (CH_{ar}), 142.3 ($C_{[2C-Imid.]}$), 145.0 (C_q), 166.3 (COO); MS (CI, CH_5^+): *m/z* (%) = 409 (1) [M^++1], 243 (100), 167 (26); HMRS (EI) calcd. for $C_{27}H_{24}N_2O_2Na_1$: 431.1736; found 431.1732.

NMR is according to the literature value [38].

40: Colourless crystals; Yield: 1.20 mg (29%); mp 193 °C. IR (KBr) ν cm^{-1}: 2991, 1721, 1606, 1490, 1445, 1424, 1227, 1203, 1128; 500 MHz ^1H NMR ($CDCl_3$): δ = 1.24 (t, *J* = 7.1 Hz, 3H, CH_3), 4.19 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 5.39 (d, *J* = 12.0 Hz, 1H, CHCOO), 5.68 (d, *J* = 12.0 Hz, 1H, *CH*CHCOO), 6.79 (s, 1H, $CH_{[5H\text{-}Imid.]}$), 7.05 (s, 1H, $CH_{[4H\text{-}Imid.]}$), 7.15–7.17 (m,6H, CH_{ar}), 7.31–7.33 (m, 9H, CH_{ar}); 100 MHz ^{13}C NMR ($CDCl_3$): δ = 14.1 (CH_3), 60.4 (CH_2CH_3), 75.3 ($C(C_6H_5)_3$), 121.8 (CH), 122.7 ($CH_{[5C\text{-}Imid.]}$), 127.3 (CH_{ar}), 127.8 (CH_{ar}), 128.0 (CH_{ar}), 130.1 ($CH_{[4C\text{-}Imid.]}$), 142.2 ($C_{[2C\text{-}Imid.]}$), 144.0 (C_q), 166.8 (COO); MS (CI, CH_5^+): *m/z* (%) = 409 (1) [M^++1], 243 (100), 167 (7); HMRS (ESI) calcd. for $C_{27}H_{24}N_2O_2Na_1$: 431.1736; found 431.1733.

*5.1.24. 2-[3-(4-Methyl-2,6,7-trioxa-bicyclo[2.2.2]octan-1-yl) propyl]-1-triphenylmethyl-1H-imidazole (**44**)*

*n*BuLi (1.5 M, 3.0 mmol, 2.0 mL) was added at −15 °C to a solution of 1-triphenylmethyl-1H-imidazole (41) (0.931 g, 3.00 mmol) in THF (36 mL) and the mixture was stirred at rt for 30 min. After cooling the mixture to −10 °C, 1-(3-iodopropyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (43) (0.904 g, 3.00 mmol) solved in THF (9 mL) was added. The reaction mixture was stirred for 25 min at −25 °C, 3 h at 0 °C, and 14 h at 10 °C. The reaction was stopped by adding H_2O (1 mL) and the solvent was evaporated. The residue was resolved in CH_2Cl_2 and washed three times with H_2O. The combined organic layers were dried over $MgSO_4$. Purification was realized by CC (*n*-Pentane/EtOAc/NEt_3 = 9/3/0.5).

Colourless crystals; Yield: 195 mg (13.5%); mp 215 °C. IR (KBr) ν cm^{-1}: 3418, 2874, 1492, 1448, 1398, 1266, 1190, 1131, 1055; 500 MHz ^1H NMR ($CDCl_3$): δ = 0.74 (s, 3H, CH_3), 1.33 (t, *J* = 7.7 Hz, 2H, $CH_2CH_2CH_2C$), 1.61 (m, 2H, $CH_2CH_2CH_2$), 1.85 (t, *J* = 8.0 Hz, 2H, $CH_2CH_2CH_2C$), 3.75 (s, 6H, OCH_2), 6.67 (d, *J* = 1.4 Hz, 1H, $CH_{[5H\text{-}Imid.]}$), 6.95 (d, *J* = 1.4 Hz, 1H, $CH_{[4H\text{-}Imid.]}$), 7.11–7.13 (m, 6H, CH_{ar}), 7.30–7.32 (m, 9H, CH_{ar}); 100 MHz ^{13}C NMR ($CDCl_3$): δ = 16.9 (CH_3), 23.4 ($CH_2CH_2CH_2C$), 32.5 ($CH_2CH_2CH_2$), 38.2 ($CH_2CH_2CH_2C$), 74.7 (OCH_2), 75.2 ($C(C_6H_5)_3$), 111.1 (C_q), 123.3 ($CH_{[5C\text{-}Imid.]}$), 128.2 ($CH_{[4C\text{-}Imid.]}$), 130.0 (CH_{ar}), 130.3 (CH_{ar}), 132.3 (CH_{ar}), 144.9 ($C_{[2C\text{-}Imid.]}$), 152.8 (C_q); MS (EI, 70 eV): *m/z* (%) = 480 (100) [M^+], 467 (12), 455 (40), 443 (55); HMRS (EI) calcd. for $C_{31}H_{32}N_2O_3$: 480.2413; found 480.2401.

