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Structure-function evaluation of imidazopyridine derivatives selective for δ subunit containing γ-aminobutyric acid type A ($GABA_A$) receptors

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$GABA_A$ receptors; δ-selectivity; DS1; DS2; structure-activity relationship

ABSTRACT: Delta selective compounds 1 and 2 (DS1, compound **22**; DS2, compound **16**) were introduced as functionally selective modulators of δ-containing GABA receptors type A ($GABA_AR$). In our hands [^{3}H]EBOB binding experiments with recombinant $GABA_AR$ using compound **22**, showed no proof of δ-selectivity, though a minimally higher preference with respect to potency for α4β3δ and α6β2/3δ receptors. In order to delineate the structural determinant for δ-preference, we synthesized 25 derivatives of DS1 and DS2, and investigated their structure-activity relationship (SAR). Four of our derivatives showed selectivity for α6β3δ receptors (**29**, **38**, **39**, **41**). All of them possess a variation at the *para*-position of the benzamide group as the major difference to compound **22**. However, two compounds (**29**, **39**), when tested in the presence of GABA, revealed an effect at several additional $GABA_AR$. The newly synthesized compounds will still serve as useful tools to investigate α6β3δ receptors.

INTRODUCTION

The main inhibitory neurotransmitter system in the brain, the γ-aminobutyric acid (GABA) system, with its predominant GABA receptors type A ($GABA_AR$) system is a target for several molecules, including benzodiazepines, neurosteroids, barbiturates, propofol, volatile anesthetics and, putatively, ethanol.[1-6] Their action leads to anxiolytic, sedative, memory-modifying, anticonvulsant, as well as hypnotic effects.

$GABA_AR$ assemble as heteropentameric complexes from a variety of subunits (α1-6, β1-3, γ1-3, δ, ε, θ, π and ρ1-3).[2] There are two different forms of inhibition mediated by $GABA_AR$. The subunit composition differs with different synaptic localization as well as type of inhibition mediated. Synaptic receptors, typically composed of two α, two β and one γ subunit, lead to phasic inhibition, whereas tonic inhibition is mediated mostly by peri- and extrasynaptic δ-containing receptors.[7] These receptors are characterized by their enhanced GABA sensitivity and reduced desensitization properties and can be regarded as prototypic extrasynaptic receptors at ambient GABA concentrations.[7] Extrasynaptic $GABA_AR$ containing the δ subunit are found in the cerebellum, thalamus, olfactory bulb, cortex and hippocampus,[8-9] where the δ subunit is frequently co-distributed with α4 and α6 subunits.[9] The $GABA_AR$ subtypes α6β2/3δ are expressed at high levels exclusively by mature cerebellar granule neurons.[10-11] In forebrain areas, e.g., in thalamic relay cells, neostriatum, dentate gyrus and some layers of the cortex, δ subunits are combined with α4 and β2/3 subunits.[12-14] The existence of α4β1δ receptors is so far only postulated and still under investigation.[15] In hippocampal interneurons tonic inhibition seems to be also conveyed via α1βδ receptors.[16] The major fraction of non-δ-containing receptors found in areas other than synapses may be constituted by α5βγ2-type receptors in the hippocampus.[7, 17]

In contrast to γ2-containing $GABA_AR$, which follow a strict arrangement order,[18-19] δ-containing receptors may assemble less stringently.[20-22] Although the occurrence of δ-containing $GABA_AR$ in different brain areas is well documented, the co-assembly and composition of these receptors, native or recombinant, as well as their detailed function is not completely understood. These receptors may be linked to several disorders like alcoholism, stroke, depression, epilepsy, schizophrenia and traumatic brain injury.[23-24] In addition to the occurrence of δ-containing $GABA_AR$ in the brain, δ subunit protein and mRNA was detected in the lamina II tissue of the spinal cord, where they could play a role in analgesia.[25-27]

Taken together, the uncertainties of δ-containing $GABA_AR$ concerning physiological processes, pharmacological interactions, assembly order and partnering subunits demand novel pharmacological tools to differentiate these receptors from other subtypes *in vitro* and *in vivo*. Although many ligands, such as general anesthetics, neurosteroids and gaboxadol show enhanced potency at δ-containing $GABA_AR$, especially when partnered with α4/6 and β3 subunits,[14] their selectivity is insufficient for detailed investigations. Later, the imidazopyridines DS1 and DS2 (Delta Selective compounds 1 and 2) were introduced by Wafford et al.

in 2009. In electrophysiological experiments, using human recombinant $GABA_AR$, the authors showed δ-selectivity for both compounds in combination with α4 and β3 subunits.[28] Later experiments revealed a GABA-enhancing effect of DS2 at additional δ-containing receptors (α1β2δ, α4β1/2/3δ, α6β2δ), while numerous other $GABA_AR$ remained unaffected.[29] However, the site of action for DS1 and DS2 is still unknown.[29]

In the present study we used [^{3}H]ethynylbicycloorthobenzoate ([^{3}H]EBOB) binding to rat recombinant $GABA_AR$ as an assay for the modulatory effect on $GABA_AR$ function of the studied compounds. [^{3}H]EBOB as well as *t*-[^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS), both so called "cage convulsants", are potent non-competitive $GABA_AR$ antagonists binding to the picrotoxinin binding site within the $GABA_AR$ ion channel.[30-31] The binding of [^{3}H]EBOB and [^{35}S]TBPS is sensitive to conformational changes in the chloride channel, e.g. being reduced by increasing concentrations of the agonist GABA.[32-35] This effect is potentiated by modulators such as benzodiazepines and neurosteroids, or in turn, reversed by inverse agonists.[33, 35-39] Allosteric modulation of [^{3}H]EBOB or [^{35}S]TBPS binding is also shown in the absence of GABA, indicating a direct effect on receptor conformation.[32, 35, 38-40]

The correlation between allosteric modulation of $GABA_AR$ binding of the cage convulsants and ionophore function has been shown numerous times, mostly for [^{35}S]TBPS,[32, 41-43] but also for [^{3}H]EBOB.[31, 33, 40] The pharmacological profile of allosteric agents for [^{35}S]TBPS and [^{3}H]EBOB binding modulation is very similar and therefore comparable.[31, 34-35]

In initial [^{3}H]EBOB binding experiments we experienced that DS1 (compound **22**, Figure 1) recognizes receptor subtypes additional to α6βδ. In electrophysiological experiments this was also shown for DS2 (compound **16**, Figure 1), although, in both cases, with higher effects on δ-containing receptors. Thus, we set out to identify the pharmacophore of these δ-preferring candidates. A systematic structure-activity relationship (SAR study) was performed by synthesis of 25 derivatives of DS1 and DS2 (Figure 1), followed by their characterization in radioligand binding assays employing the ability of these ligands to modulate the [^{3}H]EBOB binding.

We identified important positions in the chemical scaffold of DS1/DS2 for modulation of [^{3}H]EBOB binding and provide evidence that the α6 subunit is more critical than the δ subunit. Our SAR study shows that replacing the chloride of the original DS1 molecule by a fluorinated or non-fluorinated butoxy group led to novel, now truly δ-selective modulators of [^{3}H]EBOB binding using rat recombinant $GABA_AR$.

RESULTS AND DISCUSSION

Chemistry. The synthesis of *N*-[2-arylimidazo[1,2-*a*]pyridin-3-yl]amides (**16**–**36, 41**–**44**) was typically achieved in a two-step procedure. In the first step, the respective 2-aryl-imidazo[1,2-*a*]pyridin-3-amines (**1**–**11**) were synthesized by multicomponent reactions starting from commercially available 2-amino-pyridines and aromatic aldehydes, cf. Scheme 1.

Amines **1**–**7** were synthesized using a modified version of the multicomponent reaction reported by van Niel et al. for the preparation of 8-methyl-2-thiophen-2-yl-imidazo[1,2-*a*]pyridine-3-ylamine (Method A, Scheme 1).[44] The aldehyde is converted into the bisulfite adduct with $Na_2S_2O_5$ and then refluxed with the respective 2-amino-pyridine to form the corresponding Schiff base. Addition of KCN to the reflux followed by slowly cooling to room temperature precipitated the desired amines, which were collected and further purified (yield range 41-68%). Another version of this reaction was used for the synthesis of amines **10** and **11** (Method B, Scheme 1). Here, the reaction was performed in ethanol/water with added $BiCl_3$ to enhance the electrophilicity of the aldehyde. However, amine **8** could not be derived with the above procedures. The double bromination of the 2-aminopyridine starting material likely results in reduced nucleophilicity of the amine group and poor solubility in aqueous solution. In a first attempt, amine **8** was synthesized in a mixture of water/ethanol (2.25/1) and refluxing for several hours after KCN addition (Method C, Scheme 1), but yields ranged between 9% and 17%. Therefore, the approach by Bienaymé et al. was envisaged for this compound (Method D, Scheme 1).[45] Bienaymé et al. use isocyanides instead of KCN to form secondary 2-aryl-imidazo[1,2-*a*]pyridin-3-amines in methanol at room temperature. The use of *tert*-butyl isocyanide should allow acidic removal of the *tert*-butyl group afterwards. The *tert*-butyl protected intermediate was isolated in an acceptable yield (52%) and could be deprotected quantitatively to the desired amine **8** using 5 M hydrobromic acid. Amine **9** was obtained from **6** and 1,3-dibromobutane in a nucleophilic substitution step using K_2CO_3 as base (Method E, Scheme 1).

The second step in the reaction sequence was the amide bond formation (Scheme 2). The amines **1**-**11** were reacted with the respective acid chlorides in a mixture of toluene and pyridine at room temperature giving the desired *N*-[2-arylimidazo[1,2-*a*]pyridin-3-yl]amides (**16**–**36**, **41**–**44**) in good yields (60-80%). Attempts to form the amide bond via standard coupling protocols (HBTU/TBTU, PPA, IBCF) failed due to the low nucleophilicity of the aromatic amine. Compounds with a free hydroxyl group (**37**, **40**) were synthesized either from the acetoxy protected precursor **30** or from the TBDPS protected compound **36** (Scheme 3). Removal of the acetoxy group was accomplished with 5 M NaOH in THF at room temperature. Cleavage of the TBDPS group was achieved with a 1 M solution of tetrabutyl ammonium fluoride. Both reactions provided the desired products in excellent yields (88-92%). Compound **37** was used for further derivatization. It was reacted in typical Williamson ether syntheses to form products **38** and **39** with a terminal fluorine as analogues to compound **29** (Scheme 4).

Screening for δ direct acting DS derivatives. In order to identify the structural characteristics of a directly acting, δ-selective [^{3}H]EBOB modulator on $GABA_AR$, the binding assay was initially executed in the absence of GABA. Promising candidates were then tested in the presence of GABA, thus mimicking a more physiological condition.

