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Original article

Azetidine derivatives as novel γ-aminobutyric acid uptake inhibitors: Synthesis, biological evaluation, and structure–activity relationship

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Dedicated to Prof. H. Wagner with best wishes on the occasion of his 80th birthday.

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ABSTRACT

In this study azetidine derivatives representing conformationally constrained GABA or β-alanine analogs were evaluated for their potency as GABA-uptake inhibitors. The study comprised derivatives substituted in 2- as well as in 3-position with either an acetic acid moiety or a carboxylic acid function. In addition, azetidine derivatives bearing a tetrazole ring as a bioisosteric substitute for a carboxylic acid group were included. 3-Hydroxy-3-(4-methoxyphenyl)azetidine derivatives were explored as analogs of the known GABA-uptake inhibitor NNC-05-2045 exhibiting an azetidine ring instead of a piperidine ring present in the latter. Both, *N*-unsubstituted compounds as well as their *N*-alkylated lipophilic derivatives, were biologically evaluated for their affinity to the GAT-1 and GAT-3 transporters. Azetidin-2-ylacetic acid derivatives provided with a 4,4-diphenylbutenyl or 4,4-bis(3-methyl-2-thienyl)butenyl moiety as lipophilic residue were found to exhibit the highest potency at GAT-1 with IC_{50} values of 2.83 ± 0.67 μM and 2.01 ± 0.77 μM, respectively. The most potent GAT-3 inhibitor among these compounds appeared to be the β-alanine analog 1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}azetidine-3-carboxylic acid (**12d**) displaying an IC_{50} value of 15.3 ± 4.5 μM. Whereas the tetrazole derivatives showed no potency as GABA-uptake inhibitors, the 3-hydroxy-3-(4-methoxyphenyl)azetidine derivatives exhibited moderate affinity to GAT-1 (compound **18b**: IC_{50} = 26.6 ± 3.3 μM) and to GAT-3 (compound **18e**: IC_{50} = 31.0 ± 4.7 μM).

1. Introduction

Alterations in GABAergic function have been found to be involved in a number of CNS disorders, including epilepsy [1], Huntington's chorea [2], migraine [3], Parkinson disease [4], and depression [5–8]. Enhancement of the GABA function can be achieved through several mechanisms including direct receptor activation by agonists, allosteric activation by modulators such as benzodiazepines and barbiturates or inhibition of the enzymatic GABA degradation by for example vigabatrin [9]. The long-term utility of both vigabatrin and benzodiazepines as anticonvulsants is limited by the rapid development of tolerance [10,11]. An efficient, alternative strategy to palliate GABA deficiency is the inhibition of GABA transport proteins. These high-affinity carriers regulate synaptic and extra-synaptic availability of GABA by transporting the neurotransmitter from the synaptic cleft into presynaptic nerve terminals and into surrounding astrocytes [12]. GABA-uptake proteins belong to the large family of Na^+/Cl^--dependent transporter proteins, which also include the transporters for several other neurotransmitters such as dopamine, serotonin, norepinephrine, and glycine [13–16]. Recently, the three-dimensional structure of a bacterial homologue of Na^+/Cl^--dependent neurotransmitter transporters, the leucine transporter from *Aquifex aeolicus* has been elucidated by X-ray crystallographic analysis [17]. With this analysis a major advance toward the understanding of structure-function relationship in this important class of transporters has been achieved. Meanwhile, the structure of this leucine transporter has also been used, for example, as a template for the construction of three-dimensional models of the GABA transporter GAT-1 [18,19]. Molecular cloning techniques revealed the existence of four distinct GABA transporters in humans and other species. These are termed as GAT-1, GAT-2, GAT-3, and BGT-1 according to a nomenclature introduced by Borden et al. which will be used in this paper (for murine GABA transport proteins nomenclature is different) [20–22]. Our research is focused on selective targeting GABA transporter subtypes, especially GAT-1 and GAT-3, which immunocytochemical and in situ hybridization studies have shown to be the most prevalent GABA transporters in the brain. Subtype selective compounds are of great interest as they could serve as valuable tools for the characterization of the physiological function of the various GABA transporters. Moreover, inhibitors of the different GABA-uptake proteins can be expected to be of

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therapeutic benefit as well, as, for example, GAT-1 is already a proven target for the therapy of epilepsy.

A number of conformationally restricted cyclic GABA analogs such as nipecotic acid (**1**) and guvacine (**2**) were found to be inhibitors of [^{3}H]-GABA uptake in in vitro experiments (Scheme 1) [23]. β-Alanine has been reported to exhibit moderate affinity to GABA-uptake transporters with GAT-3 and GAT-2 selectivity (GAT-3: $IC_{50} = 110$ μM; GAT-2: $IC_{50} = 66$ μM) [24]. The use of these amino acids as pharmacological agents has been hampered by their poor penetration of the blood brain barrier [23,25]. Thus, considerable efforts have been made to develop selective and potent GABA transport inhibitors displaying favorable pharmacokinetic properties. Ali et al. [26] showed that adding the lipophilic 4,4-diphenylbut-3-en-1-yl moiety to the nitrogen atom of nipecotic acid (**1**) or guvacine (**2**) leading to SK&F-89976-A (**4**) ($IC_{50} = 0.20$ μM) and SK&F-100330-A (**6**) ($IC_{50} = 0.2$ μM), respectively, (Scheme 1) significantly increases the ability of these compounds to enter the brain and their potency as GABA-uptake inhibitors. To date, numerous lipophilic derivatives of nipecotic acid (**1**) and guvacine (**2**) have been synthesized and biologically evaluated for their potency as GABA-uptake inhibitors [27]. Tiagabine (**5**), which preferentially targets GAT-1, has been in clinical use since 1997 [28–33]. It is an effective and safe drug for the management of epilepsy. But, it possesses an unfavorable pharmacokinetic profile characterized by a short half-life [34]. Besides, tiagabine (**5**) exhibits moderate side effects, including dizziness, fatigue, and confusion which seem to limit its usefulness [34]. Thus, the search for GABA-uptake inhibitors with improved pharmacological properties continues.

Previously [35,36], we reported the synthesis of the pyrrolidine derivative **7** and **8**, which may be considered as analogs of tiagabine (**5**) and SK&F-89976-A (**4**), respectively, in which the nipecotic acid subunit is replaced by homoproline (**3**). Both compounds **7** and **8** show a high potency at and subtype selectivity for the GAT-1 protein (**7**: $IC_{50} = 0.396$ μM, **8**: 0.343 μM).

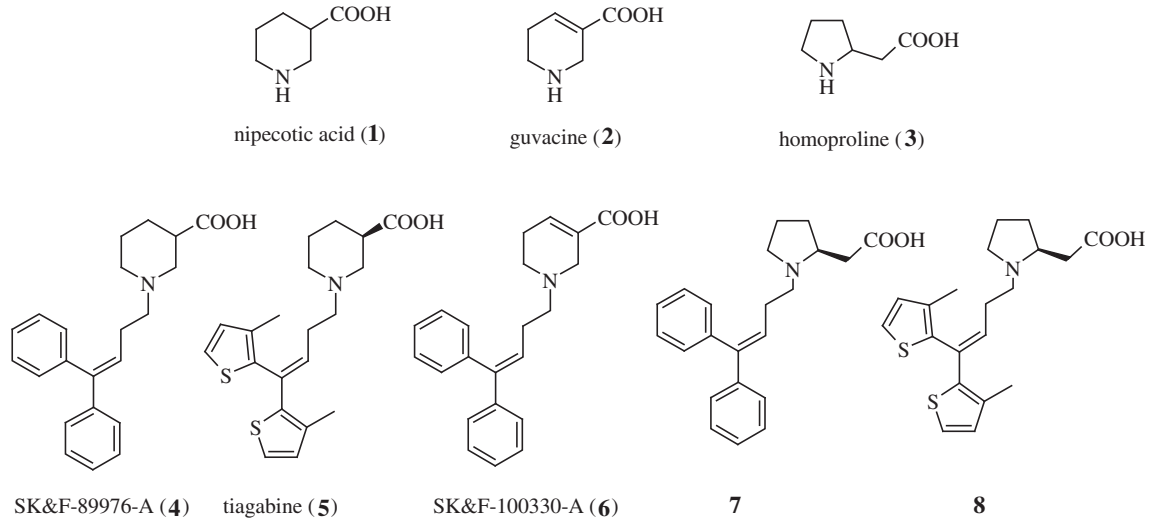
Dhar et al. [37] succeeded in the synthesis of the first inhibitor with high potency at and subtype selectivity for GAT-3, (*S*)-SNAP-5114 (**9**) (Scheme 2) with an IC_{50} value of 5 μM and a selectivity of 78:1 for GAT-3 versus GAT-1 [37]. Thomsen et al. [32,38] presented NNC-05-2045 (**10**), a 4-methoxyphenylpiperidin-4-ol derivative, which was found to be a potent inhibitor at BGT-1. But, the compound displayed only moderate subtype selectivity for this transporter (BGT-1: $K_i = 1.6 \pm 0.4$ μM; GAT-1: $K_i = 27 \pm 2$ μM; GAT-3: $K_i = 6.1 \pm 1.3$ μM) [38,39]. Replacing the nipecotic acid in (*S*)-SNAP-5114 (**9**), we found compound **11**, which compares well to (*S*)-SNAP-5114 (**9**) with respect to both, its potency at ($IC_{50} = 3.1$ μM) and subtype selectivity for GAT-3 as compared to GAT-1 (22:1) [35,36].

As part of an ongoing project with the aim to explore conformationally restricted GABA and β-alanine analogs of various ring sizes as potential subtype selective GABA-uptake inhibitors, we performed the synthesis of a series of azetidine derivatives provided with a carboxylic acid moiety and studied their in vitro GABA-uptake activity (see Scheme 3). As parent compounds the azetidine derivatives **12a**–**15a** have been selected in which the acid functionality either resides in the 2- or 3-position of the heterocyclic ring system. Variation of the position of the acid functionality was expected to shed some light on the structure–activity relationships for that class of compounds. In potent GABA-uptake inhibitors as for example in SK&F-89976-A (**4**), SK&F-100330-A (**6**), tiagabine (**5**), and (*S*)-SNAP-5114 (**9**) typically lipophilic *N*-substituent of a specific structure are present, which significantly contribute to the potency, subtype selectivity, and improved pharmacokinetic properties of these compounds. Accordingly, derivatives of the amino acids **12a**–**15a** provided with residues **b**–**d** should be included in this study as well. In addition, the azetidine derivatives **16a** and **17a**, in which the carboxylic acid moiety is replaced by the bioisosteric tetrazole group should be included in this study. Furthermore, we were interested in derivatives of **18a**, substituted with both a 4-methoxyphenyl and a hydroxy group in the 3-position of the azetidine ring, and its derivatives **18b**–**18e** as analogs of NNC-05-2045 (**10**).

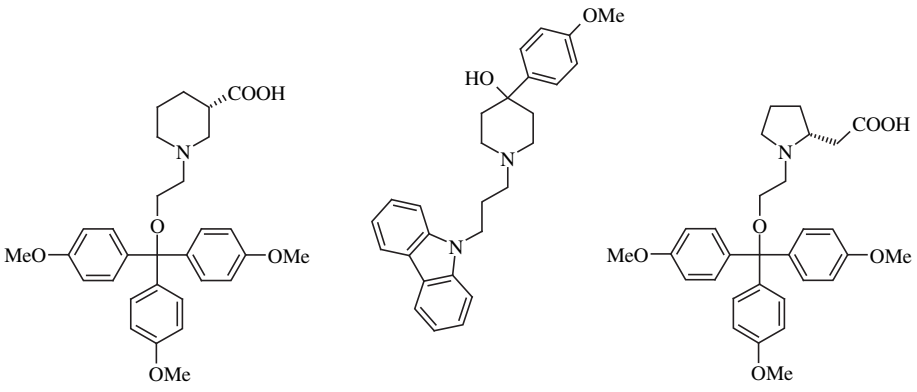
2. Chemistry

2.1. Synthesis of unsubstituted azetidine derivatives

Azetidine-3-carboxylic acid (**12a**), azetidine-3-ylacetic acid (**13a**), (*S*)-azetidine-2-carboxylic acid (**14a**), and 3-[4-methoxyphenyl]azetidine-3-ol (**18a**) (see Scheme 3) were synthesized according to literature procedures [39–42]. For the synthesis of azetidin-2-ylacetic acid (**15a**) a method has been patented by Orr [43]. It starts from γ-butyrolactone and requires seven steps. We were able to develop a more convenient synthesis leading to **15a** in only five steps (Scheme 4). The synthetic strategy is based on a method reported by Shono et al. [44] for the preparation of the



Scheme 1. Representative GAT-1 selective GABA-uptake inhibitors.