*5.1.25. (2E)-5-(1-Triphenylmethyl-1H-imidazol-2-yl)pent-2-enoic acid ethyl ester (**46**)*

According to **GP3**: Triethylphosphonoacetate (0.20 g, 0.91 mmol, 0.18 mL), NaH (18 mg, 0.70 mmol), DME (2 mL), 30 min, 3-(1-triphenylmethyl-1*H*-imidazole-2-yl)propanal (**45**) (256 mg, 0.70 mmol), DME (1 mL), 55 °C, 4 h.

Colourless crystals; Yield: 294 mg (96%); mp 122 °C. IR (KBr) ν cm^{-1}: 3408, 3057, 2977, 2926, 1711, 1656, 1489, 1447, 1405, 1365, 1315, 1274, 1234, 1203, 1157; 500 MHz ^1H NMR ($CDCl_3$): δ = 1.26 (t, *J* = 7.1 Hz, 3H, CH_3), 2.04–2.06 (m, 4H, CH_2CH_2CH), 4.13 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 5.50 (dt, *J* = 15.6/1.6 Hz, 1H, CHCOO), 6.61 (dt, *J* = 15.6/6.6 Hz, 1H, CH_2CH), 6.71 (d, *J* = 1.4 Hz, 1H, $CH_{[5H\text{-}Imid.]}$), 6.97 (d, *J* = 1.4 Hz, 1H, $CH_{[4H\text{-}Imid.]}$), 7.12–7.14 (m, 6H, CH_{ar}), 7.32–7.35 (m, 9H, CH_{ar}); 100 MHz ^{13}C NMR ($CDCl_3$): δ = 16.5 (CH_3), 31.4 (CH_2CH_2), 32.4 (CH_2CH_2CH), 62.4 (CH_2CH_3), 77.0 ($C(C_6H_5)_3$), 123.6 (*C*HCOO), 123.8 ($CH_{[5C\text{-}Imid.]}$), 127.9 ($CH_{[4C\text{-}Imid.]}$), 130.2 (CH_{ar}), 130.4 (CH_{ar}), 132.1 (CH_{ar}), 144.7 ($C_{[2C\text{-}Imid.]}$), 150.2 (CH_2CH), 151.2 (C_q), 168.9 (COO); MS (CI, CH_5^+): *m/z* (%) = 437 (2) [M^++1], 243 (100), 195 (13), 167 (7). HMRS (ESI) calcd. for $C_{29}H_{29}N_2O_2$: 437.2229; found 437.2224. Anal. Calcd. for $C_{29}H_{28}N_2O_2$ (436.56) (%): C 79.79, H 6.46, N 6.42. Found C 79.87, H 6.48, N 6.37.

*5.1.26. 4,5,6,7-Tetrahydro-1H-benzimidazol-4-carboxylic acid hydrochloride (**54**·HCl)*

According to **GP7**: 4,5,6,7-Tetrahydro-1*H*-benzimidazol-4-carboxylic acid ethyl ester (23.0 mg, 0.118 mmol) [52], HCl (1 M, 1.0 mmol, 1.0 mL).

Colourless crystals; Yield: 23.0 mg (96%); mp 200–205 °C. IR (KBr) ν cm^{-1}: 3364, 3128, 3026, 2932, 1712, 1647, 1292, 1235; 400 MHz ^1H NMR (D_2O): δ = 1.94–1.73 (m, 2H, CH_2CH_2CHCOO), 2.17–2.01 (m, 2H, CH_2CHCOO), 2.70–2.52 (m, 2H, CH_2CNH), 3.87 (t, *J* = 6.0 Hz, 1H, CHCOO), 8.48 (s, 1H, $H_{[2H\text{-}Imid.]}$); 100 MHz ^{13}C NMR (D_2O): δ = 19.34 (CH_2CNH or CH_2CH_2CHCOO), 19.45 (CH_2CNH or CH_2CH_2CHCOO), 25.33 (CH_2CHCOO), 37.36 (*C*HCOO), 123.86 ($C_{[4C\text{-}Imid.]}$), 129.24 ($C_{[5C\text{-}Imid.]}$), 131.72 ($C_{[2C\text{-}Imid.]}$), 175.73 (COO); MS (CI, CH_5^+): *m/z* (%) = 167 (100, M^+), 121 (26); HMRS (EI) calcd. for $C_8H_{10}N_2O_2$: 166.0742; found 166.0759. Anal. Calcd. for $C_8H_{10}N_2O_2 \cdot HCl + 0.5H_2O$ (211.65) (%): C 45.40, H 5.71, N 13.24. Found C 45.77, H 5.91, N 13.11.