At first, the compounds were tested on α6β3δ $GABA_AR$, one of the two δ-containing receptors in cerebellar granule cells of the mammalian brain, the other being the α6β2δ receptor.[10-11] If no [^{3}H]EBOB binding modulation in concentrations up to 10 μM was observed the compounds were not further analyzed. Promising candidates displaying effects on α6β3δ $GABA_AR$ (Table 1, data highlighted in bold) were tested at α1-, α4- and α6-containing receptors, as *in vivo* δ subunits only occur together with these three α subunits in addition to any of the three β variants.[11, 13, 15-16]

Structure-Activity Relationship. All studied compounds possess an imidazopyridine moiety as the core structure. The lead compounds **16** and **22** were modified at three sites, i.e., the thiophene group, the chlorinated benzamide group and the bromo substituents at the pyridine moiety to investigate the structure-activity relationship. A broad range of derivatives (**16**-**21**, **23**-**27**, **30**, **32**-**33**, **40**, **42**-**44**), including the published compound **16** could be dismissed after the first testing on α6β3δ $GABA_AR$ as they showed no effect in the absence of GABA.

Compounds lacking the thiophene group (**44**) or having it replaced by a phenyl (**17**, **19**), a *p*-methoxyphenyl (**24**), a bromopropoxyphenyl (**25**), a furanyl (**42**) or a methylthiophene group (**43**) showed no [^{3}H]EBOB binding modulation. This indicates an essential role for the thiophene group for [^{3}H]EBOB binding modulation.

Compounds with less than two bromide atoms (**16**, **18**, **29**, **33**) showed no or little activity, while their double-brominated counterparts (**22**, **31**) were potent modulators with IC_{50} values of 0.31 and 0.86 μM, respectively (Table 1). The bromination state seems to play a major part in the potency of [^{3}H]EBOB binding modulation (see section below). Replacement of a bromide atom by a chloride atom (**27**, **32**) led to loss of activity, indicating the crucial role of this site.

The third site in the core scaffold of DS1 investigated in more detail was the benzamide group, in particular the *para*-position of the benzamide group. Compound **26,** possessing an acetamide group instead of the benzamide group showed no effect, indicating that the benzamide group is indeed crucial for action. Interestingly, even with only one bromide substituent, compound **28** with a cyclohexylcarboxamide group instead of the benzamide group was able to modulate [^{3}H]EBOB binding, but only with a negligible potency (Table 1, IC_{50} = 10.1 ± 2.8 μM). For the group of analogs where only the *para*-position of the benzamide group is varied, a fluoro atom (**35**) but neither a methoxy (**23**) nor a fluoroethoxy (**34**) group is tolerated. However, elongating the carbon chain to a propoxy (**38**) or butoxy (**29**, **39**) group, fluorinated in two cases (**38**, **39**), seem to be beneficial for activity and selectivity for δ-containing $GABA_AR$ in [^{3}H]EBOB binding assays (Table 1). Compound **41** bears a benzoxy group at this

position and showed the lowest modulatory effect of the δ-selective compounds with no measurable IC_{50} value and an observable effect only at 10 μM (max. inhibition = 54 ± 11%), which may simply due to absence of bromide atoms. For the rest of the active compounds, **31**, **35** and **22** no δ-selectivity but a preference for most δ-containing $GABA_AR$ as revealed by their differential potency (Table 1) was observed. However, in case of compound **31** these effects were not significant.

The detailed SAR analysis revealed that a structural scaffold consisting of an imidazopyridine with at least one bromo substituent in the pyridine part and a thiophene and benzamidine group connected to the imidazo part were crucial for direct modulation of [^{3}H]EBOB binding. The chloro substituent in the *para*-position of the benzamide group proved to be exchangeable by specific moieties, but was still important for [^{3}H]EBOB binding modulation and partly responsible for δ-selectivity.

Modulation of [^{3}H]EBOB binding by compounds 22 and 16 and comparison with electrophysiological data. As we primarily searched for direct modulators, compound **16** was dismissed as a candidate for further investigation as no direct [^{3}H]EBOB modulation was observed (max. inhibition = 20 ± 10 % at 10 μM). In contrast, compound **22** showed a robust modulation of recombinant δ-containing $GABA_AR$ in [^{3}H]EBOB binding experiments (Table 1). The assumption of a direct effect by compound **22** and a GABA-modulatory effect of compound **16** is in agreement with previously published, electrophysiological data.[28] However, in [^{3}H]EBOB binding assays with additional recombinant $GABA_AR$, we experienced a preference, but no selectivity for δ-containing receptors in terms of potency (Table 1, Figure 2). Comparison of α4β3δ with other α4-containing receptors showed increased IC_{50} values of 7-fold ($p < 0.005$) for α4β3γ2 and 4-fold for α4β3 receptors (difference nearly reaching significance with $p = 0.051$) (Figure 2B). In case of α6- and β2-containing receptors the increase was 4- and 7-fold ($p < 0.005$ for α6β2 and α6β2γ2) compared to α6β2δ $GABA_AR$ (Figure 2D). The smallest difference in potency was observed for α6- and β3-containing receptors with only a 2.5- to 3-fold shift compared to α6β3δ receptors ($p < 0.005$ for α6β3, $p < 0.05$ for α6β3γ2) (Figure 2C). Although the potency of compound **22** for δ-containing $GABA_AR$ was for the most part significantly higher, no differences in efficacy between δ- and non-δ-containing receptors could be measured (Table 1, Figure 2). A slight modulation of [^{3}H]EBOB binding in α1-containing receptors by **22** was only observed at the highest concentration (10 μM) (Figure 2A). Compound **22** was the most potent modulator of [^{3}H]EBOB binding of all tested compounds, however, a 2.5-7-fold preference for δ-containing receptors might be insufficient for investigational use.

The results of [^{3}H]EBOB binding modulation by **22** contradict the reported δ-selectivity of this compound in previously published electrophysiological tests.[28] Still, recent findings from Ahring et al. support our results. These findings also indicate a lack of selectivity of **22**, as the compound modulated the activity of human recombinant α4β2γ2 receptors in the presence of GABA, as shown electrophysiologically.[46] In order to allow a better comparison between published electrophysiological data and our findings from [^{3}H]EBOB binding assays, we also performed patch-clamp experiments of the lead compounds on rat

recombinant $GABA_AR$. There is no major publication that hints to crucial differences in the function of human as compared to rat $GABA_AR$ subunits. Thus, this difference between our and previously reported data cannot be regarded as a potential pitfall. Additionally, we were able to further investigate compound **16**, which did not show any effect in [^{3}H]EBOB binding modulation. This approach supported our results from [^{3}H]EBOB binding modulation, as compound **16** was unable to sufficiently activate recombinant $GABA_AR$ in the absence of GABA (Table 2, Figure 3A). Even at a concentration of 10 μM, **16** only slightly activated α4/6β3 and α4/6β3δ $GABA_AR$. In the presence of a GABA EC_{20}, compound **16** modulated α1/4/6β3δ receptors, as well as α6β3 ones (Table 3). These results confirm the assumption of compound **16** being solely a positive allosteric modulator,[28] but also challenge the reported δ-selectivity.[29] Consistent with the [^{3}H]EBOB binding data compound **22** induced robust currents in the patch-clamp experiment in the absence of GABA (Table 2, Figure 3B). However, no δ-selectivity was observed as the compound was highly efficient at α4/6βδ receptors, as well as at α6β3 and α6β3γ2. The only discrepancy to [^{3}H]EBOB binding modulation data is the lack of activation of α4β3 and α4β3γ2 receptors in electrophysiological experiments. In the presence of a GABA EC_{20}, the effect of compound **22** on not δ-containing receptors was even more pronounced (Table 3). Especially the binary receptors α1/4/6β3 exhibited similar EC_{50} values as the corresponding δ-containing ones.

δ-selectivity of compound 29 and effect of bromo substituents on potency and efficacy. Compound **29** with its butoxy moiety attached to the benzamide group showed selectivity for α6β2/3δ receptors with IC_{50} values of 2.18 ± 0.40 μM and 2.43 ± 0.78 μM, respectively with no measurable potency on α6β2/3 or α6β2/3γ2 receptors (Figure 4A,B). However, a marginal modulation of α6β3γ2 receptors could be observed at a concentration of 10 μM (10 ± 4%). [^{3}H]EBOB binding in $GABA_AR$ containing α1 and α4 subunits was unaffected (Table 1).

Compound **31** represents the double brominated derivative of **29.** Compared to compound **29** it showed an increased potency and efficacy on α6β3δ receptors (IC_{50} = 0.86 ± 0.30 μM; max. inhibition = 71 ± 7%). However, in contrast to **29** it modulated the [^{3}H]EBOB binding of other α6- and β3-containing receptors (Table 1). Compound **33** as the non-brominated derivative of **29** was devoid of any activity. The dissimilar results for these three compounds, only differing in the bromination state, underline its crucial role in the action on $GABA_AR$.

With the butoxy moiety not being exclusively responsible for δ-selectivity and bromination probably being a key factor for potency, the selectivity of compound **29** could be explained by concentration, obscuring the effect on other receptors in the low micromolar range. Nevertheless, in [^{3}H]EBOB binding assays compound **29** remains selective up to 20 μM which approaches the solubility limit. This notion is further supported by the loss of selectivity of this compound in the presence of GABA (see section below).

Effect of GABA on δ-selectivity of DS derivatives 22, 29 and 39. [^{3}H]EBOB binding experiments were performed in the presence of GABA for three compounds (**22**, **29**, **39**). Surprisingly, the potent effect of compound **22** on [^{3}H]EBOB binding on nearly all α6- and β3-containing receptors tested was independent of GABA in the test solution (Table 4). Similar findings have previously been described in electrophysiological experiments of **22** at α4β3δ receptors.[28]

Compounds **29** and **39** were both selective for α6β3δ receptors in comparison to the α6β3 and α6β3γ2 ones in the absence of GABA. However, both lost their selectivity in the presence of GABA (Table 4). While the modulatory effect on α6β3 and α6β3γ2 receptors was enhanced in the presence of GABA, no significant effect on α6β3δ receptor modulation was observed, neither in potency nor in efficacy. In the presence of GABA, compound **29** modulated α6β3 receptors to a maximal effect of 26 ± 6% and α6β3γ2 receptors to 40 ± 5%, compound **39** modulated α6β3 receptors to a maximal effect of 57 ± 11% and α6β3γ2 receptors to 44 ± 7%. The effect of GABA on the efficacy of both compounds was highly significant (Table 4).

The fact that both compounds modulate additional receptors in presence of GABA may be explained in several ways. One possibility is that GABA activates receptors and leads to an IC_{50} shift and thus visualizes an effect that is obscured in its absence. That is a reasonable possibility for low affinity ligands like **29** where a further shift in potency between α6β3δ receptors and other α6- and β3-containing receptors would result in IC_{50} values approaching the solubility limit. Alternatively or additionally, GABA changes the receptor conformation in such a way that it enables the compounds to enter the now available binding site. As a third option, selective compounds may bind to α6β3 and α6β3γ2 receptors in the absence of GABA but lack the ability to directly activate these receptors, thus they are positive allosteric modulators solely increasing the affinity for GABA. Differentiating between these possibilities was beyond the scope of this series of experiments.