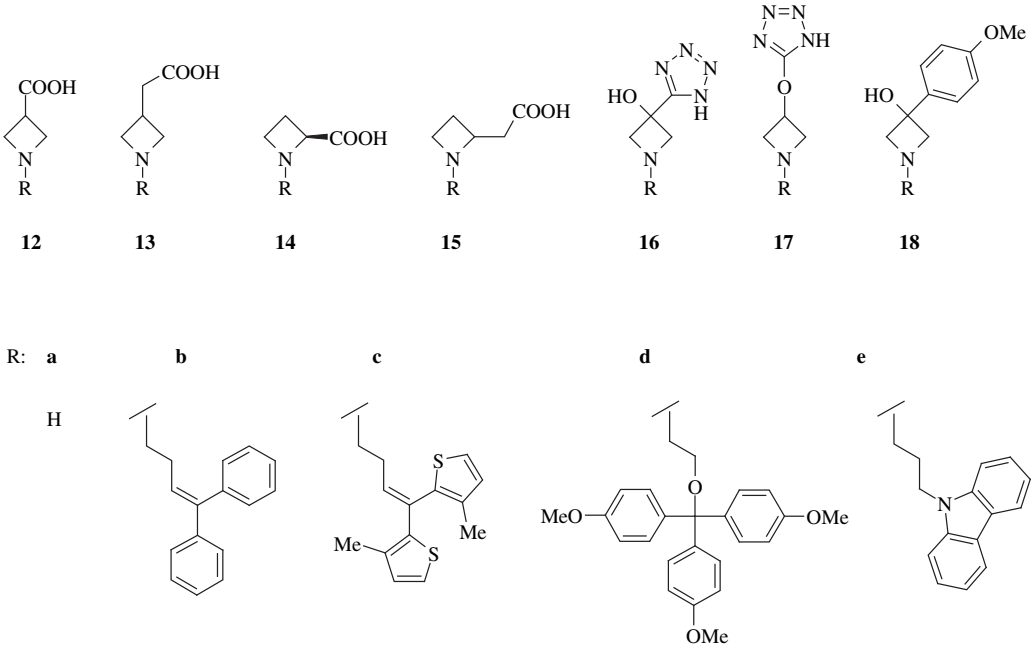


(*S*)-SNAP-5114 (**9**) NNC-05-2045 (**10**) **11**

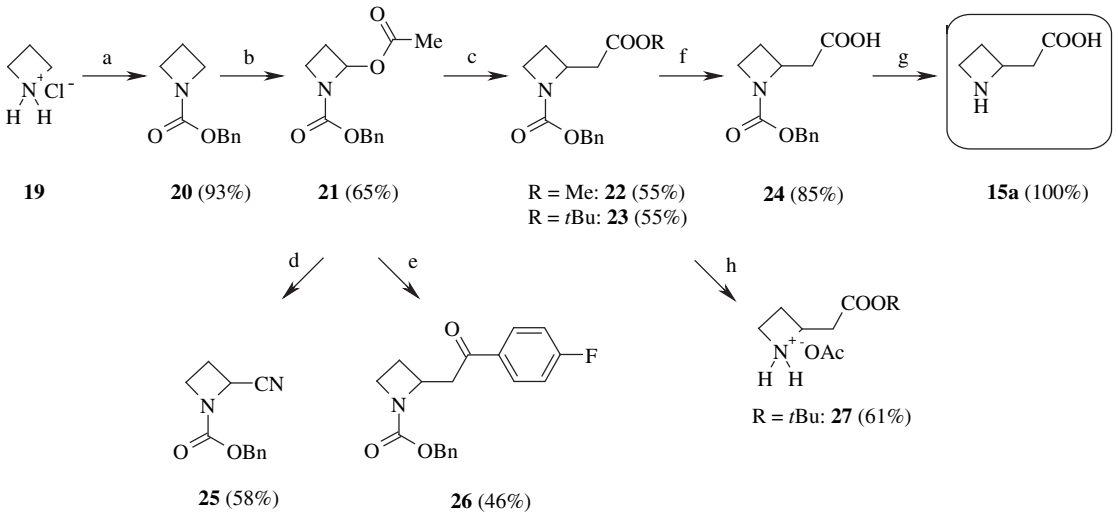
Scheme 2. Representative BGT-1 and GAT-3 selective GABA-uptake inhibitors.

2-substituted azetidines involving the anodic oxidation of a tosylated azetidine to the corresponding 2-acetoxy derivative followed by the generation of an iminium ion and subsequent trapping reaction with an appropriate nucleophile. Modifying Shono's synthetic approach, we used the benzyloxycarbonyl group instead of the tosyl group to protect the nitrogen atom of the azetidine ring. The benzyloxycarbonyl group was found to be preferable since it can be cleaved under milder reaction conditions ensuring that the azetidine ring remains intact. To access azetidin-2-ylacetic acid derivatives, we subjected the benzyloxycarbonyl group protected azetidine **20**, which we had synthesized from **19**, to anodic oxidation in the presence of sodium acetate to give the *N*-acyl iminium ion precursor **21** in 65% yield. Various transformation reactions were performed to explore the suitability of **21** for the preparation of 2-substituted azetidine derivatives. In each case **21** was treated with $TiCl_4$ at −78 °C (−70 °C for the reaction with TMSCN and [1-(4-fluorophenyl)vinyloxy]trimethylsilane) to generate the corresponding *N*-acyl iminium ion which was then reacted with different nucleophiles. The nucleophilic additions to the intermediate *N*-acyl iminium ion were successfully performed employing (1-methoxyvinyloxy)trimethylsilane, (1-*tert*-butoxyvinyloxy)trimethylsilane, TMSCN, and [1-(4-fluorophenyl)vinyloxy]trimethylsilane resulting in the formation of the addition products **22**, **23**, **25**, and **26** in 55%, 55%, 58%, and 46% yield, respectively (Scheme 4). Addition product **22** turned out to be well suited for the preparation of the required azetidin-2-ylacetic acid **15a**. Saponification of the ester functionality with NaOH in MeOH and *N*-deprotection by hydrogenation over Pd/C provided it in an overall yield of 28% (from **19**). Catalytic hydrogenation of compound **23** provided *tert*-butyl ester **27** in 61% yield, required for the synthesis of the *N*-alkylated lipophilic azetidine derivatives **15b** and **15c** (Scheme 3).

Azetidine derivative **16a**·**HCl** exhibiting a tetrazole ring in the 3-position, was synthesized starting from ketone **28** [45,46] (Scheme 5). Reaction of the lithiated tetrazole derivative **29** generated from 1-(4-methoxybenzyl)-1*H*-tetrazole according to a procedure from Satoh and Marcopulos [47] with the ketone **28** at −90 °C provided

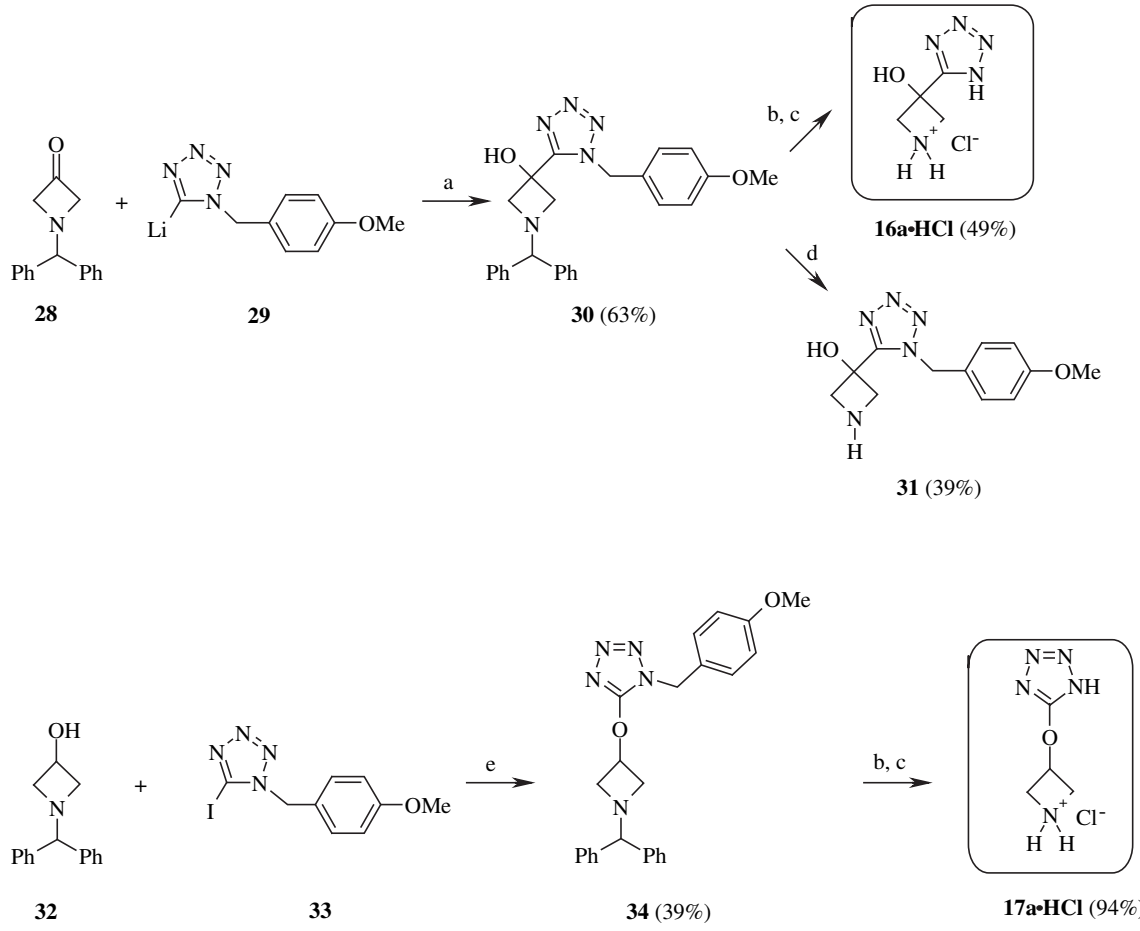


Scheme 3. Azetidine derivatives.

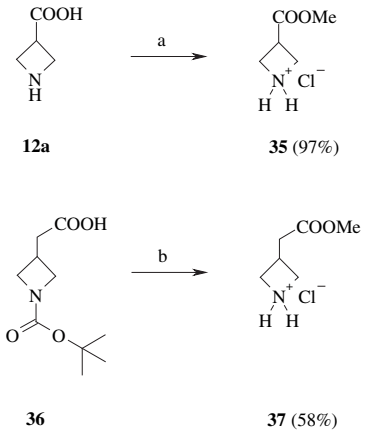


Scheme 4. Reagents and conditions: (a) benzyl chloroformate, NEt_3, CH_2Cl_2, rt; (b) anodic oxidation, AcOH, NaOAc; (c) (1-methoxyvinyloxy)trimethylsilane or (1-*tert*-butoxyvinyloxy) trimethylsilane, $TiCl_4$, CH_2Cl_2, −78 °C (2 h), 0 °C (1 h); (d) TMSCN, $TiCl_4$, CH_2Cl_2, −70 °C (2 h); (e) [1-(4-fluorophenyl)vinyloxy]trimethylsilane, $TiCl_4$, CH_2Cl_2, −70 °C (2 h); (f) 2 N NaOH, MeOH, 72 h, rt; (g) H_2, Pd/C, MeOH, 16 h, rt; (h) Pd/C, H_2, MeOH, AcOH, rt, 16 h.

after workup the addition product **30** in 63% yield. Subsequent removal of the benzhydryl and the 4-methoxybenzyl group by catalytic hydrogenation with Pearlman's catalyst [48] afforded 3-(1*H*-tetrazol-5-yl)azetidin-3-ol hydrochloride (**16a·HCl**) in 49% yield. Alternatively, selective removal of the benzhydryl group by hydrogenation over Pd/C in TFA/acetic acid provided compound **31** in 39% yield. The synthesis of the structurally related 5-(azetidin-3-yloxy)-1*H*-tetrazole hydrochloride (**17a·HCl**) was accomplished as indicated in Scheme 5. Reaction of the sodium salt of the alcohol **32** [40], generated by treatment with sodium hydride, with iodo



Scheme 5. Reagents and conditions: (a) THF/TMEDA = 10:1, −90 °C, 30 min; (b) H_2, $Pd(OH)_2$/C, MeOH, rt, 72 h; (c) 2 N HCl; (d) Pd/C, H_2, AcOH, TFA, rt, 72 h; (e) NaH, THF, rt, 4 h.



Scheme 6. Reagents and conditions: (a) $SOCl_2$, MeOH, −60 °C (30 min), rt (16 h); (b) AcCl, MeOH, rt, 16 h.

derivative **33** led to compound **34** in 39% yield. This was then transformed into the desired compound **17a**·**HCl** in 94% yield by concurrent removal of the benzhydryl and the 4-methoxybenzyl group, again accomplished by hydrogenation over Pearlman's catalyst.

2.2. Synthesis of N-alkylated azetidine derivatives

For the synthesis of the *N*-alkylated azetidine derivatives provided with a carboxylic acid moiety, in addition, the carboxylic acid esters **35** and **37** (Scheme 6) of the amino acids **12a** [49] and **13a**, (Scheme 3) had to be prepared. Azetidin-3-carboxylic acid **12a** was converted to the corresponding ester **35** [50] employing $SOCl_2$ in methanol (Scheme 6). For the synthesis of methyl azetidin-3-ylethanoate hydrochloride **37** [51], a method published by Nudelman et al. [52] was followed. This method allows a one-step conversion of *N*-Boc protected amino acids to the corresponding *N*-unprotected amino acid esters by treating the starting material with acetyl chloride in methanol. When applied to Boc protected azetidin-3-ylacetic acid **36** [40] (Scheme 6), the desired ester compound **37** was obtained in a 58% yield.

The *N*-alkylation of the amino acid esters **27**, **35**, and **37**, the tetrazole derivative **31** and the 4-hydroxy-4-phenyl derivative **18a** [42] (Scheme 7) was accomplished in analogy to a procedure published by Dhar et al. [37], which is based on alkyl bromides and an in situ Finkelstein reaction to generate alkyl iodides as alkylation agents (Scheme 7). In all cases the desired alkylation products were obtained, but yields varied considerably and were, in general, not higher than 60% (see Scheme 7).