*5.1.27. 1-Tertbutyl 6-ethyl 4,5,6,7-tetrahydro-1H-benzimidazol-1,5-dicarboxylate (**57**) and 1-tertbutyl 6-ethyl 4,5,6,7-tetrahydro-1H-benzimidazol-1,6-dicarboxylate (**58**)*

A solution of formamide (3.40 g, 75.5 mmol, 3.0 mL) and ethyl 3-bromo-4-oxocyclohexanecarboxylate (**56**) (820 mg, 3.29 mmol)

was heated at 150 °C for 2 h. The solution was then diluted with H_2O and washed with Et_2O. Having adjusted a pH-value of 8–9, the solution was extracted with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and concentrated. The obtained residue was added to a stirred suspension of NaH (91.0 mg, 3.66 mmol) and THF (12 mL) at 0 °C. After 15 min at 0 °C, the solution was warmed up to rt for 1 h. Again, the solution was cooled to 0 °C, di-*tert*-butyl dicarbonate (720 mg, 3.33 mmol) was added and it was stirred 18 h at rt. Then, the reaction mixture was washed three times with H_2O and brine. The combined aqueous layers were extracted with CH_2Cl_2 before the organic layers were concentrated and dried over $MgSO_4$. Purification was realized by CC (EtOAc = 100). Ratio: **57**:**58** = 0.53:0.47.

Colourless oil; Yield: 438 mg (44%); IR (KBr) ν cm^{-1}: 2980, 2936, 2858, 1751, 1733, 1479, 1372, 1302, 1279, 1257, 1165, 1127, 1063, 1034; 500 MHz ^{1}H NMR ($CDCl_3$): $\delta = 1.26$ (t, $J = 7.1$ Hz, 3H, CH_3), 1.59 (d, J = 3.1 Hz, 9H, $C(CH_3)_3$), 1.96–1.82 (m, 1H, CH_2CH_2CHCOO), 2.23–2.13 (m, 1H, CH_2CH_2CHCOO), 3.18–2.54 (m, 5H, CHCOO, CH_2CH_2CHCOO, CCH_2CHCOO), 4.20–4.10 (m, 2H, CH_2CH_3), 7.95 (d, J = 3.4 Hz, 1H, $H_{[2H\text{-}Imid.]}$); 100 MHz ^{13}C NMR ($CDCl_3$): δ = 14.22 (CH_3), 14.24 (CH_3), 21.97 (CH_2CH_2CHCOO), 23.15 (CCH_2CHCOO), 25.43 (CH_2CH_2CHCOO or CCH_2CHCOO), 25.57 (CH_2CH_2CHCOO or CCH_2CHCOO), 25.61 (CH_2CH_2CHCOO or CCH_2CHCOO), 26.77 (CH_2CH_2CHCOO), 27.96 ($C(CH_3)_3$), 27.97 ($C(CH_3)_3$), 39.69 (CHCOO), 39.69 (CHCOO), 60.62 (CH_2CH_3), 60.67 (CH_2CH_3), 85.11 ($C(CH_3)_3$), 85.21 ($C(CH_3)_3$), 124.12 ($C_{[4C\text{-}Imid.]}$), 124.98 ($C_{[4C\text{-}Imid.]}$), 136.57 ($C_{[2C\text{-}Imid.]}$), 136.60 ($C_{[2C\text{-}Imid.]}$), 137.20 ($C_{[5C\text{-}Imid.]}$), 138.12 ($C_{[5C\text{-}Imid.]}$), 147.74 (NCOO), 147.79 (NCOO), 174.66 (CHCOO), 174.81 (CHCOO); MS (EI, 70 eV): m/z (%) = 294 (8) [M^+], 221 (18), 194 (50) 165 (36), 120 (100); MS (CI, CH_5^+): m/z (%) = 295 (42) [M^++1], 239 (100), 195 (88); HMRS (EI) calcd. for $C_{12}H_{22}N_2O_2$: 294.1580; found 294.1570.

5.1.28. 4,5,6,7-Tetrahydro-1H-benzimidazol-5-carboxylic acid ethyl ester (**59**)

TFA (6.14 g, 53.85 mmol, 4 mL) was added to a stirred solution of **57** and **58** (58 mg, 0.20 mmol) in EtOH (1 mL). The mixture was stirred for 4 h at rt before a pH-value of 9–10 with solid Na_2CO_3 and NaOH (2 M) was adjusted. After extraction with CH_2Cl_2, the combined organic layers were dried over $MgSO_4$ and concentrated.