Question of binding site and binding properties. The modulatory effect of the DS derivatives is clearly dependent on the α variant of $GABA_AR$. Receptors containing α6 subunits are preferred by compound **22** over α4-containing ones with regard to potency (Table 1). However, this effect was only significant for α4/6β3γ2 receptors ($p = 0.008$). The δ-selective compounds **29** and **39** exclusively modulated α6-containing receptors (Table 1). None of the tested compounds was able to modulate [^{3}H]EBOB binding to α1-containing receptors. The β subunit seems to play a minor role, though for compound **39** selectivity for δ-containing receptors was only observed in combination with the β3 variant (Table 1, Figure 4C,D). For this compound the potency for α6β2δ receptors (0.27 ± 0.02 μM) was increased 3-fold ($p < 0.005$) compared to α6β3δ receptors (0.76 ± 0.08 μM). For compound **22** the effect was similar (0.15 ± 0.03 μM vs. 0.31 ± 0.04 μM) with a 2-fold higher ($p < 0.05$) potency on α6β2δ receptors. No significant difference could be measured for the α6β2/3 ($p = 0.185$) and α6β2/3γ2 ($p = 0.486$) receptor pairs. Further, compound **22** did not differentiate between β2/3-containing receptors regarding its efficacy (Table 1). Compound **29** did not distinguish between α6β2/3δ receptors, neither in potency ($p = 0.812$) nor in efficacy ($p = 0.615$). The significantly ($p < 0.05$)

increased efficacy of compound **39** for α6β2 receptors (33 ± 4%) in comparison to α6β3 receptors (11 ± 6%), and the shift in potency for δ-containing receptors by compounds **22** and **39**, indicates a slight influence of the β subunit on modulatory properties of the mentioned compounds.

Compounds **22**, **28**, **35**, **31** modulated [^{3}H]EBOB binding also in the absence of a δ subunit, but with a preference for most δ-containing receptors (Table 1), however, these effects were not significant for compound **31**. Compounds **29**, **38**, **39**, **41** showed selectivity in the absence of GABA for α6β3δ receptors and in the case of **29** for α6β2δ receptors as well (Table 1, Figure 4). These results lead to the question whether non-selective compounds address the same, an additional or a completely different binding site as δ-selective compounds. An additional binding site would explain the loss of selectivity for certain substances, with one binding site located on the δ subunit and an additional one located on another part of the receptor complex. The two binding site model could as well extend the three possibilities to explain the differences of selectivity in the absence and presence of GABA, i.e., one binding site being GABA-mimetic and thus enabling access to the modulatory site.

Regarding the high efficacy (84 ± 8%) and potency (0.31 ± 0.04 μM) of compound **22** in comparison to compound **29** (46 ± 9%; 2.18 ± 0.40 μM) at α6β3δ receptors, the question of a correlation between potency and efficacy arises. After comparing measurements of several compounds at α6β3δ receptors this assumption can be dismissed. Compound **35** has a high efficacy (87 ± 9%), combined with a modest potency (1.20 ± 0.24 μM). An even stronger phenotype is observed for compound **28**, where potency is especially low (10.1 ± 2.8 μM) but efficacy is quite high (85 ± 14%). On the contrary, compound **39** showed high potency (0.76 ± 0.08 μM), but only modest efficacy (53 ± 12%). Thus, potency and efficacy of the compounds for modulating $GABA_AR$ seem to be independently influenced by the structural composition of the molecules.

CONCLUSION

Compound **22** was originally published as a δ-selective $GABA_AR$ modulator.[28] In our hands this compound did not show selectivity but a significant preference for δ-containing receptors, which caused us to investigate the SAR and further optimize the structure to gain a selective derivative.

Four of the synthesized compounds stood out because they selectively and directly modulated the [^{3}H]EBOB binding of δ-containing $GABA_AR$. These compounds have comparable alkyl groups in *para*-position of the benzamide group, which is the major difference to compound **22** possessing a chloro substituent at this position. Two of these compounds (**29**, **39**) were further investigated because of their promising modulatory effect on [^{3}H]EBOB binding. Both compounds showed selectivity in the absence of GABA for α6β3δ receptors, compound **29** additionally for α6β2δ receptors. Besides the δ subunit selectivity, both

compounds also showed α6-selectivity. In the presence of GABA, compounds **29** and **39** lost their δ-selectivity and modulated other α6- and β3-containing receptors.

Since no correlation between potency and efficacy of the compounds for α6β3δ receptors could be observed the structural determinants are apparently different and have to be investigated by more detailed SAR studies.

Altogether we identified two promising candidates, compounds **29** and **39**. Especially with regard to the *in vitro* investigation of recombinant δ-containing $GABA_AR$, these compounds may help to further investigate the assembly of α6β2/3δ receptors.

EXPERIMENTAL SECTION

Syntheses.

General.

All reagents and solvents were of analytical-grade quality and purchased from *Sigma-Aldrich, Alfa Aesar, Acros,* or *TCI*. Commercial chemicals were used without further purification, unless otherwise noted. Solvents were purified by distillation and desiccated by standard methods if necessary. ^{1}H and ^{13}C spectra were recorded on a *Bruker Fourier AC-300,* on a *Bruker Avance* 400 MHz spectrometer equipped with a 5 mm PABBO BB)^{1}H, ^{19}F) Z-GRD probe or a *Bruker Avance* 600 MHz spectrometer equipped with a cryogenically cooled 5 mm CPDCH $^{13}C(^{1}H)$ Z-GRD probe at 300 K using TMS as internal standard. DMSO-d_6, $CDCl_3$ or CD_3OD were used as solvent. Chemical shifts δ are given in parts per million (ppm) using residual proton peaks of the solvent as reference (^{1}H / ^{13}C: DMSO: 2.50 / 39.52 ppm, $CHCl_3$: 7.26 / 77.16 ppm, MeOH: 3.31 / 49.00 ppm). Mass spectra were recorded on an *ESI-Micromass LCT* from *Micromass*. High resolution mass spectra were obtained on a *Waters* Q-TOF-Ultima 3 instrument or on a *Thermo QExactive Orbitrap* mass spectrometer *(Thermo Scientific*, Bremen, Germany) equipped with an *AP-SMALDI* 10 ion source (*TransmitMIT*, Gießen, Germany) and operated at mass resolving power 140,000@m/z200 in positive ion mode with MALDI ionization. FD-MS was measured on a *Finnigan MAT 95* instrument. Purity of the compounds was determined using analytical reverse-phase HPLC and was > 95% for all compounds. Analytical HPLC was executed with a 250*4.6 mm *Kromasil 100* C18 7µm column from *MZ-Analysentechnik*. The pump was a *PK-2080* from *Jasco*. The detector was a *UV-2075 Plus* from *Jasco*. Detection wavelength was λ = 280 nM. The flow rate was 1 mL/min. Analytical HPLC was also performed on a *Merck-Hitachi* HPLC system consisting of an L-7100 pump, an L-7200 autosampler, and an L-7400 UV detector (254 nm), using an *Chromolith* SpeedROD RP-18 column (4.6 * 50 mm) eluted at a flow rate of 4.0 mL/min. A linear gradient elution was performed with eluent A (H_2O/TFA 100:0.1) containing 0% of solvent B (MeCN/H_2O/TFA, 90:10:0.01) rising to 100% of B during 5 min. Column chromatography was performed with silica gel (0.06 – 0.02 mm or 0.040 – 0.063 mm)

purchased from *Macherey-Nagel* or *Merck*. All reactions were monitored by thin-layer chromatography using *Macherey-Nagel* ALUGRAM Xtra SIL G/UV254 silica gel 60 plates for detection at 254 nm.

Syntheses of 2-aryl-imidazo[1,2-*a*]pyridin-3-amines.

General Procedure.

0.015 mol (0.5 eq.) $Na_2S_2O_5$ were dissolved in 80 mL of distilled water. Then 0.03 mol (1.0 eq.) of the respective aldehyde was added and the mixture stirred for 30 min at rt. After addition of 0.03 mol (1.0 eq.) of the respective 2-amino-pyridine, the resulting solution was refluxed for 2 h. The solution was allowed to cool to 80 °C before 0.03 mol (1.0 eq.) KCN were added in one portion. After stirring for 1 h at 80 °C, the mixture was cooled to rt and stirred for additional 3 h. In the following, the mixture was cooled with an ice bath and the precipitate was collected by vacuum filtration. The residue was washed several times with cold water and once with 10 mL cooled (– 18 °C) ethyl acetate. Recrystallization of the crude product from H_2O/EtOH 4:1 yielded the respective 2-aryl-imidazo[1,2-*a*]pyridin-3-amines (**1**–**7**) as yellow solids.

2-(Thiophen-2-yl)imidazo[1,2-*a*]pyridin-3-amine (1)

Synthesized following the general procedure using 2.85 g Na_2S_2O5, 3.36 g thiophene-2-carbaldehyde, 2.82 g 2-amino-pyridine and 1.95 g KCN. Yield: 4.4 g (68%). R_f (ethyl acetate/petroleum ether 1:1) = 0.26. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 8.19 (dt, J = 6.9, 1.1 Hz, 1H), 7.55 (dd, J = 3.6, 1.0 Hz, 1H), 7.46 – 7.29 (m, 2H), 7.11 (dd, J = 5.1, 3.6 Hz, 1H), 7.03 (ddd, J = 9.1, 6.7, 1.2 Hz, 1H), 6.83 (td, J = 6.7, 1.1 Hz, 1H), 5.28 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 138.8, 138.5, 127.8, 125.5, 123.6, 123.2, 122.4, 121.9, 121.8, 116.2, 111.0.

2-Phenylimidazo[1,2-*a*]pyridin-3-amine (2)[47]

Synthesized following the general procedure using 2.85 g $Na_2S_2O_5$, 3.18 g benzaldehyde, 2.82 g 2-amino-pyridine and 1.95 g KCN. Yield: 4.1 g (65%). R_f (ethyl acetate/petroleum ether 1:3) = 0.47. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 8.24 (d, J = 6.9 Hz, 1H), 8.05 (d, J = 8.0 Hz, 2H), 7.46–7.36 (m, 3H), 7.23 (t, J = 7.3 Hz, 1H), 7.04 (t, J = 6.7 Hz, 1H), 6.83 (t, J = 6.7 Hz, 1H), 5.17 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 138.8, 135.2, 128.3, 127.3, 126.5, 126.1, 125.9, 122.5, 121.8, 116.6, 110.8.