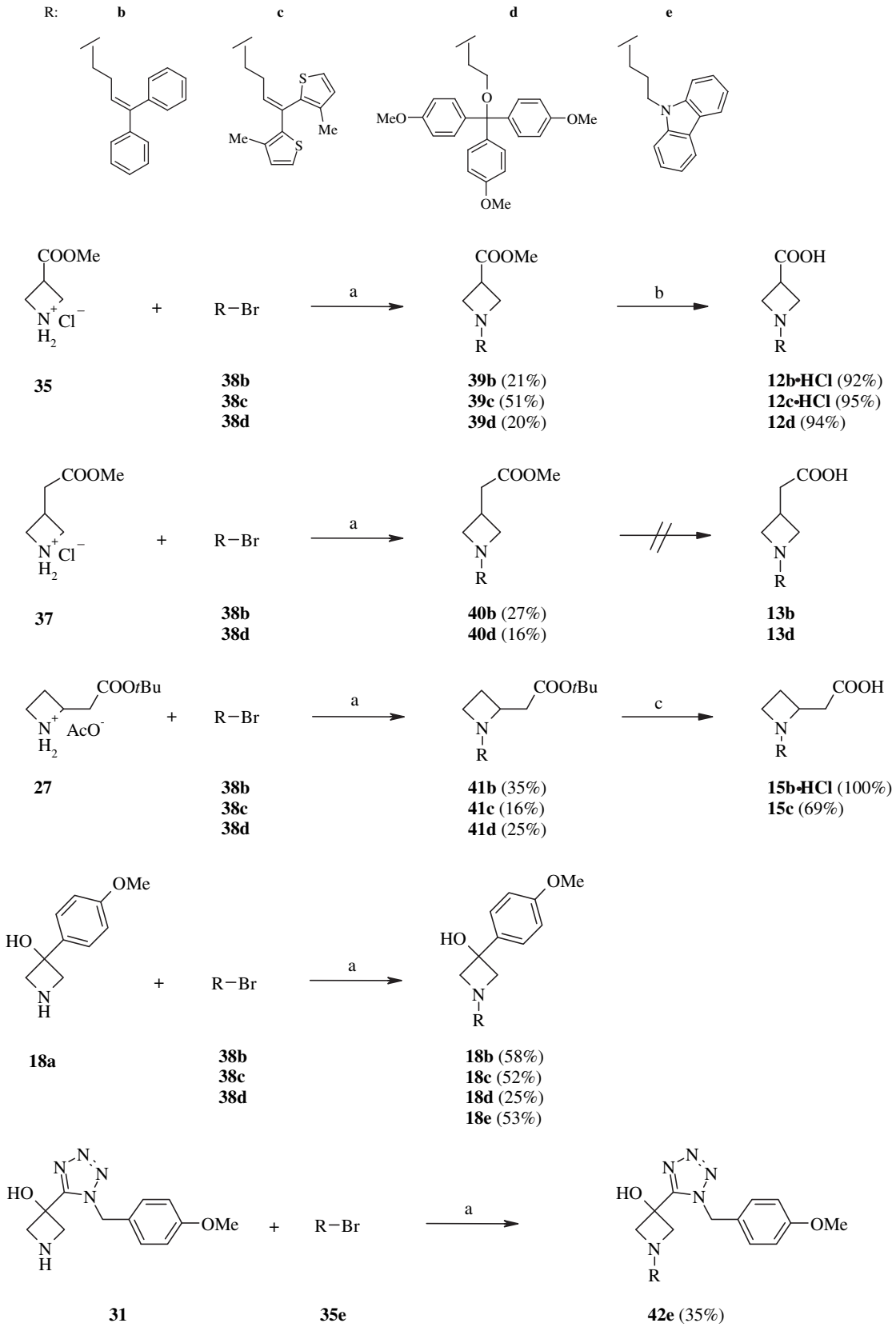
2.3. Deprotection of the azetidine derivatives

To gain access to the free amino acids, the amino acid esters **39b**–**d** and **41b**–**c** obtained from the alkylation reaction were subjected to a saponification reaction with 2 N NaOH at room temperature (see Scheme 7). In general, the resulting amino acids were isolated as their hydrochloride salts, except for **12d** provided with a trityloxyethyl moiety, which was extracted as free amino acid as the trityloxyethyl residue is known to be sensitive to acidic conditions and heat. A rearrangement to lactones occurred in case of compounds **40b** and **40d** under the conditions of the saponification reaction obviating the isolation of the desired compounds [53]. Using acidic conditions (10% HCl at 50 °C) for the hydrolysis of the ester group was not successful either, not only for compound **40d** for which it was expected because of the presence of the trityloxyethyl group, but also for **40b**, which underwent decomposition reactions under these reaction conditions. So all attempts to obtain the corresponding amino acids **13b** and **13d** failed. The cleavage of the *tert*-butyl esters in compounds **41b** and **41c**, effected by means of 20% TFA in dichloromethane at room temperature led to the amino acids **15b**·**HCl** and **15c**. All final products, thus obtained, (Scheme 7) were then studied for their inhibitory potency at the GABA transporters GAT-1 and GAT-3.

3. Biological results

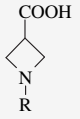
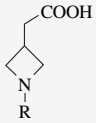
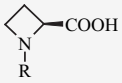
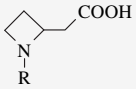
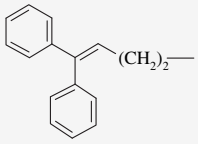
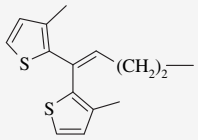
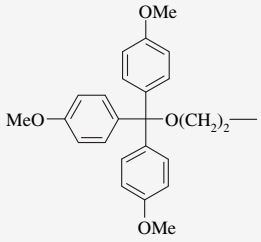
As standard assay for the characterization of GABA-uptake inhibitors, we had used a procedure based on bovine brain material [54]. Because of emerging cases of bovine spongiform encephalopathy (BSE) in German cattle during the course of this study, we were, however, forced to establish a new test system. We decided for porcine brain material as a new protein source, as this was regarded as non-hazardous. The reliability of the newly developed assay system based on porcine brain was evaluated using a series of reference compounds. Results were found to be in accordance with both, the previously used assay based on bovine brain material as well as the alternative assay systems published by other research groups. The results of the biological evaluation of the azetidine carboxylic acid and azetidinylacetic acid core structure and the corresponding *N*-alkylated derivatives as GABA-uptake inhibitors are summarized in Table 1. The values of some reference compounds can be found in Table 2. Azetidine-3-carboxylic acid (**12a**) displayed moderate GABA-uptake inhibition at GAT-3 with a selectivity of 34:1 for this transporter as compared to GAT-1 which is in the range of the GAT-3 selectivity observed for the structurally related β-alanine (GAT-3/GAT-1 81:1 [55]). Despite the affinity of **12a** to the GAT proteins, its *N*-alkylated derivatives **12b**–**c** with lipophilic residues were found to be more or less inactive at 100 μM (Table 1). Interestingly, derivative **12d** substituted with a trityloxyethyl group, showed almost no activity at GAT-1 (100 μM: 76%), but an IC_{50} value of 15.3 μM for GAT-3 inhibition. This observation resembles the affinity and selectivity of the GAT-3 selective inhibitor (*S*)-SNAP-5114 (**9**) and its homoproline analog **11** (Table 2). Azetidin-3-ylacetic acid **13a** displayed IC_{50} values of 43.3 μM for GAT-1 and 39.8 μM for GAT-3. A preliminary screening of product **13b**, that from the saponification had been obtained in impure form only, showed no activity at GAT-1 and GAT-3. Therefore, we waived further efforts in synthesizing the *N*-substituted derivatives of **13a**. The naturally occurring azetidin-2-carboxylic acid (**14a**) showed only negligible affinity to both, the GAT-1 as well as the GAT-3 transport protein. Thus, no *N*-alkylated derivatives of **14a** were prepared for biological evaluation. Among the azetidine derivatives bearing a carboxylic acid moiety, the lowest IC_{50} values were observed for the azetidin-2-ylacetic acid derivative **15c**, which is structurally related to tiagabine (**5**) and the homoproline derivative **8** (Table 2). The parent structure **15a** only showed low affinity for GAT-1 and GAT-3, but the *N*-alkylated **15c** displayed an IC_{50} value of 2.01 μM (tiagabine (**5**): 0.159 μM, **8**: 0.343 μM) with a selectivity for GAT-1 as compared to GAT-3 of 48:1. For the diphenylbutenyl derivative **15b**·**HCl** structurally related to SK&F-89976-A (**4**) and the homoproline derivative **7** a potency at GAT-1 was found (**15b**·**HCl**: $IC_{50} = 2.83$ μM, **4**: 1.18 μM, **7**: 0.396 μM; Table 2) similar to that of **15c**, but its selectivity for GAT-1 as compared to GAT-3 was distinctly lower (11:1).

The IC_{50} values of both, of the tetrazole and tetrazoloxy substituted azetidine compounds as well as of the 4-hydroxy-4-(4-methoxyphenyl)azetidine derivatives are reported in Table 3. The tetrazole derivatives **16a**·**HCl** and **17a**·**HCl** showed even at high concentration (1000 μM and 100 μM, respectively) no or only negligible inhibition of GAT-1 and GAT-3. 4-Hydroxy-4-(4-methoxyphenyl)azetidine (**18a**), which was synthesized as an



Scheme 7. Reagents and conditions: (a) K_2CO_3, NaI; (b) 2 N NaOH, H_2O, MeOH, rt, 48 h; (c) TFA, CH_2Cl_2, rt, 2 h. Where the term "HCl" has been added to the compound number, the compound has been isolated as hydrochloride salt though only the neutral form is listed.

Table 1
Uptake inhibition [IC_{50} (μM)] at GAT-1 and GAT-3 exhibited by azetidine carboxylic acid derivatives.

								
	IC_{50} [μM]		IC_{50} [μM]		IC_{50} [μM]		IC_{50} [μM]	
R	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3
H	1550 ± 150^a **12a**	45.5 ± 3.5^b **12a**	43.3 ± 34.8^a **13a**	39.8 ± 17.5^b **13a**	1950 ± 300^a **14a**	706 ± 26^b **14a**	111 ± 71^a **15a**	312 ± 120^b **15a**
	101%[a] ($n = 1$) **12b·HCl**	81.6%[b] ($n = 1$) **12b·HCl**	–*	–*	–	–	2.83 ± 0.67^a **15b·HCl**	31.4 ± 11.5^f **15b·HCl**
	95.6%[a] ($n = 1$) **12c·HCl**	96.6%[b] ($n = 1$) **12c·HCl**	–	–	–	–	2.01 ± 0.77^e **15c**	96.6 ± 30.9^f **15c**
	76.10%[a] ($n = 1$) **12d**	15.3 ± 4.5^b **12d**	–	–	–	–	–	–

If not stated otherwise the results are given as means of IC_{50}-values ± SEM of three independent experiments each performed in triplicate, $n = 1$ denotes value from a single experiment, percentages represent specific binding remaining in presence of 100 μM inhibitor. The abbreviations a–f specify the used biological material: [a]bfcP2B, [b]bbsP2C, [c]cfcP2B, [d]cbsP2C, [e]pfcP2B, [f]pbsP2C (see Experimental section).
Where "HCl" has been added to the compound number the compound has been tested as hydrochloride of the drawn neutral structure.
* Preliminary screening of the impure saponification product **13b** showed no activity.

N-unsubstituted analog of NNC-05-2045 (**10**) with reduced ring size was devoid of any significant activity at GAT-1 as well as GAT-3. However, *N*-alkylated derivatives of **18a** exhibited reasonable potencies as GABA-uptake inhibitors. The highest affinity was observed for the diphenylbutenyl derivative **18b** showing IC_{50} values of 26.6 μM and 48.5 μM at GAT-1 and GAT-3, respectively. Compound **18e**, which is a direct analog of **10** with a reduced ring size, was found to display an IC_{50} value of 31.0 μM at GAT-3 (**10**: $IC_{50} = 3.34$ μM) with a moderate preference for this transporter.

4. Conclusion

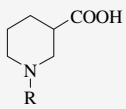
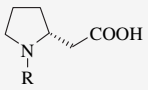
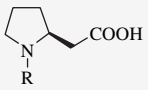
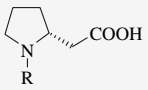
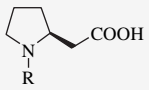
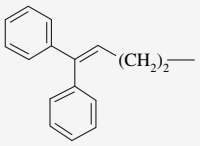
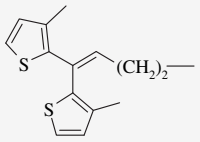
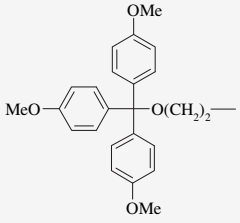
Azetidine derivatives were synthesized as structural analogs of the known GABA-uptake inhibitors tiagabine (**5**), SK&F-89976-A (**4**), (*S*)-SNAP-5114 (**9**), and NNC-05-2045 (**10**). The activity of the azetidine derivatives was found to be strongly dependent on both the position of the carboxylic acid moiety and on the nature of the lipophilic groups. Among the investigated compounds, the highest affinity at GAT-1 was observed for the azetidin-2-ylacetic acid analog **15c** of tiagabine (**5**) displaying an IC_{50} value of 2.01 μM and a selectivity of 48:1 for GAT-1 over GAT-3. The azetidine-3-carboxylic acid derivative **12d** was the most potent inhibitor of GAT-3 with an IC_{50} value of 15.3 μM and a selectivity greater than 7:1 (GAT-3/GAT-1). The tetrazole substituted azetidine derivatives **16a·HCl** and **17a·HCl** appeared to be inactive as GABA-uptake inhibitors when tested at a concentration of 100 μM or 1000 μM, respectively. Compound **18e** as an analog of NNC-05-2045 (**10**) of a smaller ring size, showed only a moderate potency at and subtype selectivity for GAT-3 ($IC_{50} = 31.0$ μM).