Colourless crystals; Yield: 51.6 mg (94%); mp 145–150 °C. IR (KBr) ν cm^{-1}: 3149, 3045, 2925, 2858, 2822, 2674, 1716, 1665, 1446, 1383, 1299, 1206, 1172, 1132; 500 MHz ^{1}H NMR ($CDCl_3$): $\delta = 1.27$ (t, $J = 7.1$ Hz, 3H, CH_3), 2.00 (ddt, $J = 14.2/9.6/5.9$ Hz, 1H, CH_2CH_2CHCOO), 2.25 (ddt, J = 14.2/5.1/3.0 Hz, 1H, CH_2CH_2CHCOO), 2.79–2.63 (m, 2H, CH_2CH_2CHCOO), 2.87–2.79 (m, 1H, CHCOO), 2.92 (d, $J = 6.9$ Hz, 2H, CCH_2CHCOO), 4.17 (q, $J = 7.1$ Hz, 2H, CH_2CH_3), 7.97 (s, 1H, $H_{[2H\text{-}Imid.]}$); 125 MHz ^{13}C NMR ($CDCl_3$): δ = 14.17 (CH_3), 20.07 (CH_2CH_2CHCOO), 23.68 (CCH_2CHCOO), 25.24 (CH_2CH_2CHCOO), 39.35 (CHCOO), 60.99 (CH_2CH_3), 127.25 ($C_{[4C\text{-}Imid.]}$), 127.73 ($C_{[5C\text{-}Imid.]}$), 132.17 ($C_{[2C\text{-}Imid.]}$), 173.91 (COO); MS (CI, CH_5^+): m/z (%) = 195 (100) [M^++1], 120 (4); HMRS (EI) calcd. for $C_{10}H_{14}N_2O_2$: 194.1055; found 194.1100.

5.1.29. 4,5,6,7-Tetrahydro-1H-benzimidazol-5-carboxylic acid hydrochloride (**55**·HCl)

According to **GP7**: **59** (51.6 mg, 0.266 mmol), HCl (2 M, 6.0 mmol, 3.0 mL).

Colourless crystals; Yield: 48.8 mg (90%); mp 248–250 °C. IR (KBr) ν cm^{-1}: 3424, 3150, 3007, 2855, 1707, 1654, 1497, 1459, 1412, 1331, 1246, 1190, 1087; 500 MHz ^{1}H NMR (D_2O): δ = 2.00 (ddt, J = 13.8/9.3/7.0 Hz, 1H, CH_2CH_2CHCOO), 2.21 (ddt, J = 13.8/5.6/3.3 Hz, 1H, CH_2CH_2CHCOO), 2.69 (t, $J = 6.0$ Hz, 2H, CH_2CH_2CHCOO), 2.85 (ddt, J = 16.2/8.0/1.6 Hz, 2H, CCH_2CHCOO), 2.92 (dd, J = 16.2/5.5 Hz, 1H, CCH_2CHCOO), 3.02–2.95 (m, 1H, CHCOO), 8.43 (s, 1H, $H_{[2H\text{-}Imid.]}$); 125 MHz ^{13}C NMR (D_2O): δ = 18.19 (CH_2CH_2CHCOO), 21.80 (CCH_2CHCOO), 23.89 (CH_2CH_2CHCOO), 38.06 (CHCOO), 125.48 ($C_{[4C\text{-}Imid.]}$), 126.50 ($C_{[5C\text{-}Imid.]}$), 131.22 ($C_{[2C\text{-}Imid.]}$), 178.14 (COO); MS (EI, 70 eV): m/z (%) = 166 (40) [M^+], 119 (35), 94 (100); HMRS (EI) calcd. for $C_8H_{10}N_2O_2$: 166.0742; found 166.0765; Anal. Calcd. for $C_8H_{10}N_2O_2$ (166.18) (%): C 46.05, H 5.64, N 5.83, N 13.43. Found C 46.21, H 5.44, N 13.16.

NMR and melting point (lit.: 248–250 °C) are according to literature values [59].

6. Pharmacological methods

6.1. [^{3}H]GABA uptake

[^{3}H]GABA uptake was studied as described (A. Kragler, G. Höfner, K. T. Wanner, synthesis of aminomehtylphenol derivatives as inhibitors of the murine GABA transporters mGAT1–mGAT4, Eur. J. Med. Chem., 43, 2404–2411, 2008).

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