6-Bromo-2-(thiophen-2-yl)imidazo[1,2-*a*]pyridin-3-amine (3)

Synthesized following the general procedure using 2.85 g $Na_2S_2O_5$, 3.36 g thiophene-2-carbaldehyde, 5.19 g 2-amino-5-bromo-pyridine and 1.95 g KCN. Yield: 4.9 g (56%). R_f (ethyl acetate/petroleum ether 1:1) = 0.65. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 8.50 (s, 1H), 7.55 (d, J = 3.4 Hz, 1H), 7.42 (d, J = 5.0 Hz, 1H), 7.37 (d, J = 9.5 Hz, 1H), 7.18–7.03 (m, 2H), 5.49 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 138.2, 136.7, 127.9, 126.2, 124.2, 124.0, 123.5, 122.2, 122.2, 117.3, 105.2.

6-Bromo-2-phenylimidazo[1,2-*a*]pyridin-3-amine (4)[48]

Synthesized following the general procedure using 2.85 g $Na_2S_2O_5$, 3.18 g benzaldehyde, 5.19 g 2-amino-5-bromo-pyridine and 1.95 g KCN. Yield: 4.5 g (52%). R_f (ethyl acetate/petroleum ether 1:1) = 0.62. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 8.54 (s, 1H), 8.02 (d, J = 7.7 Hz, 2H), 7.53–7.35 (m, 3H), 7.24 (t, J = 7.3 Hz, 1H), 7.12 (dd, J = 9.5, 1.5 Hz, 1H), 5.38 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 136.9, 134.7, 128.4, 127.6, 127.3, 126.1, 124.2, 122.3, 117.7, 105.1.

6-Bromo-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridin-3-amine (5)

Synthesized following the general procedure using 2.85 g $Na_2S_2O_5$, 4.08 g 4-methoxy-benzaldehyde, 5.19 g 2-amino-5-bromo-pyridine and 1.95 g KCN. Yield: 3.9 g (41%). R_f (ethyl acetate/petroleum ether 1:1) = 0.51. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 8.50 (s, 1H), 7.97 (d, J = 8.7 Hz, 2H), 7.38 (d, J = 9.4 Hz, 2H), 7.10 (d, J = 5.5 Hz, 1H), 6.99 (d, J = 8.7 Hz, 2H), 5.20 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 157.9, 136.9, 128.6, 127.5, 127.2, 126.0, 123.9, 122.2, 117.4, 113.8, 104.9, 55.1.

4-(3-Amino-6-bromoimidazo[1,2-*a*]pyridin-2-yl)phenol (6)

Synthesized following the general procedure using 2.85 g $Na_2S_2O_5$, 3.66 g 4-hydroxybenzaldehyde, 5.19 g 2-amino-5-bromo-pyridine and 1.95 g KCN. Yield: 3.7 g (43%). R_f (ethyl acetate/petroleum ether 1:1) = 0.16. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 9.45 (s, 1H), 8.48 (s, 1H), 7.85 (d, J = 8.5 Hz, 2H), 7.36 (d, J = 9.4 Hz, 1H), 7.09 (d, J = 9.4 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 5.13 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 156.1, 136.9, 129.2, 127.6, 125.6, 123.7, 122.1, 117.3, 115.2, 104.8.

6-Chloro-2-(thiophen-2-yl)imidazo[1,2-*a*]pyridin-3-amine (7)

Synthesized following the general procedure using 2.85 g $Na_2S_2O_5$, 3.36 g thiophene-2-carbaldehyde, 3.86 g 2-amino-5-chloro-pyridine and 1.95 g KCN. Yield: 4.3 g (58%). R_f (ethyl acetate/petroleum ether 1:1) = 0.64. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 8.44 (s, 1H), 7.56 (d, J = 3.6 Hz, 1H), 7.48–7.39 (m, 2H), 7.12 (t, J = 8.7 Hz, 1H), 7.04 (d, J = 11 Hz, 1H), 5.50 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 138.2, 136.7, 127.9, 126.4, 124.0, 123.8, 122.19, 122.18, 120.1, 118.3, 117.1.

Synthesis of 6,8-dibromo-2-(thiophen-2-yl)imidazo[1,2-*a*]pyridin-3-amine (8)

Procedure 1. 0.01 mol (0.5 eq.) $Na_2S_2O_5$ were dissolved in 90 mL distilled water and 0.02 mol (1.0 eq.) thiophene-2-carbaldehyde were added. The resulting solution was stirred at rt for 30 min. Then 36 mL EtOH and 0.02 mol (1.0 eq.) 2-amino-3,5-dibromopyridine were added and the mixture was refluxed for 2 h. 0.02 mol (1.0 eq.) KCN were added in small portions to the refluxing mixture. After heating at reflux for 2 h, the mixture was allowed to cool to rt and stirred for additional 3 h. EtOH was removed under reduced pressure and the remaining aqueous solution was cooled in an ice bath. The precipitate was collected by vacuum filtration and washed several times with cold water and once with cooled (– 18 °C) ethyl acetate. Pure **8** was obtained after recrystallization from H_2O/MeOH 4:1 as brown-yellow solid (1.6 g, 17%).

Procedure 2. 2-Amino-3,5-dibromopyridine (1.00 g, 3.97 mmol) was dissolved in methanol (10 mL). Thiophene-2-carbaldehyde (557 µL, 5.96 mmol), *tert*-butylisonitrile (517 µL, 4.57 mmol) and a 1 M solution of perchloric acid in methanol (500 µL) were added before the solution was stirred for 24 h at room temperature. After additional isonitrile was added (135 µL, 1.20 mmol), the reaction mixture was again stirred for 24 h before it was diluted with dichloromethane (50 mL) and washed with aqueous $NaHCO_3$ (2 × 50 mL) and brine (50 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated *in vacuo*. The obtained residue was purified by column chromatography (cyclohexane:ethyl acetate, 30:1 + 3% triethylamine) to yield 6,8-dibromo-*N*-(*tert*-butyl)-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-amine as yellow crystals (888 mg, 2.07 mmol), yield 52%. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 8.57 (d, J = 1.7 Hz, 1H), 7.82 (dd, J = 3.6, 1.2 Hz, 1H), 7.74 (d, J = 1.7 Hz, 1H), 7.53 (dd, J = 5.1, 1.2 Hz, 1H), 7.12 (dd, J = 5.1, 3.6 Hz, 1H), 4.83 (s, 1H), 1.10 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 137.4, 136.9, 135.7, 128.7, 127.5, 126.0, 125.7, 125.2, 123.8, 110.9, 104.4, 56.6, 30.1. This compound (716 mg, 1.67 mmol) was suspended in 5 M hydrobromic acid (20 mL) and stirred at 110 °C for 2 h, then at 80 °C for 1.5 h. After alkalization with 5 M solution of NaOH and extraction with ethyl acetate (2 × 50 mL), the organic layer was dried with Na_2SO_4. Removal of the solvent *in vacuo* yielded **8** as yellow solid (610 mg, 1.64 mmol), yield 98%. R_f (ethyl acetate/petroleum ether 1:3) = 0.47. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 8.57 (s, 1H), 7.57 (d, J = 3.6 Hz, 1H), 7.52 (s, 1H), 7.47 (d, J = 5.1 Hz, 1H), 7.14 (t, J = 8.6 Hz, 1H), 5.68 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 137.6, 134.3, 128.0, 125.6, 124.4, 123.7, 122.7, 121.9, 110.4, 104.1.

Synthesis of 6-bromo-2-[4-(3-bromopropoxy)phenyl]-imidazo[1,2-*a*]pyridin-3-amine (9)

0.3 g (1 mmol, 1.0 eq.) **6**, 0.4 g (0.2 mL, 2 mmol, 2.0 eq.) 1,3-dibromopropane and 0.17 g (1.2 mmol, 1.2 eq.) K_2CO_3 were refluxed in 10 mL acetone for 8 h. Acetone was removed *in vacuo* and the residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate 1:1) yielding **9** as yellow solid. Yield: 0.31 g (74%). R_f (ethyl acetate/petroleum ether 1:1) =

0.60. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 8.51 (s, 1H), 7.96 (d, J = 8.6 Hz, 2H), 7.38 (d, J = 9.4 Hz, 1H), 7.11 (d, J = 9.5 Hz, 1H), 7.01 (d, J = 8.6 Hz, 2H), 5.22 (s, 2H), 4.12 (t, J = 5.9 Hz, 2H), 3.69 (t, J = 6.5 Hz, 2H), 2.27 (quint, J = 6.1 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 157.0, 136.9, 128.3, 127.6, 127.4, 126.1, 124.1, 122.2, 117.4, 114.4, 105.0, 65.3, 31.9, 31.3.

Synthesis of 2-(furan-2-yl)imidazo[1,2-*a*]pyridin-3-amine (10)

To a solution of furan-2-carbaldehyde (0.2 mL, 2.41 mmol) in a mixture of H_2O (0.5 mL) and EtOH (0.5 mL) was added 2-aminopyridine (229 mg, 2.43 mmol), KCN (164 mg, 2.52 mmol) and $BiCl_3$ (37 mg, 0.117 mmol). H_2O (1 mL) was additionally added and the mixture was stirred at 110 °C for 1.5 h. The resulting dark brown mixture was evaporated directly on celite and purified by flash column chromatography (7:3 EtOAc/heptane) yielding the product as an orange semi-solid (147 mg, 31%). ^{1}H NMR (400 MHz; DMSO-d_6): δ [ppm] = 8.16—8.13 (m, 1H), 7.69—7.68 (m, 1H), 7.35 (dt, J = 9.1, 1.1 Hz, 1H), 7.04—7.00 (m, 1H), 6.83—6.79 (m, 1H), 6.69 (dd, J = 3.3, 0.7 Hz, 1H), 6.58 (dd, J = 3.3, 1.8 Hz, 1H), 5.42—5.35 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 151.0, 141.6, 139.1, 127.5, 122.8, 122.1, 118.9, 116.9, 111.9, 111.4, 104.8.

Synthesis of 2-(5-methylthiophene-2-yl)imidazo[1,2-*a*]pyridin-3-amine (11)

To a solution of 5-methylthiophene-2-carbaldehyde (0.87 mL, 7.93 mmol) in EtOH (10 mL) was added $BiCl_3$ (250 mg, 0.792 mmol) to form a suspension, which was stirred at ambient temperature for 20 min followed by addition of 2-aminopyridine (761 mg, 8.08 mmol). The reaction was refluxed at 95 °C for 1.5 h after which KCN (1.03 g, 15.9 mmol) was added along with H_2O (5 mL) and heating was continued at 107 °C for 6 h. The dark mixture was quenched with NaOH (1 M, 6 mL) and extracted with EtOAc (3 x 200 mL). The combined organic phase was dried over $MgSO_4$ and purified by flash column chromatography (8:2 EtOAc/heptane). The product was precipitated from EtOAc/heptane to afford an orange solid (315 mg, 17%). ^{1}H NMR (400 MHz; CD_3OD): δ [ppm] = 8.15 (dt, J = 6.9, 1.2 Hz, 1H), 7.38 (dt, J = 9.1, 1.1 Hz, 1H), 7.32 (d, J = 3.6 Hz, 1H), 7.17 (ddd, J = 9.1, 6.7, 1.3 Hz, 1H), 6.90—6.86 (m, 1H), 6.78 (dt, J = 3.5, 1.1 Hz, 1H), 2.52—2.50 (m, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ [ppm] = 140.2, 138.6, 134.4, 125.5, 123.4, 123.3, 122.1, 115.2, 111.6, 13.7.