5. Experimental section

5.1. Methods and materials

Anhydrous reactions were carried out in vacuum dried glassware under nitrogen atmosphere. THF, Et_2O, NEt_3, DME, diisopropylamine, 1,4-dioxane, and toluene were freshly distilled from sodium metal/benzophenone ketyl, CH_2Cl_2, DMF, and acetonitrile from CaH_2 and CH_3OH from Mg prior to use. Other common solvents for recrystallization and column chromatography were always distilled before use. Purchased chemical reagents were used without further purification. TLC plates were made from silica gel 60 F_{254} on aluminum sheets (Merck). Compounds were stained with 5% $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 0.2% $Ce(SO_4)_2\cdot 4H_2O$, and 5%

Table 2
Uptake inhibition [IC_{50} (μM)] at GAT-1 and GAT-3 for some reference compounds.

										
	IC_{50} [μM]		IC_{50} [μM]		IC_{50} [μM]		IC_{50} [μM]		IC_{50} [μM]	
R	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3
H	4.77 ± 1.17^b (*rac*)-**1**	17.3 ± 2.36^b (*rac*)-**1**	2.15 ± 0.11^b (*R*)-**1**	18.5 ± 5.6^b (*R*)-**1**	32.6 ± 6.5^b (*S*)-**1**	218 ± 54^b (*S*)-**1**	214 ± 16^b (*R*)-**3**	602 ± 59^b (*R*)-**3**	482 ± 39^b (*S*)-**3**	74.2 ± 6.2^b (*S*)-**3**
	1.18 ± 0.24^b **4**	290 ± 151^b **4**	–	–	–	–	–	–	0.396 ± 0.026^b **7**	64.8 ± 12.1^b **7**
	–	–	0.159 ± 0.029^b **5**	483 ± 83^b **5**	–	–	–	–	0.343 ± 0.044^b **8**	26.6 ± 4.3^b **8**
	–	–	–	–	83.3 ± 15.7^b **9**	1.08 ± 0.24^b **9**	67.8 ± 19.0^b **11**	3.10 ± 0.45^b **11**	–	–

If not stated otherwise the results are given as means of IC_{50}-values ± SEM of three independent experiments each performed in triplicate, percentages represent specific binding remaining in presence of 100 μM inhibitor. The abbreviation b specifies the used biological material: bbsP2C (see experimental section).

conc. H_2SO_4. If nothing else is stated, Merck silica gel (mesh 230–400) was used as stationary phase for flash chromatography. Optical rotations: Polarimeter 241 MC at λ 589 cm^{-1}. Melting points: mp (uncorrected) were determined with a Büchi 510 Melting Point apparatus. Elementary analysis: Elementaranalysator Rapid (Heraeus). IR spectroscopy: FT-IR Spectrometer 1600 and Paragon 1000 (Perkin Elmer), oily samples as film, solid samples as pellets for measurements. Mass spectroscopy: Mass Spectrometer 5989 A with 59980 B particle beam LC/MS interface (Hewlett Packard). NMR spectroscopy: NMR spectra were recorded on JNMR-GX (Jeol, 400 MHz and 500 MHz) with TMS as internal standard and integrated with the program of NMR-software Nuts (2D Version 5.097, Acorn NMR, 1995). If nothing else is stated, measurements were performed at 400 MHz at room temperature.

5.1.1. General procedure 1 (GP1)

2 N NaOH was added to the respective ester compound dissolved in alcohol. The reaction mixture was stirred for 48 h. Following acidification to pH 1–2 using HCl (0.01 N) and extraction with CH_2Cl_2 (3×), the organic layers were dried ($MgSO_4$) and concentrated in vacuo.

5.1.2. General procedure 2 (GP2)

2 N NaOH was added to the respective ester compound dissolved in alcohol. The reaction mixture was stirred for 48 h. The mixture was acidified using HCl (0.01 N) until the solution turned cloudy (pH 5–6) and extracted with CH_2Cl_2 (3×). The organic layers were dried ($MgSO_4$) and concentrated in vacuo.

5.1.3. General procedure 3 (GP3)

A solution of the respective alkyl bromide was added to a solution of the respective amine. K_2CO_3 and NaI were added and the reaction mixture was stirred for the time given under nitrogen and under exclusion of light. The solvent was removed in vacuo. The crude product was extracted with EtOAc (30 ml). The organic layer was washed with water (15 ml), dried ($NaSO_4$), and concentrated in vacuo.

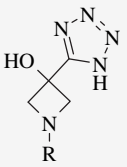
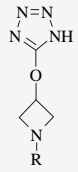
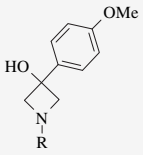
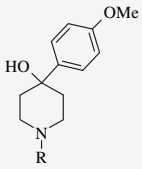
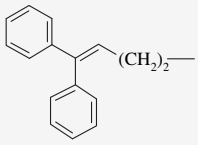
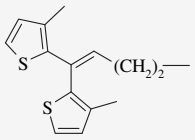
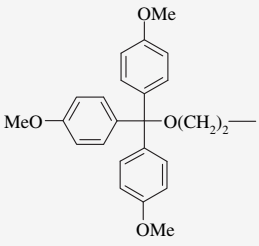
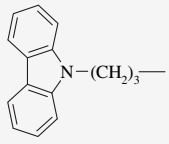
5.1.4. General procedure 4 (GP4)

Acetyl chloride (2.5 equiv) was added to the respective amino acid in MeOH at 0 °C. The reaction mixture was allowed to warm to rt and was stirred for 24 h. The solvents and excess of acetyl chloride were removed in vacuo.

*5.1.5. 1-(4,4-Diphenylbut-3-en-1-yl)azetidine-3-carboxylic acid hydrochloride (**12b**·**HCl**)*

Hydrolysis according to GP1 starting from **39b** (71 mg, 0.22 mmol) in 1 ml MeOH and 2 N NaOH (0.275 ml, 0.55 mmol). Yield: 69 mg (92%); colorless solid, mp 64 °C. ^1H NMR ($CDCl_3$): $\delta = 2.39$ ppm (q, 2 H, $J = 7.4$ Hz), 3.04 (t, 2 H, $J = 7.4$ Hz), 3.18–3.23 (m, 2 H), 3.25 (m, 1 H), 3.44–3.49 (m, 2 H), 6.05 (t, 1 H, $J = 7.6$ Hz), 7.14–7.38 (m, 10 H). IR (KBr): $\tilde{\nu}$ = 2952 cm^{-1}, 2924, 2849, 2566, 1718, 1495, 1443, 1379, 1074, 761, 699. MS (CI): *m*/*z* (%): 308

Table 3
Uptake inhibition [IC_{50} (μM)] at GAT-1 and GAT-3 exhibited by azetidine derivatives.

								
	IC_{50} [μM]		IC_{50} [μM]		IC_{50} [μM]		IC_{50} [μM]	
R	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3
H	95.6%[c] ($n = 1$) **16a·HCl**	106%[d] ($n = 1$) **16a·HCl**	112%[e] ($n = 1$) **17a·HCl**	95.1%[f] ($n = 1$) **17a·HCl**	102%[a] ($n = 1$) **18a**	90.7%[b] ($n = 1$) **18a**	–	–
	–	–	–	–	26.6 ± 3.3[a] **18b**	48.5 ± 12.8[b] **18b**	–	–
	–	–	–	–	39.5 ± 3.2[a] **18c**	63.0 ± 28.5[b] **18c**	–	–
	–	–	–	–	77.5 ± 4.3[a] **18d**	60.0 ± 3.0[b] **18d**	–	–
	–	–	–	–	61.3%[e] ($n = 1$) **18e**	31.0 ± 4.7[f] **18e**	51.2[e] ($n = 1$) **10**	3.34[f] ($n = 1$) **10**

If not stated otherwise the results are given as means of IC_{50}-values ± SEM of three independent experiments each performed in triplicate, $n = 1$ denotes value from a single experiment, percentages represent specific binding remaining in presence of 100 μM inhibitor (1 mM in the case of **17a·HCl**). The abbreviations a–f specify the used biological material (see Experimental section).
Where "HCl" has been added to the compound number the compound has been tested as hydrochloride of the drawn neutral structure.

[$M^+ + 1 - HCl$] (20), 264 (100). $C_{20}H_{22}ClNO_2$ (343.8): calcd C 69.86, H 6.45, N 4.07; found C 69.65, H 6.59, N 4.14.

5.1.6. 1-[4,4-Bis(3-methylthien-2-yl)but-3-en-1-yl]azetidine-3-carboxylic acid hydrochloride (*12c·HCl*)

Hydrolysis according to GP1 starting from **39c** (108 mg, 0.299 mmol) in 2 ml MeOH and 2 N NaOH (0.371 ml, 0.75 mmol). Yield: 109 mg (95%); yellowish solid, mp 148 °C. ^{1}H NMR ($CDCl_3$): $\delta = 1.93$ ppm (s, 3 H), 1.99 (s, 3 H), 2.40 (q, 2 H, $J = 7.4$ Hz), 3.08 (t, 2 H, $J = 7.4$ Hz), 3.38 (qui, 1 H, $J = 8.2$ Hz), 3.73–3.94 (m, 2 H), 4.05–4.28 (m, 2 H), 5.94 (t, 1 H, $J = 7.4$ Hz), 6.73 (d, 1 H, $J = 4.9$ Hz), 6.83 (d, 1 H, $J = 4.9$ Hz), 7.05 (d, 1 H, $J = 4.9$ Hz), 7.21 (d, 1 H, $J = 4.9$ Hz). ^{13}C NMR APT ($CDCl_3$): $\delta = 14.3$ ppm, 14.8, 25.2, 34.5, 54.6, 56.3, 123.2, 124.8, 127.9, 129.8, 131.0, 131.4, 133.8, 134.3, 135.8, 138.4, 176.0. IR (KBr): $\tilde{\nu} = 3423$ cm^{-1}, 2921, 2853, 1601, 1383, 1221, 1083, 1005, 932, 834, 714. MS (CI): *m*/*z* (%): 348 [$M^+ + 1 - HCl$] (27), 114(100). $C_{18}H_{22}ClNO_2S_2$ (383.9): calcd C 56.31, H 5.78, N 3.65; found C 55.97, H 6.10, N 3.38.

5.1.7. 1-{2-[Tris(4-methoxyphenyl)methoxy]ethyl}azetidine-3-carboxylic acid hydrochloride (*12d*)

Hydrolysis according to GP2 starting from **39d** (53 mg, 0.11 mmol) in 0.5 ml MeOH and 2 N NaOH (0.138 ml, 0.27 mmol). Reaction time: 72 h. Yield: 50 mg (94%); colorless solid, mp 92 °C. ^{1}H NMR ($CDCl_3$): $\delta = 2.96$–3.01 ppm (m, 2 H), 3.37–3.42 (m, 2 H), 3.28–3.38 (m, 1 H), 3.34–3.40 (m, 2 H), 3.55–3.61 (m, 2 H), 3.76 (s, 9 H), 6.78–6.83 (m, 6 H), 7.25–7.30 (m, 6 H). IR (KBr): $\tilde{\nu} = 3429$ cm^{-1}, 2953, 2835, 1607, 1508, 1463, 1249, 1175, 1033, 827, 583. MS (CI): *m*/*z* (%): 333 (100), 227 (100), 145 (11), 101 (7). $C_{28}H_{31}NO_6$ (477.6): calcd C 70.42, H 6.54, N 2.93; found C 70.21, H 6.69, N 2.99.

*5.1.8. (RS)-Azetidin-2-ylacetic acid (**15a**) [44]*

20% Pd/C (8 mg) was added to a solution of **24** (37 mg, 0.15 mmol) in 1 ml MeOH. The mixture was subjected to hydrogen under ambient pressure at rt for 16 h and then filtrated. The solvent was evaporated. Purification by ion exchange chromatography (Dowex 50 W x 8, Fluka, eluent – saturated aqueous NH_3) and evaporation in vacuo afforded **15a**. Yield: 17 mg (~100%); colorless crystals. ^{1}H NMR (D_2O): δ = 2.18–2.30 ppm (m, 1 H), 2.39–2.51 (m, 1 H), 2.58–2.72 (m, 2 H), 3.78 (ddd, 1 H, J = 15.6, 10.1, 5.5), 3.89–3.99 (m, 1 H), 4.56–4.65 (m, 1 H). IR (Film): $\tilde{\nu}$ = 3424 cm^{-1}, 2971, 1573, 1400, 1264, 1075. MS (CI): m/z (%): 116 [M^+ + 1] (58), 100 (100), 98 (46).