Syntheses of acid chlorides.

4-Butoxybenzoyl chloride (12)[49-50]

5.0 g (0.033 mol, 1.0 eq.) methyl-4-hydroxybenzoate, 6.75 g (0.05 mol, 1.5 eq.) 4-bromobutane and 5.5 g (0.04 mol, 1.2 eq.) K_2CO_3 were refluxed in 65 mL acetone for 10 h. The solution was cooled to rt and the precipitated inorganic salts were filtered

off before acetone was removed under reduced pressure. The residue was purified by column chromatography (SiO_2, petroleum/ethyl acetate 9:1) yielding methyl-4-butoxybenzoate as colorless oil (4.40 g, 64%). R_f (ethyl acetate/petroleum ether 1:1) = 0.71. ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.97 (d, J = 8.8 Hz, 2H), 6.90 (d, J = 8.8 Hz, 2H), 4.01 (t, J = 6.5 Hz, 2H), 3.88 (s, 3H), 1.78 (quint, J = 6.6 Hz, 2H), 1.49 (sext, J = 7.4 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm] = 167.0, 163.1, 131.7, 122.4, 114.2, 68.0, 51.9, 31.3, 19.3, 13.9. 1.0 g (5 mmol, 1.0 eq.) methyl-4-butoxybenzoate and 1.0 g (25 mmol, 5 eq.) NaOH in 10 mL distilled water were refluxed for 2 h. After cooling to rt, the solution was acidified to pH 1 by addition of 1 M HCl. The mixture was extracted three times with ethyl acetate, the combined organic layers were dried with Na_2SO_4 and the solvent was evaporated to yield 4-butoxybenzoic acid as colorless solid (0.93 g, 96%). R_f (ethyl acetate/petroleum ether 3:1) = 0.23. ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm] = 8.06 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 4.03 (t, J = 6.5 Hz, 2H), 1.80 (quint, J = 6.6 Hz, 2H), 1.51 (sext, J = 7.3 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm] = 172.1, 163.8, 132.5, 121.5, 114.3, 68.1, 31.3, 19.3, 14.0. 0.7 g 4-butoxybenzoic acid was dissolved in 3 mL $SOCl_2$ and refluxed for 2 h. Then $SOCl_2$ was removed *in vacuo* yielding **12** as light-yellow oil, which was used directly in the next step without further purification or characterization.

4-(2-fluoroethoxy)benzoyl chloride (13)[51]

4-Hydroxybenzoic acid (200 mg, 1.45 mmol) and 40% aqueous *n*-Bu_4POH solution (2.02 mL, 2.896 mmol) was dissolved in dry THF (3 mL). After the mixture was cooled in an ice bath, 1-bromo-2-fluoroethanol (184 mg, 1.45 mmol) was added dropwise and the mixture was stirred for 16 h at room temperature. The solvent was removed under vacuum and the crude 4-(2-fluoroethoxy)benzoic acid was purified by column chromatography (methanol:dichloromethane, 1:40 + 3% formic acid) to give a colorless solid (95 mg, 0.52 mmol), yield 36%. ^{1}H NMR (300 MHz, CD_3OD) δ [ppm] = 7.95 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 9.0 Hz, 2H), 4.79 (m, 1H), 4.63 (m, 1H), 4.31 (m, 1H), 4.21 (m, 1H). Spectral data match that reported in literature.[51] Thionyl chloride (0.50 mL, 6.9 mmol) was added dropwise to 4-(2-fluoroethoxy)benzoic acid (85 mg, 0.46 mmol) under inert atmosphere. After heating at 85 °C for 4 h the excess of thionyl chloride was removed under high vacuum to give the title compound as light yellow oil. It was used without further purification or characterization.

4-Fluorobenzoyl chloride (14)[52]

Thionyl chloride (77.7 µL, 1.07 mmol) was added dropwise to 4-fluorobenzoic acid (50.0 mg, 0.357 mmol) under inert atmosphere. After heating at 85 °C for 1.5 h the excess of thionyl chloride was removed under high vacuum to give the desired product **14** as colorless oil at room temperature, which sets under cooling. It was used without further purification or characterization in the next step.

4-((tert-Butyldiphenylsilyl)oxy)benzoyl chloride (15)[53]

To a solution of 4-hydroxybenzaldehyde (1.00 g, 8.20 mmol) and imidazole (1.45 g, 31.3 mmol) in DMF (15 mL) *tert*-butylchlorodiphenylsilane (2.77 mL, 10.7 mmol) was added and the mixture was stirred for 42 h at room temperature. After repeated addition of TBDPS-Cl (640 µL, 2.46 mmol), the solution was stirred for another 24 h before saturated aqueous NH_4Cl (20 mL) was added and extracted with diethyl ether (3 × 50 mL). The combined organic extracts were washed with brine (50 mL) and dried with Na_2SO_4 before the solvent was removed *in vacuo*. Purification of the crude product by column chromatography (cyclohexan:ethyl acetate, 30:1) gave 4-((*tert*-butyldiphenylsilyl)oxy)benzaldehyde as colorless crystals (2.13 g, 5.92 mmol), yield 72%. ^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 9.81 (s, 1H), 7.74 – 7.67 (m, 4H), 7.67 – 7.61 (m, 2H), 7.49 – 7.34 (m, 6H), 6.90 – 6.82 (m, 2H), 1.11 (s, 9H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ [ppm] = 191.1, 161.3, 135.5, 132.0, 131.9, 130.4, 128.1, 127.9, 120.4, 26.5, 19.6.[53] 4-((*tert*-Butyldiphenylsilyl)oxy)benzaldehyde (102 mg, 0.283 mmol) was dissolved in a mixture of dichloromethane/water (4 mL, ratio 1:1) before tetrabutylammonium hydrogensulfate (8.00 mg, 0.0248 mmol) was added. $KMnO_4$ (44.7 mg, 0.283 mmol) was subsequently added portion wise under ice cooling. The reaction mixture was stirred for 3 h at room temperature before dichloromethane (15 mL) and 2 M hydrochloric acid (15 mL) were added and separated. After extraction of the aqueous phase with dichloromethane (2 × 20 mL) the organic layers were dried with Na_2SO_4 and the solvent removed *in vacuo*. Purification by column chromatography (cyclohexane:ethyl acetate, 9:1 + 3% formic acid) gave 4-((*tert*-butyldiphenylsilyl)oxy)benzoic acid as colorless solid (74 mg, 0.197 mmol), yield 70%. ^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.92 – 7.81 (m, 2H), 7.78 – 7.66 (m, 4H), 7.50 – 7.33 (m, 6H), 6.86 – 6.75 (m, 2H), 1.12 (s, 9H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ [ppm] = 171.8, 160.7, 135.5, 132.2, 130.3, 128.1, 122.2, 119.8, 26.5, 19.6.[54] 4-((*tert*-Butyldiphenylsilyl)oxy)benzoic acid (374 mg, 0.99 mmol) was combined with oxalyl chloride (1 mL) under inert atmosphere and stirred for 3 h at room temperature. Excess oxalyl chloride was removed under high vacuum to yield **15** as slightly yellow liquid, which was used directly in the next step without further purification or characterization.

Syntheses of *N*-[2-arylimidazo[1,2-*a*]pyridin-3-yl]amides.

General Procedure.

1.7 mmol (1.0 eq.) of the respective 2-aryl-imidazo[1,2-*a*]pyridin-3-amine (**1**–**9**) were dissolved in 8 mL dry toluene and 4 mL dry pyridine. Then 1.9 mmol (1.1 eq.) of the specified acid chloride were added in one portion and the resulting mixture stirred for 1h at rt. 6 mL of distilled water were added and stirred for additional 15 min. The solution was cooled with an ice bath and the precipitate was collected by vacuum filtration, then washed several times with water and once with cooled (– 18 °C) acetone. The

residue was purified by column chromatography (SiO_2) to yield the respective *N*-[2-arylimidazo[1,2-*a*]pyridin-3-yl]amides (**16**–**33**) as colorless solids.

4-Chloro-*N*-[2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]benzamide (16)[44]

Synthesized following the general procedure using 0.37 g **1** and 0.33 g 4-chlorobenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50 to 100:0. Yield: 0.43 g (71%). R_f (ethyl acetate/petroleum ether 1:1) = 0.14. ^{1}H NMR (300 MHz, DMSO-d_6), δ [ppm] = 10.75 (s, 1H); 8.18-8.14 (m, 3H); 7.71 (d, J = 8.5 Hz, 2H); 7.62 (d, J = 9.0 Hz, 1H); 7.55 (d, J = 5.0 Hz, 1H); 7.49 (d, J = 3.6 Hz, 1H); 7.34 (t, J = 8.6 Hz, 1H); 7.13 (t, J = 8.6 Hz, 1H); 6.96 (t, J = 6.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6), δ [ppm] = 165.7, 142.2, 137.3, 136.2, 134.1, 131.8, 130.0, 128.8, 127.9, 126.2, 125.6, 124.4, 123.9, 116.6, 114.1, 112.4. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{12}ClN_3OS$: 354.05, found: 354.06. RP-HPLC: R_t (MeCN/H_2O 1:1) = 9.48 min, purity > 99%. Spectral data match that reported in literature.[44]

4-Chloro-*N*-(2-phenylimidazo[1,2-*a*]pyridin-3-yl)benzamide (17)

Synthesized following the general procedure using 0.36 g **2** and 0.33 g 4-chlorobenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 25:75 to 50:50. Yield: 0.40 g (67%). R_f (ethyl acetate/petroleum ether 3:1) = 0.46. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.79 (s, 1H), 8.23–8.09 (m, 3H), 7.98 (d, J = 8.0 Hz, 2H), 7.78–7.60 (m, 3H), 7.44 (t, J = 7.6 Hz, 2H), 7.39–7.29 (m, 2H), 6.96 (t, J = 6.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 165.7, 142.1, 137.8, 137.3, 133.5, 131.8, 130.0, 128.8, 128.6, 127.7, 126.6, 125.3, 123.9, 116.9, 115.2, 112.3. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{14}ClN_3O$: 348.08, found: 348.09. RP-HPLC: R_t (MeCN/H_2O 1:1) = 10.68 min, purity: 98%.

***N*-[6-Bromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]-4-chlorobenzamide (18)**

Synthesized following the general procedure using 0.50 g **3** and 0.33 g 4-chlorobenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50 to 100:0. Yield: 0.57 g (79%). R_f (ethyl acetate/petroleum ether 1:1) = 0.49. ^{1}H NMR (300 MHz, DMSO-d_6), δ [ppm] = 10.74 (s, 1H), 8.63 (s, 1H), 8.15 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.63-7.56 (m, 2H), 7.50-7.54 (m, 2H), 7.13 (t, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 165.8, 140.6, 137.2, 135.7, 134.8, 131.9, 130.2, 128.7, 128.5, 128.0, 126.6, 124.8, 124.1, 117.7, 114.8, 106.5. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{11}BrClN_3OS$: 431.95, found: 431.92. RP-HPLC: R_t (MeCN/H_2O 1:1) = 18.07 min, purity: 98%.