*5.1.9. (RS)-1-(4,4-Diphenylbut-3-en-1-yl)azetidin-2-ylacetic acid hydrochloride (**15b·HCl**)*

TFA (1.2 ml) was added to a solution of **41b** (66 mg, 0.17 mmol) in 3 ml CH_2Cl_2 at 0 °C. The reaction mixture was stirred for 2 h at rt and acidified with HCl (0.6 ml, 2 N) followed by evaporation of the solvent. The crude product was washed with Et_2O (3 × 5 ml). Yield: 61 mg (~100%); colorless solid, mp 61 °C. ^{1}H NMR ($CDCl_3$): δ = 2.31–2.56 ppm (m, 4 H), 2.73–2.83 (m, 1 H), 2.84–2.97 (m, 1 H), 3.29–3.41 (m, 1 H), 3.42–3.54 (m, 1 H), 3.64–3.74 (m, 1 H), 4.15–4.25 (m, 1 H), 4.39–4.52 (m, 1 H), 6.02 (t, 1 H, J = 7.3 Hz), 7.11–7.16 (m, 2 H), 7.17–7.25 (m, 4 H), 7.31–7.49 (m, 4 H), 11.6 (s br, 1 H). ^{13}C NMR ($CDCl_3$): δ = 22.1 ppm, 24.8, 37.2, 50.7, 54.9, 65.8, 121.8, 127.2, 127.5, 128.2, 128.6, 129.5, 139.0, 141.4, 145.6, 171.3. IR (KBr): $\tilde{\nu}$ = 3425 cm^{-1}, 3023, 1725, 1671, 1494, 1444, 1199, 1136, 765, 702. MS (CI): m/z (%): 322 [M^+ + 1 – HCl] (6), 304 (54), 224 (100), 215 (76). HRMS (ESI–) for $C_{21}H_{22}NO_2$: calcd 320.1651; found 320.1663.

*5.1.10. (RS)-1-[4,4-Bis(3-methyl-2-thienyl)but-3-en-1-yl]azetidin-2-ylacetic acid (**15c**)*

TFA was added dropwise to a solution of **41c** (26 mg, 0.06 mmol) in 2 ml CH_2Cl_2 at 0 °C until a concentration of 20% (v/v) TFA was reached. The reaction mixture was stirred for 2 h at rt followed by evaporation of the solvent. Purification by CC (CH_2Cl_2/EtOH = 50:50) afforded **15c**. Yield: 16 mg (69%), colorless solid. TLC: R_f = 0.08 (CH_2Cl_2/EtOH = 50:50). ^{1}H NMR ($CDCl_3$): δ = 1.93 ppm (s, 3 H), 1.99 (s, 3 H), 2.36–2.56 (m, 4 H), 2.80–2.89 (m, 1 H), 2.98–3.10 (m, 1 H), 3.16–3.25 (dd, 1 H, J = 18.5, 9.2 Hz), 3.51–3.69 (m, 2 H), 4.23–4.34 (m, 1 H), 4.44–4.55 (m, 1 H), 5.92 (t, 1 H, J = 7.5 Hz), 6.75 (d, 1 H, J = 5.0 Hz), 6.86 (d, 1 H, J = 5.0 Hz), 7.07 (d, 1 H, J = 5.0 Hz), 7.23 (d, 1 H, J = 5.0 Hz). ^{13}C NMR APT ($CDCl_3$): δ = 14.2 ppm, 14.8, 21.8, 24.9, 51.3, 55.0, 65.7, 123.5, 124.8, 126.4, 130.2, 131.6, 132.1, 133.4, 134.7, 136.3. IR (KBr): $\tilde{\nu}$ = 3429 cm^{-1}, 2924, 2857, 2615, 1728, 1668, 1418, 1199, 834, 798, 721. MS (CI): m/z (%): 362 [M^+ + 1] (100), 344 (26), 218 (26), 184 (27), 128 (68). HRMS (CI) for $C_{19}H_{23}NO_2S_2$: calcd 361.1170; found 361.1195.

*5.1.11. 3-(1H-Tetrazol-5-yl)azetidin-3-ol hydrochloride (**16a·HCl**)*

Compound **30** (1.83 g, 4.28 mmol) was dissolved in 50 ml of MeOH. $Pd(OH)_2$/C (732 mg, 40%) was added. The mixture was stirred for 72 h at rt under hydrogen atmosphere. Following filtration and washing with water, the combined organic and water phase was concentrated. The residue was acidified using HCl (2 N). Following extraction with Et_2O, the water phase was concentrated to yield compound **16a·HCl**. Yield: 375 mg (49%); colorless solid, mp 174 °C. ^{1}H NMR (D_2O): δ = 4.41 ppm (d, 2 H, J = 12.5 Hz), 4.63 (d, 2 H, J = 12.5 Hz). ^{13}C NMR ($CDCl_3$): δ = 57.3 ppm, 66.1, 157.6. IR (KBr): $\tilde{\nu}$ = 2994 cm^{-1}, 2833, 2709, 1769, 1583, 1202, 1026, 696. MS (CI): m/z (%): 142 [M^+ + 1 – HCl] (100). $C_4H_8ClN_5O$ (177.6): calcd C 27.05, H 4.54, N 39.43; found C 26.82, H 4.50, N 39.43.

*5.1.12. 5-(Azetidin-3-yloxy)-1H-tetrazole hydrochloride (**17a·HCl**)*

As described for compound **16a·HCl** starting from **34** (0.22 g, 0.51 mmol) in 10 ml MeOH and 85 mg $Pd(OH)_2$/C (40%). Yield: 85.5 mg (94%); colorless solid, mp 130 °C. ^{1}H NMR (MeOH-d_4): δ = 4.30 ppm (dd, 2 H, J = 12.8, 4.7 Hz), 4.58 (dd, 2 H, J = 12.8, 6.7 Hz), 5.50 (tt, 1 H, J = 6.7/4.7 Hz). ^{13}C NMR (MeOH-d_4): δ = 52.0 ppm (CH_2), 70.3 (CH), 162.0. IR (KBr): $\tilde{\nu}$ = 3035 cm^{-1}, 2919, 2812, 2628, 2488, 1718, 1621, 1357, 1146, 1066, 733. MS (CI): m/z (%): 142 [M^+ + 1 – HCl] (100). HRMS (ESI+) for $C_4H_8N_5O$: calcd 142.0729; found 142.0713.

*5.1.13. Benzyl azetidine-1-carboxylate (**20**)*

Azetidine hydrochloride (**19**) (5.25 g, 56.1 mmol) was suspended in 100 ml CH_2Cl_2 and cooled to 0 °C. NEt_3 (23.4 ml, 168.3 mmol) was added followed by the addition of benzyl chloroformate (15.9 ml, 112.2 mmol). The reaction mixture was stirred for 72 h, acidified using HCl (2 N) and extracted with CH_2Cl_2 (4 × 100 ml). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by CC (n-heptane/EtOAc = 80:20) afforded compound **20**. Yield: 9.97 g (93%), colorless oil. TLC: R_f = 0.22 (n-heptane/EtOAc = 80:20). ^{1}H NMR ($CDCl_3$): δ = 2.23 ppm (quint, 2 H, J = 7.6 Hz), 4.03 (t, 4 H, J = Hz), 5.09 (s, 2 H), 7.27–7.39 (m, 5 H). IR (KBr): $\tilde{\nu}$ = 2958 cm^{-1}, 2888, 1709, 1415, 1354, 1128, 1174, 982, 751, 698. MS (CI): m/z (%): 192 [M^+ + 1] (31), 91(100). $C_{11}H_{13}NO_2$ (191.2): calcd C 69.09, H 6.85, N 7.32; found C 69.14, H 6.96, N 7.32.

*5.1.14. (RS)-Benzyl 2-(acetyloxy)azetidine-1-carboxylate (**21**)*

NaOAc (11.55 g) was dissolved in 200 ml of glacial acetic acid. Compound **20** (4.90 g, 25.6 mmol) was added. The reaction mixture was subjected to anodic oxidation for 40 h at a constant current of 0.6 A under exclusion of air (surface of Pt-electrode: 2 × 50 cm^2) while keeping the reaction at a constant temperature of 20 °C using water cooling. The reaction mixture was poured into water (100 ml) and extracted with CH_2Cl_2 (5 × 100 ml). The combined organic layers were washed with water (50 ml), dried ($MgSO_4$), and concentrated in vacuo. Purification by CC (n-heptane/EtOAc = 80:20) afforded compound **21**. Yield: 4.15 g (65%); colorless oil. TLC: R_f = 0.16 (n-heptane/EtOAc = 80:20). ^{1}H NMR ($CDCl_3$): δ = 2.07 ppm (s, 3 H), 2.16–2.26 (m, 1 H), 2.61–2.71 (m, 1 H), 3.83 (ddd, 1 H, J = 8.5/8.0/5.7 Hz), 3.97 (ddd, 1 H, J = 8.9/8.0/5.5 Hz), 5.10 (d, 1 H, J = 12.4 Hz), 5.17 (d, 1 H, J = 12.4 Hz), 6.36 (dd, 1 H, J = 6.4/3.6 Hz), 7.29–7.40 (m, 5 H). IR (KBr): $\tilde{\nu}$ = $\tilde{\nu}$ = 2970 cm^{-1} 2899, 1750, 1717, 1411, 1351, 1232, 1039, 753, 699. MS (CI): m/z (%): 250 [M^+ + 1] (1), 190 (100), 91 (73). $C_{13}H_{15}NO_4$ (249.3): calcd C 62.64, H 6.06, N 5.62; found C 62.64, H 5.97, N 5.58.

*5.1.15. (RS)-Benzyl (2-methoxycarbonylmethyl)azetidine-1-carboxylate (**22**)*

$TiCl_4$ (205 µl, 2.05 mmol, 1 M in CH_2Cl_2) was added dropwise to **21** (511 mg, 2.05 mmol) in 5 ml CH_2Cl_2 at −78 °C and the reaction mixture was stirred for 10 min. (1-Methoxy-1-vinyloxy)trimethylsilane (750 mg, 5.12 mmol) was added dropwise. The reaction mixture was stirred for 2 h at −78 °C and for 1 h at 0 °C. The resulting solution was transferred via syringe to a vigorously stirred saturated $NaHCO_3$ solution at 0 °C. The reaction mixture was extracted with CH_2Cl_2 (3 × 10 ml), dried ($MgSO_4$), and concentrated in vacuo. Purification by CC (n-heptane/EtOAc = 80:20) afforded compound **22**. Yield: 298 mg (55%); colorless oil. TLC: R_f = 0.12 (n-heptane/EtOAc = 80:20). ^{1}H NMR ($CDCl_3$): δ = 1.99–2.10 ppm (m, 1 H), 2.39–2.50 (m, 1 H), 2.69 (dd, 1 H, J = 15.9, 8.6 Hz), 2.94–3.06 (m, 1 H), 3.64 (s, 3 H), 3.86–4.00 (m, 2 H), 4.56–4.65 (m, 1 H), 5.09 (s, 2 H), 7.29–7.39 (m, 5 H). IR (KBr): $\tilde{\nu}$ = 2954 cm^{-1}, 2893, 1731, 1705, 1413, 1352, 1253, 1135, 753, 698. MS (CI): m/z (%): 264 [M^+ + 1] (80), 220 (63), 91 (100). $C_{14}H_{17}NO_4$ (263.3): calcd C 63.87, H 6.51, N 5.32; found C 63.50, H 6.66, N 5.25.

*5.1.16. (RS)-Benzyl (2-tert-butoxycarbonylmethyl)azetidine-1-carboxylate (**23**)*

As described for compound **22** starting from $TiCl_4$ (500 µl, 0.50 mmol, 1 M in CH_2Cl_2), **21** (250 mg, 1.00 mmol) in 2 ml

CH_2Cl_2 and (1-*tert*-butoxy-1-vinyloxy)trimethylsilane (450 mg, 2.83 mmol). Purification by CC (*n*-heptane/EtOAc = 80:20) afforded compound **23**. Yield: 168 mg (55%); colorless oil. TLC: $R_f = 0.22$ (*n*-heptane/EtOAc = 80:20). ^{1}H NMR (nitrobenzene d_5, 140 °C): $\delta = 1.47$ ppm (s, 9 H), 2.08–2.19 (m, 1 H), 2.34–2.45 (m, 1 H), 2.69 (dd, 1 H, $J = 15.4$, 8.6 Hz), 2.94 (dd, 1 H, $J = 15.4$, 4.5 Hz), 3.91–3.96 (m, 2 H), 4.57–4.65 (m, 1 H), 5.16 (s, 2 H), 7.18–7.42 (m, 5 H). IR (KBr): $\tilde{\nu} = 2975\ cm^{-1}$, 2892, 1726, 1709, 1411, 1351, 1148, 753, 698. MS (CI): *m*/*z* (%): 306 [$M^+ + 1$] (1), 250 (39), 206 (63), 91 (100). $C_{17}H_{23}NO_4$ (305.4): calcd C 66.86, H 7.59, N 4.59; found C 66.75, H 7.41, N 4.62.

5.1.17. (RS)-1-[(Benzyloxy)carbonyl]azetidin-2-ylacetic acid (**24**)

NaOH (1.25 ml, 2.5 mmol, 2 N) was added dropwise to **22** (0.26 g, 0.97 mmol) in 4 ml MeOH at 0 °C. The reaction mixture was allowed to warm to rt and was stirred for 72 h. The solvent was removed in vacuo. The crude product was suspended in water and extracted with Et_2O (5 ml). The water layer was acidified using HCl (2 N) and extracted with CH_2Cl_2 (3 × 10 ml). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Purification by CC (*n*-heptane/EtOAc = 80:20) afforded compound **24**. Yield: 205 mg (85%); colorless oil. TLC: $R_f = 0.13$ (*n*-heptane/EtOAc = 80:20). ^{1}H NMR ($CDCl_3$): $\delta = 2.00$–2.12 ppm (m, 1 H), 2.40–2.51 (m, 1 H), 2.73 (dd, 1 H, $J = 16.2$, 8.1 Hz), 2.98–3.11 (m, 1 H), 3.88–4.00 (m, 2 H), 4.56–4.65 (m, 1 H), 5.09 (s, 2 H), 7.28–7.39 (m, 5 H). IR (KBr): $\tilde{\nu} = 3319\ cm^{-1}$, 2963, 1706, 1540, 1420, 1355, 1283, 1139, 751, 697. MS (CI): *m*/*z* (%): 250 [$M^+ + 1$] (5), 206 (23), 91 (100). $C_{13}H_{15}NO_4$ (249.3): calcd C 62.64, H 6.07, N 5.62; found C 63.07, H 6.07, N 5.73.

5.1.18. (RS)-Benzyl 2-cyanoazetidine-1-carboxylate (**25**)

$TiCl_4$ (38 mg, 0.20 mmol) in 0.5 ml CH_2Cl_2 was added dropwise to a mixture of **21** (50 mg, 0.20 mmol) and TMSCN (60 mg, 0.56 mmol) in 1.5 ml CH_2Cl_2 at −70 °C. The reaction mixture was stirred for 2 h and allowed to warm up to rt. Workup and purification as described for compound **22** afforded compound **25**. Yield: 19.7 mg (46%); colorless oil. TLC: $R_f = 0.07$ (*n*-heptane/EtOAc = 80:20). ^{1}H NMR ($CDCl_3$): $\delta = 2.55$–2.72 ppm (m, 2 H), 4.03 (ddd, $J = 9.0/8.6/6.4$ Hz, 1 H), 4.13 (ddd, 1 H, $J = 9.0/8.3/5.9$ Hz), 4.83 (dd, 1 H, $J = 9.0/5.9$ Hz), 5.17 (s, 2 H), 7.30–7.41 (m, 5 H). ^{13}C NMR ($CDCl_3$): $\delta = 21.5$ ppm, 48.0, 67.5, 117.5, 135.7, 155.0, 128.1, 128.3, 128.5. IR (KBr): $\tilde{\nu} = 2968\ cm^{-1}$, 2895, 1715, 1409, 1349, 1136, 769, 698. MS (CI): *m*/*z* (%): 217 [$M^+ + 1$] (18), 190 (11), 91 (100). $C_{12}H_{12}N_2O_2$ (216.2): calcd C 66.65, H 5.59, N 12.95; found C 66.24, H 5.62, N 12.48.

5.1.19. (RS)-Benzyl 2-[2-(4-fluorophenyl)-2-oxoethyl]azetidine-1-carboxylate (**26**)

As described for compound **25** starting from $TiCl_4$ (75 mg, 0.40 mmol) in 0.1 ml CH_2Cl_2, **21** (100 mg, 0.40 mmol) and [1-(4-fluorophenyl)vinyloxy]trimethylsilane (0.17 g, 0.8 mmol) in 4 ml CH_2Cl_2. Workup and purification as described for compound **22** afforded compound **26**. Yield: 76 mg (58%); colorless oil. TLC: $R_f = 0.09$ (*n*-heptane/EtOAc = 80:20). ^{1}H NMR ($CDCl_3$): $\delta = 1.97$–2.09 ppm (m, 1 H), 2.49–2.61 (m, 1 H), 3.24 (dd, 1 H, $J = 16.7$, 9.5 Hz), 3.27–3.86 (m, 1 H), 3.90–4.07 (m, 2 H), 4.74–4.82 (m, 1 H), 5.09 (s, 2 H), 7.05–7.14 (m, 2 H), 7.28–7.40 (m, 5 H), 7.87–8.01 (m, 2 H). IR (KBr): $\tilde{\nu} = 2958\ cm^{-1}$, 2883, 1705, 1680, 1597, 1410, 1351, 1135, 834, 752, 698. MS (CI): *m*/*z* (%): 328 [$M^+ + 1$] (7), 177 (20), 91 (100). $C_{19}H_{18}FNO_3$ (327.4): calcd C 69.71, H 5.54, N 4.28; found C 69.35, H 5.54, N 4.19.

5.1.20. (RS)-tert-Butyl azetidin-2-ylacetate hydroacetate (**27**)

20% Pd/C (80 mg) and acetic acid (0.2 ml) were added to a solution of 20 (0.40 g, 1.3 mmol) in 5 ml MeOH. The mixture was subjected to hydrogen under ambient pressure at rt for 16 h and then filtrated. The solvent was evaporated. Yield: 184 mg (61%); colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 1.45$ ppm (s, 9 H), 2.04 (s, 3 H), 2.30–2.41 (m, 1 H), 2.51–2.61 (m, 1 H), 2.78 (dd, 1 H, J = 17.1, 6.8 Hz), 2.95 (dd, 1 H, J = 17.1, 7.2 Hz), 3.82 (ddd, 1 H, J = 15.1, 9.7, 5.1 Hz), 3.92–4.01 (m, 1 H), 4.63 (qui, 1 H, J = 7.6 Hz), 9.47 (s br, 2 H). ^{13}C NMR (APT) ($CDCl_3$): $\delta = 22.5$ ppm, 24.9, 28.0, 38.8, 42.3, 55.5, 81.8, 168.8, 177.2. IR (KBr): $\tilde{\nu} = 3414\ cm^{-1}$, 2980, 1722, 1556, 1410, 1370, 1161, 1016, 845. MS (CI): *m*/*z* (%): 231 [$M^+ + 1$] (16), 213 (100), 172 (6), 145 (8), 130 (42), 116 (81).

5.1.21. 1-Diphenylmethyl-3-[1-(4-methoxybenzyl)-1H-tetrazol-5-yl]azetidin-3-ol (**30**)

1-(4-Methoxybenzyl)tetrazol (1826 mg, 9.60 mmol) was dissolved in 48 ml of THF/TMEDA (10:1). *n*BuLi (6.0 ml, 1.6 M in hexane) was added dropwise at −90 °C to generate lithium compound **29** and the reaction mixture was stirred for 5 min. Following the addition of compound **28** (2278 mg, 9.60 mmol) in 10 ml THF and stirring for 30 min, the mixture was warmed to rt. The reaction was quenched with NH_4Cl_{sat} (20 ml). The mixture was extracted with EtOAc (3 × 100 ml), the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by CC (petrol ether/EtOAc = 70:30) afforded compound **30**. Yield: 2577 mg (63%); colorless solid, mp 143 °C. TLC: $R_f = 0.26$ (petrol ether/EtOAc = 70:30). ^{1}H NMR (benzene-d_6): $\delta = 3.18$ ppm (s, 3 H), 3.23 (d, 2 H, $J = 9.1$ Hz), 3.42–3.49 (s br, 1 H), 3.67 (d, 2 H, $J = 9.1$ Hz), 4.31 (s, 1 H), 5.11 (s, 2 H), 6.52–6.58 (m, 2 H), 6.95–7.03 (m, 4 H), 7.05–7.13 (m, 4 H), 7.32–7.38 (m, 4 H). IR (KBr): $\tilde{\nu} = 3269\ cm^{-1}$, 3025, 2955, 2835, 1733, 1612, 1514, 1252, 1029, 746, 704. MS (CI): *m*/*z* (%): 428 [$M^+ + 1$] (21), 238 (8), 167 (79), 121 (100). $C_{25}H_{25}N_5O_2$ (427.5): calcd C 70.24, H 5.89, N 16.38; found C 70.07, H 5.97, N 16.26.

5.1.22. 3-[1-(4-Methoxybenzyl)-1H-tetrazol-5-yl]azetidin-3-ol (**31**)

Compound **30** (0.21 g, 0.48 mmol) was dissolved in 10 ml AcOH. Pd/C (82.0 mg, 40%) and TFA (1 ml) were added. The mixture was stirred for 72 h at rt under hydrogen. Following filtration and washing with water, the combined methanol and water phases were concentrated and acidified with HCl (2 N). The water phase was extracted with CH_2Cl_2 (3 × 10 ml). Following alkalization using NaOH (2 N) and extraction with CH_2Cl_2 (3 × 10 ml), the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to afford compound **31**. Yield: 49.0 mg (39%); colorless solid, mp 158 °C. ^{1}H NMR ($CDCl_3$): $\delta = 3.50$–3.57 ppm (m, 2 H), 3.77 (s, 3 H,), 4.27–4.34 (m, 2 H), 5.67 (s, 2 H), 6.79–6.87 (m, 2 H), 7.15–7.22 (m, 2 H). IR (KBr): $\tilde{\nu} = 3263\ cm^{-1}$, 2977, 2943, 1613, 1515, 1202, 1253, 1031, 846, 776. MS (CI): *m*/*z* (%): 262 [$M^+ + 1$] (37), 121 (100). HRMS (ESI+) for $C_{12}H_{16}N_5O_2$: calcd 262.13039; found 262.1303. $C_{12}H_{15}N_5O_2$ (261.2): calcd C 55.16, H 5.79, N 26.80; found C 55.19, H 6.09, N 24.36.

5.1.23. 5-(1-Diphenylmethylazetidin-3-yloxy)-1-(4-methoxybenzyl)-1H-tetrazol (**34**)

Compound **32** (685 mg, 2.86 mmol) was dissolved in 25 ml THF. NaH (110 mg, 4.58 mmol, 1.6 equiv) was added and the mixture was stirred at rt until gas development ceased (approximately 2 h). A solution of **33** (904.8 mg, 2.86 mmol) in 5 ml THF was added dropwise and the mixture was stirred for 4 h at rt. The reaction was quenched with ice water (20 ml) and extracted with Et_2O (3 × 50 ml). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by CC (petrol ether/acetone = 80:20). Yield: 471 mg (39%); highly viscous oil. TLC: $R_f = 0.15$ (petrol ether/acetone = 80:20). ^{1}H NMR ($CDCl_3$): $\delta = 3.13$–3.19 ppm (m, 2 H), 3.68–3.74 (m, 2 H), 3.83 (s, 3 H), 4.39 (s, 1 H), 5.25 (s, 2 H), 5.29–5.35 (m, 1 H), 6.90–6.93 (m, 2 H), 7.19–7.24 (m, 2 H), 7.27–7.32 (m, 6 H), 7.39–7.43 (m, 4 H). IR (KBr): $\tilde{\nu} = 3025\ cm^{-1}$, 2954, 2835, 1609, 1563, 1514, 1250, 1029, 747, 704. MS (CI): *m*/*z* (%): 428 [$M^+ + 1$] (45), 222 (65), 167 (100). HRMS (EI+)

for $C_{25}H_{25}N_5O_2$: calcd 427.2008; found 427.1992. $C_{25}H_{25}N_5O_2$ (427.5): calcd C 70.24, H 5.89, N 16.38; found C 69.66, H 5.97, N 16.07.

*5.1.24. Methyl azetidin-3-carboxylate (**35**) [50]*

Thionylchloride (20.5 g, 172 mmol) was added to MeOH (50 ml) at −60 °C. After 30 min azetidin-3-carboxylic acid (250 mg, 2.47 mmol) was added and the resulting mixture was stirred for 16 h at rt. Removement of the solvent in vacuo afford compound **35** [50]. Yield: 364 mg (97%); yellow solid. ^{1}H NMR (CD_3OD): $\delta = 3.72-3.77$ ppm (m, 4 H), 4.19–4.31 (m, 4 H). IR (KBr): $\tilde{\nu} = 3443$ cm^{-1}, 2922, 2618, 2460, 1732, 1586, 1440, 1383, 1304, 1214, 1191, 1110, 1065, 1006, 920, 821, 741. MS (CI): *m*/*z* (%): 116 [$M^+ + 1$-HCl] (100).

*5.1.25. Methyl azetidin-3-ylacetate hydrochloride (**37**) [51]*

Esterification according to GP4 starting from 2-(1-*tert*-butyloxycarbonylazetidine-3-yl)acetic acid (**36**) [40] (100 mg, 0.46 mmol) in 2 ml MeOH and acetyl chloride (36 mg, 0.46 mmol). Purification by CC (CH_2Cl_2/EtOH = 50:50 + 0.5% AcOH). Yield: 44 mg (58%); colorless, hygroscopic solid. ^{1}H NMR ($CDCl_3$): $\delta = 2.80$ ppm (d, 2 H, $J = 7.8$ Hz), 3.25 (sep, 1 H, $J = 7.8$ Hz), 3.70 (s, 3 H), 3.83–3.93 (m, 2 H), 4.22–4.32 (m, 2 H), 9.71 (s br, 2 H). IR (KBr): $\tilde{\nu} = 3422$ cm^{-1}, 3092, 2977, 2954, 1725, 1541, 1432, 1294, 1210, 994, 869. MS (CI): *m*/*z* (%): 130 [$M^+ + 1 -$ HCl] (100).

*5.1.26. Methyl 1-(4,4-diphenylbut-3-en-1-yl)azetidin-3-carboxylate (**39b**)*

N-alkylation according to GP3 starting from **35** (158 mg, 1.04 mmol) in 5 ml THF, 4-bromo-1,1-diphenylbuten (**38b**) (300 mg, 1.04 mmol), K_2CO_3 (577 mg, 4.16 mmol) and NaI (5 mg, 0.03 mmol). The reaction mixture was refluxed for 48 h. Purification by CC (acetone/*n*-heptane = 30:70). Yield: 71 mg (21%); colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 2.13$ ppm (q, 2 H, $J = 7.4$ Hz), 2.52 (t, 2 H, $J = 7.4$ Hz), 3.18–3.23 (m, 2 H), 3.25 (qui, 1 H), 3.44–3.49 (m, 2 H), 3.69 (s, 3 H), 6.05 (t, 1 H, $J = 7.6$ Hz), 7.14–7.38 (m, 10 H). IR (KBr): $\tilde{\nu} = 2950$ cm^{-1}, 2837, 1736, 1493, 1436, 1199, 760, 701. MS (CI): *m*/*z* (%): 322 [$M^+ + 1$] (100). $C_{21}H_{23}NO_2$ (321.42): calcd C 78.47, H 7.21, N 4.36; found C 78.55, H 7.41, N 4.08.