***N*-(6-Bromo-2-phenylimidazo[1,2-*a*]pyridin-3-yl)-4-chlorobenzamide (19)**

Synthesized following the general procedure using 0.49 g **4** and 0.33 g 4-chlorobenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 40:60 to 90:10. Yield: 0.44 g (61%). R_f (ethyl acetate/petroleum ether 1:1) = 0.53. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.78 (s, 1H), 8.60 (s, 1H), 8.14 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 7.6 Hz, 2H), 7.77–7.59 (m, 3H), 7.51–7.38 (m, 3H), 7.33 (t, J = 7.3 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 165.8, 140.6, 138.6, 137.2, 133.1, 131.8, 130.2, 128.6, 128.3, 128.0, 126.7, 124.1, 118.1, 115.9, 106.5. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{13}BrClN_3O$: 425.99 found: 426.02. RP-HPLC: R_t (MeCN/H_2O 1:1) = 20.49 min, purity > 99%.

4-Methoxy-*N*-[2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]benzamide (20)[44]

Synthesized following the general procedure using 0.37 g **1** and 0.32 g 4-methoxybenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50 to 100:0. Yield: 0.39 g (66%). R_f (ethyl acetate/petroleum ether 1:1) = 0.18. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.50 (s, 1H), 8.22–8.00 (m, 3H), 7.61 (d, J = 9.0 Hz, 1H), 7.54 (d, J = 4.2 Hz, 1H), 7.49 (d, J = 3.6 Hz, 1H), 7.33 (t, J = 7.9 Hz, 1H), 7.19–7.08 (m, 3H), 6.95 (t, J = 6.8 Hz, 1H), 3.88 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.0, 162.5, 142.1, 136.4, 134.1, 130.1, 127.8, 126.1, 125.4, 125.1, 124.3, 123.8, 116.6, 114.6, 113.9, 112.3, 55.6. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{15}N_3O_2S$: 350.09, found: 350.09. RP-HPLC: R_t (MeCN/H_2O 1:1) = 6.75 min, purity > 99%. Spectral data match that reported in literature.[44]

***N*-[6-Bromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]-4-methoxybenzamide (21)**

Synthesized following the general procedure using 0.50 g **3** and 0.32 g 4-methoxybenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 60:40 to 90:10. Yield: 0.54 g (75%). R_f (ethyl acetate/petroleum ether 1:1) = 0.36. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.51 (s, 1H), 8.50 (s, 1H), 8.12 (d, J = 8.7 Hz, 2H), 7.65–7.54 (m, 2H), 7.51–7.40 (m, 2H), 7.19–7.09 (m, 3H), 3.88 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.1, 162.5, 140.5, 135.8, 134.8, 130.2, 128.3, 127.9, 126.5, 125.2, 124.7, 123.9, 117.7, 115.3, 113.8, 106.4, 55.6. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{14}BrN_3O_2S$: 428.00, found: 427.99. RP-HPLC: R_t (MeCN/H_2O 1:1) = 10.27 min, purity > 99%.

4-Chloro-*N*-[6,8-dibromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]benzamide (22)[44]

Synthesized following the general procedure using 0.63 g **8** and 0.33 g 4-chlorobenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 75:25 to 100:0. Yield: 0.51 g (59%). R_f (ethyl acetate/petroleum ether 3:1) = 0.43. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.84 (s, 1H), 8.75 (s, 1H), 8.15 (d, J = 8.5 Hz, 2H), 7.92 (s, 1H), 7.70 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 4.9 Hz, 1H), 7.53 (d, J = 3.4 Hz, 1H), 7.14 (t, J = 8.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 165.8, 138.5, 137.3, 135.4, 135.0, 131.8, 130.2, 130.1, 128.6, 128.0, 127.0, 125.3, 124.0, 116.5, 110.7, 105.6. ESI-MS (*m/z*): $[M+H]^+$ calcd for

$C_{18}H_{10}Br_2ClN_3OS$: 509.87, found: 509.89. RP-HPLC: R_t (MeCN/H_2O 1:1) = 38.73 min, purity > 99%. Spectral data match that reported in literature.[44]

***N*-[6,8-Dibromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]-4-methoxybenzamide (23)[44]**

Synthesized following the general procedure using 0.63 g **8** and 0.32 g 4-methoxybenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50 to 90:10. Yield: 0.53 g (62%). R_f (ethyl acetate/petroleum ether 1:3) = 0.27. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.59 (s, 1H), 8.62 (s, 1H), 8.12 (d, J = 8.6 Hz, 2H), 7.91 (s, 1H), 7.60 (d, J = 5.0 Hz, 1H), 7.52 (d, J = 3.6 Hz, 1H), 7.27–6.99 (m, 3H), 3.88 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.1, 162.6, 138.4, 135.4, 135.2, 130.3, 130.0, 128.0, 126.9, 125.2, 125.1, 123.8, 117.0, 113.8, 110.4, 105.5, 55.6. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{13}Br_2N_3O_2S$: 505.91 found: 505.95. RP-HPLC: R_t (MeCN/H_2O 1:1) = 22.03 min, purity: 98%. Spectral data match that reported in literature.[44]

***N*-[6-Bromo-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridin-3-yl]-4-chlorobenzamide (24)**

Synthesized following the general procedure using 0.54 g **5** and 0.33 g 4-chlorobenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50 to 100:0. Yield: 0.60 g (78%). R_f (ethyl acetate/petroleum ether 1:1) = 0.38. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.73 (s, 1H), 8.55 (s, 1H), 8.14 (d, J = 8.3 Hz, 2H), 7.89 (d, J = 8.6 Hz, 2H), 7.69 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 9.5 Hz, 1H), 7.43 (d, J = 9.5 Hz, 1H), 7.01 (d, J = 8.6 Hz, 2H), 3.77 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 165.8, 159.2, 140.5, 138.7, 137.2, 131.9, 130.1, 128.6, 128.0, 125.5, 123.9, 117.8, 115.0, 114.1, 106.2, 55.2. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{15}BrClN_3O_2$: 456.00, found: 456.03. RP-HPLC: R_t (MeCN/H_2O 1:1) = 20.45 min, purity > 99%.

***N*-[6-Bromo-2-[4-(3-bromopropoxy)phenyl]imidazo[1,2-*a*]pyridin-3-yl]-4-chloro-benzamide (25)**

Synthesized following the general procedure using 0.72 g **9** and 0.33 g 4-chlorobenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50 to 100:0. Yield: 0.68 g (71%). R_f (ethyl acetate/petroleum ether 1:1) = 0.51. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.73 (s, 1H), 8.56 (s, 1H), 8.14 (d, J = 8.5 Hz, 2H), 7.88 (d, J = 8.7 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 9.4 Hz, 1H), 7.44 (d, J = 9.5 Hz, 1H), 7.03 (d, J = 8.7 Hz, 2H), 4.10 (t, J = 5.9 Hz, 2H), 3.66 (t, J = 6.5 Hz, 2H), 2.24 (quint, J = 6.2 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 165.8, 158.3, 140.5, 138.7 137.2, 131.8, 130.1, 128.6, 128.1, 125.7, 123.9, 117.8, 115.0, 114.7, 106.2, 65.3, 31.8, 31.2. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{23}H_{18}Br_2ClN_3O_2$: 561.95, found: 561.98. RP-HPLC: R_t (MeCN/H_2O 1:1) = 35.54 min, purity: 97%.

***N*-[6-Bromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]acetamide (26)**

Synthesized following the general procedure using 0.50 g **3** and 0.15 g acetyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 40:60 to 80:20. Yield: 0.44 g (78%). R_f (ethyl acetate/petroleum ether 1:1) = 0.30. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.15 (s, 1H), 8.44 (s, 1H), 7.63–7.50 (m, 3H), 7.42 (d, J = 9.5 Hz, 1H), 7.16 (t, J = 8.7 Hz, 1H), 2.24 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 170.5, 140.3, 135.9, 134.2, 128.2, 128.0, 126.4, 124.7, 123.9, 117.7, 115.1, 106.3, 22.9. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{13}H_{10}BrN_3OS$: 335.97, found: 335.98. RP-HPLC: R_t (MeCN/H_2O 1:1) = 4.28 min, purity > 99%.

4-Chloro-*N*-[6-chloro-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]benzamide (27)[44]

Synthesized following the general procedure using 0.42 g **7** and 0.33 g 4-chlorobenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50 to 100:0. Yield: 0.48 g (73%). R_f (ethyl acetate/petroleum ether 1:1) = 0.52. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.76 (s, 1H), 8.58 (s, 1H), 8.16 (d, J = 8.4 Hz, 2H), 7.75–7.63 (m, 3H), 7.57 (d, J = 5.0 Hz, 1H), 7.50 (d, J = 3.6 Hz, 1H), 7.39 (d, J = 5.5 Hz, 1H), 7.13 (t, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 165.8, 140.6, 137.2, 135.7, 135.1, 131.8, 130.2, 128.7, 128.0, 126.6, 126.4, 124.8, 122.1, 119.6, 117.5, 115.0. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{18}H_{11}Cl_2N_3OS$: 380.00 found: 388.01. RP-HPLC: R_t (MeCN/H_2O 1:1) = 16.19 min, purity > 99%.

***N*-[6-Bromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]cyclohexancarboxamide (28)**

Synthesized following the general procedure using 0.50 g **3** and 0.28 g cyclohexanecarbonyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 40:60 to 50:50. Yield: 0.46 g (67%). R_f (ethyl acetate/petroleum ether 1:3) = 0.45. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.03 (s, 1H), 8.21 (s, 1H), 7.66–7.54 (m, 2H), 7.49 (d, J = 3.2 Hz, 1H), 7.43 (d, J = 9.5 Hz, 1H), 7.16 (t, J = 8.4 Hz, 1H), 2.59 (t, J = 5.5 Hz, 1H), 2.06 (d, J = 6.0 Hz, 2H), 1.81 (d, J = 6.0 Hz, 2H), 1.68 (d, J = 5.5 Hz, 1H), 1.57–1.12 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 176.0, 140.4, 135.9, 134.4, 128.2, 127.9, 126.5, 124.7, 123.5, 117.8, 115.0, 106.3, 43.7, 28.9, 25.4, 25.3. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{18}H_{18}BrN_3OS$: 404.04, found: 404.04. RP-HPLC: R_t (MeCN/H_2O 1:1) = 15.03 min, purity > 99%.