*5.1.27. Methyl 1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl] azetidine-3-carboxylate (**39c**)*

N-alkylation according to GP3 starting from **35** (200 mg, 1.32 mmol) in 2 ml THF, 2-[4-bromo-1-(3-methylthien-2-yl)but-1-enyl]-3-methylthiophene (**38c**) (844 mg, 2.58 mmol) in 2 ml THF, K_2CO_3 (950 mg, 6.9 mmol) and NaI (5 mg, 0.03 mmol). The reaction mixture was refluxed for 16 h and subsequently stirred for 32 h at rt. CH_2Cl_2 (4 ml) was added. The precipitate was filtered off and washed with CH_2Cl_2. The filtrate was concentrated in vacuo. Purification by CC (*n*-heptane/EtOAc = 80:20 + 1% $NEtMe_2$). Yield: 242 mg (51%); yellowish oil. TLC: $R_f = 0.08$ (*n*-heptane/EtOAc = 80:20 + 1% $NEtMe_2$). ^{1}H NMR (benzene-d_6): $\delta = 1.96$ ppm (s, 3 H), 1.97 (s, 3 H), 2.12 (q, 2 H, $J = 7.1$ Hz), 2.25 (t, 2 H, $J = 7.1$ Hz), 2.92–3.00 (m, 1 H), 3.13–3.23 (m, 4 H), 3.29 (s, 3 H), 6.10 (t, 1 H, $J = 7.1$ Hz), 6.57 (d, 1 H, $J = 5.4$ Hz), 6.61 (d, 1 H, $J = 5.4$ Hz), 6.75 (d, 1 H, $J = 5.4$ Hz), 6.86 (d, 1 H, $J = 5.4$ Hz). IR (KBr): $\tilde{\nu} = 2949$ cm^{-1}, 2835, 1736, 1436, 1364, 1199, 1174, 712. MS (CI): *m*/*z* (%): 362 [$M^+ + 1$] (17), 128 (100). $C_{19}H_{23}NO_2S_2$ (361.5): calcd C 63.12, H 6.41, N 3.87, S 17.74; found C 62.83, H 6.38, N 3.87, S 17.65.

*5.1.28. Methyl 1-{2-tris(4-methoxyphenyl)methoxy]ethyl} azetidine-3-carboxylate (**39d**)*

N-alkylation according to GP3 starting from **35** (100 mg, 0.662 mmol) in 1.5 ml of CH_2Cl_2, 1-{(2-bromoethoxy)[bis(4-methoxyphenyl)]methyl}-4-methoxybenzene (**38d**) (347 mg, 0.76 mmol) in 1.5 ml of CH_2Cl_2 and K_2CO_3 (228 mg, 1.65 mmol). Reaction temperature: rt; reaction time: 48 h. Purification by CC (*n*-heptane/EtOAc = 20:80). Yield: 64 mg (20%); colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 2.65$ ppm (t, 2 H, $J = 5.5$ Hz), 3.08 (t, 2 H, $J = 5.5$ Hz), 3.27–3.36 (m, 1 H), 3.34–3.40 (m, 2 H), 3.55–3.61 (m, 2 H), 3.72 (s, 3 H), 3.79 (s, 9 H), 6.78–6.83 (m, 6 H), 7.28–7.33 (m, 6 H). IR (KBr): $\tilde{\nu} = 2951$ cm^{-1}, 2835, 1734, 1607, 1507, 1249,1174, 1034, 827, 583. MS (CI): *m*/*z* (%): 333 (84), 227 (100). $C_{29}H_{33}NO_6$ (491.6): calcd C 70.86, H 6.77, N 2.85; found C 70.59, H 6.99, N 2.89.

*5.1.29. Methyl 1-(4,4-diphenylbut-3-en-1-yl)azetidin-3-ylacetate (**40b**)*

N-alkylation according to GP3 starting from **37** (375 mg, 2.26 mmol) in 2.5 ml of DME, (4-bromo-1-phenylbut-1-enyl) benzene (**38b**) (800 mg, 2.80 mmol, 1.25 equiv) in 2.5 ml of DME, K_2CO_3 (1.25 g, 9.05 mmol, 4.0 equiv) and NaI (10 mg). Reaction temperature: rt; reaction time: 120 h. CH_2Cl_2 (10 ml) was added. The precipitate was filtered off and washed with CH_2Cl_2. The filtrate was extracted with water (3 × 5 ml). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by CC (*n*-heptane/EtOAc = 60:40 + 1% $NEtMe_2$). Yield: 204 mg (27%); colorless oil. TLC: $R_f = 0.12$ (*n*-heptane/EtOAc = 60:40 + 1% $NEtMe_2$). ^{1}H NMR ($CDCl_3$): $\delta = 2.13$ ppm (q, 2 H, $J = 7.6$ Hz), 2.50 (t, 2 H, $J = 7.6$ Hz), 2.56 (d, 2 H, $J = 7.1$ Hz), 2.72–2.82 (m, 3 H), 3.36–3.46 (m, 2 H), 3.64 (s, 3 H), 6.05 (t, 1 H, $J = 7.6$ Hz), 7.14–7.38 (m, 10 H). IR (KBr): $\tilde{\nu} = 2950$ cm^{-1}, 2814, 1737, 1496, 1437, 1193, 1173, 769, 702. MS (CI): *m*/*z* (%): 336 [$M^+ + 1$] (100), 142 (55). $C_{22}H_{25}NO_2$ (335.5): calcd C 78.77, H 7.51, N 4.18; found C 78.30, H 7.39, N 4.14.

*5.1.30. Methyl 1-{2-[tris(4-methoxyphenyl)methoxy]ethyl} azetidin-3-ylacetic acid (**40d**)*

N-alkylation according to GP3 starting from **37** (246 mg, 1.48 mmol) in 2.5 ml of THF, 1-{(2-bromoethoxy)[bis(4-methoxyphenyl)]methyl}-4-methoxybenzene (**38d**) (1.35 mg, 2.96 mmol) in 2.5 ml of THF, K_2CO_3 (821 mg, 5.94 mmol), and NaI (10 mg). Reaction temperature: rt; reaction time: 116 h. Workup as described for compound **39c**. Purification by CC (*n*-heptane/EtOAc = 60:40 + 1% $NEtMe_2$). Yield: 119 mg (16%); colorless oil. ^{1}H NMR (benzene-d_6): $\delta = 2.36$ ppm (d, 2 H, $J = 7.8$ Hz), 2.58 (t, 2 H, $J = 5.8$ Hz), 2.60–2.70 (m, 1 H), 2.82–2.86 (m, 2 H), 3.27 (s, 3 H), 3.28 (t, 2 H, $J = 5.8$ Hz), 3.31 (s, 9 H), 3.35–3.39 (m, 2 H), 6.78–6.73 (m, 6 H), 7.56–7.61 (m, 6 H). ^{13}C APT NMR (benzene-d_6): $\delta = 28.1$ ppm, 38.4, 50.9, 54.7, 59.7, 61.1, 63.1, 86.3, 113.4, 130.3, 137.6, 158.9, 172.2. IR (KBr): $\tilde{\nu} = 2951$ cm^{-1}, 2834, 1735, 1607, 1507, 1249, 1175, 1035, 827, 583. MS (CI): *m*/*z* (%): 506 [$M^+ + 1$] (68), 474 (100). $C_{30}H_{35}NO_6$ (505.6): calcd C 71.27, H 6.98, N 2.77; found C 71.38, H 7.47, N 2.69.

*5.1.31. (RS)-tert-Butyl 1-(4,4-diphenylbut-3-en-1-yl)azetidin-2-ylacetate (**41b**)*

N-alkylation according to GP3 starting from **27** (183 mg, 0.788 mmol) in 2.0 ml of THF, (4-bromo-1-phenylbutenyl)benzene (**38b**) (452 mg, 1.59 mmol) in 2.0 ml of THF, K_2CO_3 (444 mg, 3.21 mmol) and NaI (5 mg). Reaction temperature: rt; reaction time: 120 h. The solvent was evaporated. The crude product was extracted with CH_2Cl_2 (3 × 20 ml). The combined organic layers were washed with water (10 ml), dried ($MgSO_4$), and concentrated in vacuo. Purification by CC (*n*-heptane/EtOAc = 80:20 + 1% $NEtMe_2$). Yield: 104 mg (35%); colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 1.36$ ppm (s, 9 H), 1.72–1.82 (m, 1 H), 1.83–1.91 (m, 1 H), 2.14–2–28 (m, 3 H), 2.36 (dd, 1 H, $J = 15.0$, 7.3 Hz), 2.39–2.46 (m, 1 H), 2.50 (dd, 1 H, $J = 15.0$, 5.8 Hz), 2.68 (dt, 1 H, $J = 11.0$, 7.3 Hz), 3.14–3.21 (m, 1 H), 3.27–3.26 (m, 1 H), 6.17 (t, 1 H, $J = 7.3$ Hz), 7.02–7.14 (m, 4 H), 7.17–7.22 (m, 4 H), 7.30–7.34 (m, 2 H). ^{13}C NMR (benzene-d_6): $\delta = 23.9$, 27.8, 28.7, 42.8, 51.6, 58.3, 62.8, 79.4, 126.8, 128.3, 130.1, 140.5, 142.5, 143.1, 170.4. IR (KBr): $\tilde{\nu} = 2975$ cm^{-1}, 2929, 1725, 1493, 1444, 1367, 1153, 761, 701. MS (CI): *m*/*z* (%): 378

[$M^+ + 1$] (19), 322 (39), 262 (3), 184 (100), 128 (57). $C_{25}H_{31}NO_2$ (377.5): calcd C 79.54, H 8.28, N 3.71; found C 79.18, H 8.38, N 3.69.

*5.1.32. (RS)-tert-Butyl 1-[4,4-bis(3-methylthien-2-yl)but-3-en-1-yl]azetidin-2-ylacetate (**41c**)*

N-alkylation according to GP3 starting from **27** (200 mg, 0.86 mmol) in 2.0 ml of THF, 2-[4-bromo-1-(3-methylthien-2-yl) butenyl]-3-methylthiophene (**38c**) (573 mg, 1.75 mmol) in 2.0 ml THF, K_2CO_3 (484 mg, 3.51 mmol), and NaI (5 mg, 0.03 mmol). Reaction temperature: rt; reaction time: 48 h. Workup as described for compound **39c**. Purification by (*n*-heptane/EtOAc = 90:10 + 1% $NEtMe_2$). Yield: 57 mg (16%); yellowish oil. ^{1}H NMR (benzene-d_6): δ = 1.37 ppm (s, 9 H), 1.71–1.82 (m, 1 H), 1.83–1.92 (m, 1 H), 1.99 (s, 3 H), 2.00 (s, 3 H), 2.14–2.26 (m, 3 H), 2.36 (dd, 1 H, J = 15.2, 7.4 Hz), 2.37–2.47 (m, 1 H), 2.54 (dd, 1 H, J = 15.2, 5.8 Hz), 2.59–2.69 (m, 1 H), 3.15–3.21 (m, 1 H), 3.27–3.37 (m, 1 H), 6.17 (t, 1 H, J = 7.2 Hz), 6.59 (d, 1 H, J = 5.1 Hz), 6.62 (d, 1 H, J = 5.1 Hz), 6.76 (d, 1 H, J = 5.1 Hz), 6.87 (d, 1 H, J = 5.1 Hz). ^{13}C NMR APT (benzene-d_6): δ = 14.5 ppm, 14.9, 24.2, 28.1, 28.9, 43.1, 51.8, 58.0, 63.0, 79.6, 123.0, 124.6, 129.7, 131.3, 133.7, 134.4, 170.4. IR (KBr): $\tilde{\nu}$ = 2920 cm^{-1}, 2851, 1728, 1641, 1560, 1458, 1367, 1156, 711. MS (CI): *m*/*z* (%): 418 [$M^+ + 1$] (27), 362 (4), 184 (100), 128 (8). $C_{23}H_{31}NO_2S_2$ (417.6): calcd C 66.15, H 7.48, N 3.35, S 15.35; found C 66.24, H 7.40, N 3.33, S 15.06.