***N*-[6-Bromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl]-4-butoxybenzamide (29)**

Synthesized following the general procedure using 0.50 g **3** and 0.28 g **12**. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50 to 90:10. Yield: 0.45 g (57%). R_f (ethyl acetate/petroleum ether 1:3) = 0.42. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.49 (s, 1H), 8.48 (s, 1H), 8.10 (d, J = 8.6 Hz, 2H), 7.64–7.53 (m, 2H), 7.51–7.41 (m, 2H), 7.16–7.09 (m, 3H), 4.10 (t, J = 6.4 Hz, 2H), 1.75 (quint, J = 6.6 Hz, 2H), 1.47 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.1, 162.0, 140.5, 135.8, 134.8, 130.2, 128.3, 127.9, 126.5, 124.9, 124.7, 123.9, 117.7, 115.3, 114.2, 106.4, 67.5,

30.6, 18.7, 13.7. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{20}BrN_3O_2S$: 470.05, found: 470.04. RP-HPLC: R_t (MeCN/H_2O 1:1) = 8.45 min, purity > 99%.

4-((2-(Thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl)carbamoyl)phenyl acetate (30)

Synthesized following the general procedure using 197 mg **1** and 200 mg 4-acetoxybenzoyl chloride. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50 to 100:0. Yield: 0.23 g (65%). R_f (ethyl acetate/petroleum ether 1:1) = 0.45. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.69 (s, 1H), 8.25 – 8.11 (m, 3H), 7.66 – 7.47 (m, 3H), 7.43 – 7.29 (m, 3H), 7.13 (t, *J* = 6.7 Hz, 1H), 6.95 (t, *J* = 6.7 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 169.0, 165.9, 153.6, 142.1, 136.26, 134.1, 130.5, 129.7, 127.9, 126.1, 125.5, 124.4, 123.9, 122.2, 116.6, 114.2, 112.4, 20.9. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{15}N_3O_3S$: 378.0907, found: 378.0888. RP-HPLC: R_t (MeCN/H_2O 1:1) = 9.26 min, purity: 98%.

4-Butoxy-*N*-(6,8-dibromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl)benzamide (31)

Synthesized following the general procedure using 200 mg **8** and 150 mg of **12**. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50. Yield: 0.21 g (62%). R_f (ethyl acetate/petroleum ether 1:1) = 0.55. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.57 (s, 1H), 8.60 (d, *J* = 1.6 Hz, 1H), 8.10 (d, J = 8.8 Hz, 2H), 7.90 (d, *J* = 1.6 Hz, 1H), 7.60 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.52 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.21 – 7.05 (m, 3H), 4.10 (t, *J* = 6.5 Hz, 2H), 1.74 (dq, *J* = 8.3, 6.5 Hz, 2H), 1.56 – 1.36 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.1, 162.0, 138.4, 135.4, 135.2, 130.3, 130.0, 128.0, 126.9, 125.2, 124.8, 123.7, 116.9, 114.2, 110.7, 105.5, 67.6, 30.6, 18.7, 13.7. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{19}Br_2N_3O_2S$: 547.9637, found: 547.9618. RP-HPLC: R_t (MeCN/H_2O 1:1) = 9.88 min, purity: 97%.

4-Butoxy-*N*-(6-chloro-2-(thiophene-2-yl)-imidazol[1,2-*α*]pyridin-3-yl)benzamide (32)

Synthesized following the general procedure using 0.50 g **7** and 0.64 g **12**. Column chromatography: SiO_2, ethyl acetate/petroleum 50:50. Yield: 0.50 g (66%). R_f (ethyl acetate/petroleum ether 1:1) = 0.38. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.56 (s, 1H), 8.50 (d, *J* = 1.3 Hz, 1H), 8.11 (d, *J* = 8.8 Hz, 2H), 7.75 – 7.66 (m, 1H), 7.59 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.53 (dd, *J* = 3.6, 1.0 Hz, 1H), 7.44 (dd, *J* = 9.5, 1.3 Hz, 1H), 7.18 – 7.08 (m, 3H), 4.10 (t, *J* = 6.5 Hz, 2H), 1.81 – 1.68 (m, 2H), 1.47 (h, *J* = 7.6 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.1, 162.0, 140.1, 135.0, 134.4, 130.3, 127.9, 126.9, 126.8, 125.1, 124.9, 122.1, 119.9, 117.1, 115.6, 114.3, 67.6, 30.6, 18.7, 13.7. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{20}ClN_3O_2S$: 426.10, found: 426.11. RP-HPLC: R_t (MeCN/H_2O 1:1) = 6.18 min, purity: 98%.

4-Butoxy-*N*-(-2-(thiophene-2-yl)-imidazol[1,2-*α*]pyridin-3-yl)benzamide (33)

Synthesized following the general procedure using 0.30 g **1** and 0.45 g **12**. Column chromatography: SiO_2, ethyl acetate/petroleum 60:40. Yield: 0.24 g (60%). R_f (ethyl acetate/petroleum ether 2:1) = 0.45. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.73 (s, 1H), 8.32 (d, J = 6.8 Hz, 1H), 8.20 – 8.05 (m, 2H), 7.84 – 7.53 (m, 4H), 7.26 – 7.06 (m, 4H), 4.11 (t, J = 6.5 Hz, 2H), 1.75 (m, 2H), 1.55 – 1.36 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.3, 162.2, 140.4, 132.7, 131.1, 130.3, 128.7, 128.7, 127.9, 127.7, 126.1, 124.6, 115.3, 114.9, 114.4, 114.2, 67.6, 30.6, 18.7, 13.7. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{21}N_3O_2S$: 392.1427 found: 392.1415. R_t (MeOH) = 4.82 min, purity: 98%.

Synthesis of *N*-(6,8-dibromo-2-(thiophene-2-yl)imidazo[1,2-a]pyridin-3-yl)-4-(2-fluoroethoxy)benzamide (34)

To a solution of **8** (132.0 mg, 0.355 mmol) in a mixture of dry toluene (1.5 mL) and dry pyridine (0.9 mL) under inert atmosphere **13** (93.4 mg, 0.461 mmol) was added and the mixture was stirred for 16 h at room temperature. Water (0.5 mL) was added and the mixture was stirred for 10 min at room temperature. After cooling in an ice bath the precipitate was filtered off and washed with cold water. Purification of the crude product by column chromatography (gradient hexane:ethyl acetate, 1:1 to hexane:ethyl acetate, 0:1) yielded **34** as colorless solid (132.6 mg, 0.25 mmol), yield 70%. ^{1}H NMR: (300 MHz, DMSO-d_6) δ [ppm] = 10.62 (s, 1H), 8.64 (s, 1H), 8.12 (d, J = 8.0 Hz, 2H), 7.92 (s, 1H), 7.61 (d, J = 5.0 Hz, 1H), 7.53 (d, J = 1.2, 3.6 Hz, 1H), 7.17 (m, 3H), 4.88 (m, 1H), 4.72 (m, 1H), 4.43 (m, 1H), 4.34 (m, 1H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ [ppm] = 166.5, 161.9, 138.9, 135.9, 135.6, 130.8, 130.5, 128.4, 127.4, 125.8, 125.7, 124.2, 117.3, 114.8, 111.2, 106.0, 83.3, 81.7. FD-MS (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{14}N_3O_2SBr_2F$: 539.9, found: 539.9.

***N*-(6,8-dibromo-2-(thiophene-2-yl)imidazo[1,2-a]pyridin-3-yl)-4-fluorobenzamide (35)**

Compound **8** (74.6 mg, 0.200 mmol) was dissolved in a mixture of dry toluene (0.5 mL) and dry pyridine (0.3 mL) under inert atmosphere before **14** (60.0 µL, 0.508 mmol) was added. The mixture was stirred for 69 h, then dichloromethane (10 mL) was added. The organic phase was washed with equal volumina of water, 1 M aqueous NaOH and 1 M aqueous hydrochloric acid before it was dried over Na_2SO_4 and concentrated *in vacuo*. The obtained residue was purified by column chromatography (gradient cyclohexane:ethyl acetate, 6:1 + 3% triethylamine to cyclohexane:ethyl acetate, 1:1 + 3% triethylamine) to yield **35** as colorless solid (89.8 mg, 0.181 mmol), yield 91%. ^{1}H NMR: (300 MHz, DMSO-d_6) δ [ppm] = 10.78 (s, 1H), 8.73 (d, J = 1.6 Hz, 1H), 8.26 – 8.16 (m, 2H), 7.92 (d, J = 1.6 Hz, 1H), 7.61 (dd, J = 5.1, 1.2 Hz, 1H), 7.53 (dd, J = 3.7, 1.2 Hz, 1H), 7.50 – 7.41 (m, 2H), 7.15 (dd, J = 5.0, 3.6 Hz, 1H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ [ppm] = 165.7, 164.6 (d, J = 250 Hz), 138.6, 135.4, 135.0, 131.1 (d, J = 9.3 Hz), 130.2, 129.5 (d, J = 2.8 Hz), 128.0, 127.0, 125.3, 123.9, 116.5, 115.6 (d, J = 22 Hz), 110.7, 105.7. HR-MS (ESI): $[M+H]^+$ calcd for $C_{18}H_{11}N_3OSBr_2F$: *m/z* = 493.8974, found 493.8981.

Synthesis of 4-((*tert*-Butyldiphenylsilyl)oxy)-*N*-(6,8-dibromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl)benzamide (36)

Compound **8** (185 mg, 0.496 mmol) was dissolved in a mixture of dry toluene (3 mL) and dry pyridine (1.5 mL) before it was added to **15** (353 mg, 0.893 mmol) under inert atmosphere. The solution was stirred for 43 h. Ethyl acetate (30 mL) was added and the organic layer was washed with 1 M aqueous hydrochloric acid and 1 M aqueous NaOH, dried over Na_2SO_4 and concentrated *in vacuo*. Purification of the crude product by column chromatography (cyclohexane:ethyl acetate, 8:1) gave **36** as colorless solid (271 mg, 0.370 mmol), yield 75%. ^{1}H NMR: (300 MHz, DMSO-d_6) δ [ppm] = 10.54 (s, 1H), 8.63 (d, J = 1.6 Hz, 1H), 7.99 – 7.91 (m, 2H), 7.89 (d, J = 1.6 Hz, 1H), 7.77 – 7.67 (m, 4H), 7.59 (dd, J = 5.1, 1.2 Hz, 1H), 7.56 – 7.44 (m, 7H), 7.12 (dd, J = 5.1, 3.6 Hz, 1H), 6.94 – 6.87 (m, 2H), 1.08 (s, 9H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ [ppm] = 166.0, 158.5, 138.4, 135.4, 135.1, 135.0, 131.5, 130.5, 130.2, 130.0, 128.2, 128.00, 126.9, 126.0, 125.3, 123.8, 119.2, 116.7, 110.7, 105.5, 26.2, 19.0.