*5.1.33. (RS)-tert-Butyl 1-{2-[tris(4-methoxyphenyl)methoxy]ethyl} azetidin-2-ylacetate (**41d**)*

N-alkylation according to GP3 starting from **27** (300 mg, 1.29 mmol) in 6.0 ml of THF, 1-{(2-bromoethoxy)[bis(4-methoxyphenyl)]methyl}-4-methoxybenzene (**38d**) (1.6 g, 3.4 mmol), K_2CO_3 (1.21 g, 8.75 mmol), and NaI (10 mg, 0.060 mmol). Reaction temperature: rt; reaction time: 48 h. Workup as described for compound **39c**. Purification by (*n*-heptane/EtOAc = 90:10 + 1% $NEtMe_2$). Yield: 179 mg (25%); colorless oil. ^{1}H NMR ($CDCl_3$): δ = 1.37 (s, 9 H, C(CH_3)$_3$), 1.80–1.91 (m, 1 H, CH_2CH_2CHN), 1.91–2.00 (m, 1 H, CH_2CH_2CHN), 2.40 (dd, 1 H, J = 15.2/7.4 Hz, CHCH_2CO), 2.52–2.63 (m, 2 H, CHCH_2CO, NCH_2CH_2O), 2.73–2.81 (m, 1 H, NCH_2CH_2CH), 2.84–2.91 (m, 1 H, NCH_2CH_2O), 3.31 (s, 9 H, PhOCH_3), 3.22–3.37 (m, 11 H, NCH_2CH_2O, PhOCH_3), 3.42–3.52 (m, 2 H, NCH_2CH_2CH, CHCH$_2$CO), 6.77–6.84 (m, 6 H, CH_{ar}CO), 7.52–7.58 (m, 6 H, CH_{ar}CC). ^{13}C NMR APT (benzene-d_6): δ = 24.7 (CH_2CH_2CHN), 28.0 (C(CH_3)$_3$), 42.9 (CHCH$_2$CO), 53.4 (NCHCH$_2$CH$_2$), 54.6 (PhOCH_3), 58.3 (NCH_2CH_2O), 63.1 (NCH), 63.2 (NCH_2CH_2O), 113.2 (CH_{ar}CO), 130.1 (CH_{ar}CC), 137.4 (C_q), 158.7 (C_q), 170.5 (C=O). IR (KBr): $\tilde{\nu}$ = 2930 cm^{-1}, 2832, 1726, 1608, 1508, 1251,1175, 1153, 1037, 828. MS (CI, CH_5^+): *m*/*z* (%): 333 (100), 227 (9). $C_{33}H_{41}NO_6$ (547.7): calcd C 72.37, H 7.55, N 2.56; found C 72.94, H 8.17, N 2.54.

*5.1.34. 1-(3-Carbazol-9-ylpropyl)-3-[1-(4-methoxybenzyl)-1H-tetrazol-5-yl]azetidin-3-ol (**42e**)*

N-alkylation according to GP3 starting from **30** (275 mg, 0.92 mmol) in 5.0 ml of THF, 9-(3-bromopropyl)-9*H*-carbazole (**38e**) (266 mg, 0.92 mmol, 1.0 equiv), K_2CO_3 (382 mg, 2.76 mmol, 3 equiv), and NaI (10 mg). Reaction temperature: rt; reaction time: 96 h. Workup as described for compound **39c**. Purification by (petrol ether/acetone = 60:40). Yield: 149.5 mg (35%); colorless solid, mp 155 °C. ^{1}H NMR ($CDCl_3$): δ = 1.90 ppm (qui, 2 H, J = 7.1 Hz), 2.50 (t, 2 H, J = 7.1 Hz), 3.14 (d, 2 H, J = 9.4 Hz), 3.54 (d, 2 H, J = 9.4 Hz), 3.71 (s, 3 H), 4.33 (t, 2 H, J = 7.1 Hz), 5.39 (s, 2 H), 6.64–6.69 (m, 2 H), 7.21 (t, 2 H, J = 7.5 Hz), 7.35 (d, 2 H, J = 8.0 Hz), 7.42 (t, 2 H, J = 7.5 Hz), 8.06 (d, 2 H, J = 8.0 Hz). IR: $\tilde{\nu}$ = 3309 cm^{-1}, 3054, 2934, 2829, 1612, 1594, 1514, 1484, 1452, 1326, 1251, 1176, 1031, 825, 751, 724. MS (CI): *m*/*z* (%): 469 [$M^+ + 1$] (1), 279 (43), 121 (100). $C_{27}H_{28}N_6O_2$ (468.6): calcd C 69.21, H 6.02, N 17.94; found C 69.24, H 6.13, N 17.51.

*5.1.35. 1-(4,4-Diphenylbut-3-en-1-yl)-3-(4-methoxyphenyl) azetidin-3-ol (**18b**)*

N-alkylation according to GP3 starting from **18a** (183 mg, 1.02 mmol) in 5.0 ml of THF, (4-bromo-1-phenylbutenyl)benzene (**38b**) (293 mg, 1.02 mmol), K_2CO_3 (422 mg, 3.06 mmol), and NaI (5 mg, 0.03 mmol). Reaction temperature: rt; reaction time: 48 h. Workup as described for compound **39c**. Purification by (*n*-heptane/EtOAc = 50:50 + 5% $NEtMe_2$). Yield: 230 mg (58%); colorless solid, mp 53 °C. ^{1}H NMR ($CDCl_3$): δ = 2.22 ppm (q, 4 H, J = 7.4 Hz), 2.64 (t, 2 H, J = 7.3 Hz), 3.00 (s br, 1 H), 3.36 (d, 2 H, J = 8.7 Hz), 3.55 (d, 2 H, J = 8.7 Hz), 3.80 (s, 3 H), 6.09 (t, 1 H, J = 7.6 Hz), 6.87–6.92 (m, 2 H), 7.15–7.38 (m, 10 H), 7.40–7.45 (m, 2 H). IR (KBr): $\tilde{\nu}$ = 3052 cm^{-1}, 2947, 2832, 1610, 1581, 1513, 1442, 1245, 1173, 1032, 830, 758, 701. MS (CI): *m*/*z* (%): 386 [$M^+ + 1$] (34), 370 (100), 236 (79), 176 (25). $C_{26}H_{27}NO_2$ (385.5): calcd C 81.01, H 7.06, N 3.63; found C 80.89, H 7.17, N 3.64.

*5.1.36. 1-[4,4-Bis(3-methylthien-2-yl)but-3-en-1-yl]-3-(4-methoxyphenyl)azetidin-3-ol (**18c**)*

N-alkylation according to GP3 starting from **18a** (200 mg, 1.12 mmol) in 4.0 ml of THF, 2-[4-bromo-1-(3-methylthien-2-yl) butenyl]-3-methylthiophene (**38c**) (548 mg, 1.67 mmol), K_2CO_3 (463 mg, 3.4 mmol), and NaI (5 mg, 0.03 mmol). Reaction temperature: reflux; reaction time: 24 h. Workup as described for compound **39c**. Purification by (*n*-heptane/EtOAc = 60:40 + 1% $NEtMe_2$). Yield: 247 mg (52%); colorless solid, mp 93 °C. ^{1}H NMR (benzene-d_6): δ = 1.98 ppm (s, 3 H), 2.00 (s, 3 H), 2.25 (q, 2 H, J = 7.2 Hz), 2.43 (t, 2 H, J = 7.2 Hz), 3.15 (d, 2 H, J = 8.4 Hz), 3.34 (s, 3 H), 3.46 (d, 2 H, J = 8.4 Hz), 6.16 (t, 1 H, J = 7.2 Hz), 6.58 (d, 1 H, J = 5.1 Hz), 6.62 (d, 1 H, J = 5.1 Hz), 6.76 (d, 1 H, J = 5.1 Hz), 6.84–6.89 (m, 3 H), 7.59–7.64 (m, 2 H). IR (KBr): $\tilde{\nu}$ = 3412 cm^{-1}, 2930, 2833, 1611, 1514, 1456, 1247, 1177, 1034, 832, 713. MS (CI): *m*/*z* (%): 426 [$M^+ + 1$] (12), 408 (4), 192 (100). $C_{24}H_{27}NO_2S_2$ (425.6): calcd C 67.73, H 6.39, N 3.29, S 15.07; found C 67.78, H 6.32, N 3.29, S 14.96.

*5.1.37. 3-(4-Methoxyphenyl)-1-{2-[tris(4-methoxyphenyl) methoxy]ethyl}azetidin-3-ol (**18d**)*

N-alkylation according to GP3 starting from **18a** (100 mg, 0.56 mmol) in 5.0 ml of THF, 1-{(2-bromoethoxy)[bis(4-methoxyphenyl)]methyl}-4-methoxybenzene (**38d**) (0.26 mg, 0.56 mmol), K_2CO_3 (231 mg, 1.68 mmol), and NaI (5 mg, 0.03 mmol). Reaction temperature: reflux; reaction time: 48 h. Workup as described for compound **39c**. Purification by (*n*-heptane/EtOAc = 60:40 + 5% $NEtMe_2$). Yield: 92 mg (25%); colorless solid, mp 64 °C. ^{1}H NMR ($CDCl_3$): δ = 2.77 ppm (t, 2 H, J = 5.7 Hz), 3.15 (t, 2 H, J = 5.7 Hz), 3.50 (d, 2 H, J = 8.7 Hz), 3.73 (d, 2 H, J = 8.7 Hz), 3.77 (s, 9 H), 3.81 (s, 3 H), 6.78–6.83 (m, 6 H), 6.90–6.95 (m, 2 H), 7.30–7.35 (m, 6 H), 7.47–7.52 (m, 2 H). IR (KBr): $\tilde{\nu}$ = 3396 cm^{-1}, 2931, 2833, 1608, 1582, 1508, 1463, 1248, 1175, 1033, 827, 582. MS (CI): *m*/*z* (%): 180 [$M^+ + 1$] (60), 162 (100), 151 (17). $C_{34}H_{37}NO_6$ (555.7): calcd C 73.49, H 6.71, N 2.52; found C 73.29, H 6.93, N 2.36.

*5.1.38. 1-(3-Carbazol-9-ylpropyl)-3-(4-methoxyphenyl)azetidin-3-ol (**18e**)*

N-alkylation according to GP3 starting from **18a** (1.00 g, 5.58 mmol) in 10 ml of THF, 9-(3-bromopropyl)-9*H*-carbazole (**38e**) (1.61 mg, 5.58 mmol), K_2CO_3 (2.31 g, 16.7 mmol), and NaI (10 mg, 0.07 mmol). Reaction temperature: rt; reaction time: 72 h. Workup as described for compound **39c**. Purification by (*n*-heptane/EtOAc = 60:40 + 1% $NEtMe_2$). Yield: 1150 mg (53%); colorless solid, mp 47 °C. ^{1}H NMR (benzene-d_6): δ = 1.52 ppm (qui, 2 H, J = 6.5 Hz), 1.90–2.02 (s br, 1 H), 2.10 (t, 2 H, J = 6.5 Hz), 2.96 (d, 2 H, J = 7.9 Hz), 3.34 (s, 3 H), 3.41 (d, 2 H, J = 7.9 Hz), 3.98 (t, 2 H, J = 6.5 Hz), 6.88–6.92 (m, 2 H), 7.23 (t, 2 H, J = 7.3 Hz), 7.35 (d, 2 H, J = 8.3 Hz),

7.42 (t, 2 H, $J = 7.3$ Hz), 7.59–7.63 (m, 2 H), 8.06 (d, 2 H, $J = 8.3$ Hz). ^{13}C NMR (benzene-d_6): $\delta = 28.8$ ppm, 41.8, 56.4, 57.7, 70.7, 73.0, 110.7, 115.5, 120.8, 122.2, 125, 127.4, 128.0, 138.7, 142.6, 161.0. IR: $\tilde{\nu} = 3380\ cm^{-1}$, 3048, 2932, 2829, 1606, 1596, 1512, 1484, 1452, 1326, 1246, 1030, 830, 748, 723. MS (CI); m/z (%): 387 [$M^+ + 1$] (100), 369 (90), 236 (47), 151 (35). $C_{25}H_{26}N_2O_2$ (386.5): calcd C 77.69, H 6.78, N 7.25; found C 77.78, H 6.93, N 7.13.

5.1.39. Preparation of subcellular membrane suspensions

Two subcellular membrane pellets, termed bfcP2B (from bovine frontal cortex)[a] and bbsP2C (from bovine brain stem)[b], respectively, were prepared according to literature.[54] Their suspensions were prepared and measured as described by *Bradford*. [56] cfcP2B (from calf frontal cortex)[c] and cbsP2C (from calf brain stem)[d], pfcP2B (from porcine frontal cortex)[e] and pbsP2C (from porcine brain stem)[f] were applied alternatively instead of bfcP2B and bbsP2C, respectively.

5.1.40. Inhibition of GAT-1 mediated GABA-uptake

Aliquots of about 50–100 μg protein bfcP2B (alternatively cfcP2B or pfcP2B) were preincubated with 10 μM aminooxyacetic acid and a test compound in 200 μl of buffer (119 mM NaCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.7 mM KCl, 11 mM glucose and 25 mM Tris-HCl pH 7.2) for 10 min at 37 °C. Following the addition of 25 μl of 12.5 nM [^{3}H] GABA and 25 μl of 250 nM GABA, the sample was incubated at 37 °C for 4 min. The incubation was terminated by filtration in a Brandel M-24R Harvester through Whatman GF/C filters, which had been immersed in 0.9% NaCl for 1 h. The filters were washed with 0.9% NaCl (4 × 2 ml) and then measured in 3 ml of Rotiszint Eco Plus by the use of a Packard TriCarb 1600 Counter. Specific uptake was defined as difference between entire uptake and non-specific uptake, which was determined with identical samples lacking NaCl.

5.1.41. Inhibition of GAT-3 mediated GABA-uptake

Aliquots of about 50–100 μg protein bbsP2C (alternatively cbsP2C or pbsP2C) were preincubated with 10 μM aminooxyacetic acid, 10 μM NNC-711 and a test compound in 200 μl of buffer (119 mM NaCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.7 mM KCl, 11 mM glucose and 25 mM Tris-HCl pH 7.2) for 10 min at 37 °C. The addition of 25 μl of 50 nM [^{3}H] GABA and 25 μl of 1 μM GABA was followed.

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