Synthesis of *N*-(6,8-dibromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl)-4-hydroxybenzamide (37)

Compound **36** (129 mg, 0.176 mmol) was dissolved in freshly distilled tetrahydrofuran (2 mL) before a 1 M solution of tetrabutylammonium fluoride in THF (264 µL, 0.264 mmol) was added. The reaction mixture was stirred for 24 h. After removal of the solvent under reduced pressure the crude product was obtained. Purification by column chromatography (gradient cyclohexane:ethyl acetate, 4:1 + 3% formic acid until 100% ethyl acetate + 3% formic acid) yielded product **37** as colorless solid (76.6 mg, 0.155 mmol), yield 88%. ^{1}H NMR: (300 MHz, DMSO-d_6) δ [ppm] = 10.49 (s, 1H), 10.28 (s, 1H), 8.57 (d, J = 1.7 Hz, 1H), 8.07 – 7.95 (m, 2H), 7.91 (d, J = 1.6 Hz, 1H), 7.60 (dd, J = 5.0, 1.2 Hz, 1H), 7.51 (dd, J = 3.7, 1.2 Hz, 1H), 7.14 (dd, J = 5.1, 3.6 Hz, 1H), 6.99 – 6.88 (m, 2H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ [ppm] = 166.2, 161.3, 138.4, 135.4, 135.2, 130.4, 130.0, 128.0, 126.9, 125.2, 123.7, 123.4, 117.1, 115.1, 110.8, 105.5. HR-MS (ESI): $[M+H]^+$ calcd for $C_{18}H_{12}N_3O_2SBr_2$: m/z = 491.9017, found 491.9023.

Synthesis of *N*-(6,8-dibromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl)-4-(3-fluorpropoxy)benzamide (38)

To a solution of 3-fluoropropan-1-ol (324 µL, 4.00 mmol) in dichloromethane (4 mL) was added 4-methylbenzenesulfonyl chloride (839 mg, 4.40 mmol) portion-wise and pyridine (360 µL, 4.40 mmol, 1.10 eq.) dropwise under ice cooling. The reaction mixture was then stirred for 7 h at room temperature. After addition of water (20 mL) and saturated aqueous NH_4Cl solution (10 mL) the mixture was extracted with ethyl acetate (3 × 60 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* before purification of the crude product by column chromatography (cyclohexane:ethyl acetate, 7:1) which yielded 3-fluoropropyl 4-methylbenzenesulfonate as highly viscous, colorless oil (684 mg, 2.95 mmol), yield 74%. ^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.90 – 7.71 (m, 2H), 7.43 – 7.30 (m, 2H), 4.48 (dt, J = 47, 5.7 Hz, 2H), 4.16 (t, J = 6.1 Hz, 2H),

2.45 (s, 3H), 2.16 – 1.91 (m, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ [ppm] = 145.1, 132.9, 130.1, 128.0, 79.7 (d, J = 166 Hz), 66.3 (d, J = 4.9 Hz), 30.2 (d, J = 20 Hz), 21.8. This compound (31.5 mg, 0.134 mmol) was added to a solution of **37** (50.0 mg, 0.103 mmol) and K_2CO_3 (18.5 mg, 0.134 mmol) in DMF (4 mL). The resulting mixture was heated to 40 °C and stirred for 24 h under inert atmosphere. Next ethyl acetate (40 mL) was added and the organic phase washed with 1 M aqueous NaOH (3 × 30 mL) and dried over Na_2SO_4 before the solvent was removed under reduced pressure. Purification by column chromatography (cyclohexane:ethyl acetate, 5:1) yielded the title compound as colorless solid (17.5 mg, 0.0316 mmol), yield 31%.^{1}H NMR: (300 MHz, DMSO-d_6) δ [ppm] = 10.60 (s, 1H), 8.63 (d, J = 1.6 Hz, 1H), 8.20 – 8.04 (m, 2H), 7.91 (d, J = 1.6 Hz, 1H), 7.60 (dd, J = 5.1, 1.2 Hz), 7.52 (dd, J = 3.7, 1.2 Hz, 1H), 7.25 – 7.09 (m, 3H), 4.64 (dt, J = 47, 5.9 Hz, 2H), 4.21 (t, J = 6.3 Hz, 2H), 2.16 (dp, J = 26, 6.1 Hz, 2H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ [ppm] = 166.1, 161.7, 138.5, 135.4, 135.2, 130.3, 130.1, 128.0, 127.0, 125.2, 125.1, 123.8, 116.9, 114.3, 110.8, 105.6, 80.8 (d, J = 162 Hz), 64.0 (d, J = 5.4 Hz), 29.7 (d, J = 20 Hz). HR-MS (ESI): $[M+H]^+$ calcd for $C_{21}H_{17}N_3O_2FSBr_2$: m/z = 551.9392, found 551.9404.

Synthesis of *N*-(6,8-dibromo-2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl)-4-(4-fluorbutoxy)benzamide (39)

Compound **37** (38.7 mg, 0.0785 mmol) was dissolved in acetone (2 mL) and K_2CO_3 (14.1 mg, 0.102 mmol) was added. The mixture was stirred shortly before 1-bromo-4-fluorobutane (11.0 µL, 0.102 mmol) and a catalytic amount of KI were added. The reaction mixture was then stirred for 24 h and heated under reflux, whereupon additional alkyl halogenide (0.0306 mmol) in acetone (0.5 mL) was added. The reaction mixture was finally stirred and heated under reflux for another 23 h before ethyl acetate (30 mL) was added and the organic phase was washed with 1 M aqueous NaOH solution (3 × 30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was then purified by column chromatography (cyclohexane:ethyl acetate, 6:1 + 3% triethylamine) to yield **39** as colorless solid (15.3 mg, 0.0270 mmol), yield 35%. ^{1}H NMR: (300 MHz, DMSO-d_6) δ [ppm] = 10.59 (s, 1H), 8.62 (d, J = 1.7 Hz, 1H), 8.15 – 8.06 (m, 2H), 7.91 (d, J = 1.6 Hz, 1H), 7.60 (dd, J = 5.1, 1.2 Hz, 1H), 7.52 (dd, J = 3.7, 1.2 Hz, 1H), 7.17 – 7.10 (m, 3H), 4.52 (dt, J = 47, 5.9 Hz, 2H), 4.14 (t, J = 6.0 Hz, 2H), 1.93 – 1.72 (m, 4H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ [ppm] = 166.1, 161.9, 138.5, 135.4, 135.2, 130.3, 130.0, 128.0, 127.0, 125.2, 124.9, 123.8, 116.9, 114.3, 110.8, 105.6, 83.6 (d, J = 162 Hz), 67.4, 26.6 (d, J = 19 Hz), 24.6 (d, J = 5.3 Hz). HR-MS (ESI)**:** $[M+H]^+$ calcd for $C_{22}H_{19}N_3O_2SBr_2F$: m/z = 565.9549, found 565.9551.

Synthesis of 4-hydroxy-*N*-(2-(thiophene-2-yl)imidazo[1,2-a]pyridin-3-yl)benzamide (40)

0.15 g of compound **30** were dissolved in 3 mL THF and 3 mL 5 M NaOH were added. The biphasic mixture was vigorously stirred for 3 h at rt. THF was removed under reduced pressure and the residue was diluted with 10 mL H_2O. The solution was acidified to pH 1 by addition of 3 M HCl. The precipitate formed was collected by vacuum filtration and dried *in vacuo*. The

product was obtained as colorless solid (0.13 g, 92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.78 (s, 1H), 10.41 (s, 1H), 8.53 (d, J = 6.7 Hz, 1H), 8.04 (d, J = 8.7 Hz, 2H), 7.96 – 7.72 (m, 4H), 7.37 (t, J = 6.7 Hz, 1H), 7.25 (dd, J = 4.9, 3.9 Hz, 1H), 6.97 (d, J = 8.7 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.6, 161.7, 144.7, 130.5, 129.3, 129.3, 128.0, 125.4, 122.9, 116.04, 115.98, 115.3, 113.40, 113.37, 108.8, 105.6. Purity by HPLC: 99%. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{13}N_3O_2S$: 336.0801, found: 336.0788.

4-(Benzyloxy)-*N*-(2-(thiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl)benzamide (41)

Compound **1** was reacted with 4-benzyloxybenzoylchloride following the general procedure. Yield: 90 mg (63%). ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 10.49 (s, 1H), 8.18 – 8.04 (m, 3H), 7.60 (d, J = 9.1 Hz, 1H), 7.57 – 7.28 (m, 8H), 7.22 (d, J = 8.8 Hz, 2H), 7.12 (dd, J = 4.9, 3.7 Hz, 1H), 7.00 – 6.87 (m, 1H), 5.25 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.1, 161.6, 139.8, 138.4, 136.6, 135.4, 135.2, 130.3, 128.5, 128.00, 127.98, 127.8, 127.0, 125.3, 123.8, 116.5, 114.7, 112.5, 110.7, 105.5, 69.4. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{19}N_3O_2S$: 425.13, found: 425.14. RP-HPLC: R_t (MeCN/H_2O 1:1) = 4.35 min, purity 98%.

Synthesis of 4-chloro-*N*-(2-(furan-2-yl)imidazo[1,2-*a*]pyridin-3-yl)benzamide (42)

Compound **10** (122 mg, 0.612 mmol) was dissolved in dry THF (20 mL). Pyridine (0.74 mL, 9.17 mmol) was added under nitrogen atmosphere and the reaction mixture was stirred for 5 min followed by addition of 4-chlorobenzoyl chloride (0.086 mL, 0.673 mmol). The reaction was stirred at ambient temperature for 1 h and subsequently quenched with H_2O (15 mL) and extracted with EtOAc (2 x 100 mL). The combined organic phase was washed with H_2O and brine, the organic layer was dried over Na_2SO_4 and reduced *in vacuo* to afford a light brown solid which was purified by flash column chromatography (8:2 EtOAc/heptane + 0.1% AcOH) to afford a brown film (162 mg). The product was precipitated from DCM/EtOAc (1:1, 30 mL) as a light brown solid, which was filtered, washed with EtOAc and dried (82.6 mg, 40%). ^{1}H-NMR (600 MHz; DMSO-d_6): δ [ppm] = 10.69 (s, 1H), 8.15—8.12 (m, 3H), 7.75 (dd, J = 1.7, 0.8 Hz, 1H), 7.70—7.68 (m, 2H), 7.60 (dt, J = 9.1, 1.0 Hz, 1H), 7.34 (ddd, J = 9.1, 6.7, 1.3 Hz, 1H), 6.96 (td, J = 6.8, 1.1 Hz, 1H), 6.80 (dd, J = 3.3, 0.8 Hz, 1H), 6.60 (dd, J = 3.4, 1.8 Hz, 1H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 166.1, 149.1, 143.5, 142.8, 137.6, 132.4, 131.4, 130.4, 129.2, 125.9, 124.4, 117.2, 115.2, 112.8, 112.1, 108.3. Purity by HPLC: 99%, HRMS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{12}ClN_3O_2$: 338.0691; found: 338.0693.

Synthesis of 4-chloro-*N*-(2-(5-methylthiophene-2-yl)imidazo[1,2-*a*]pyridin-3-yl)benzamide (43)

Compound **11** (315 mg, 1.37 mmol) was dissolved in dry THF (20 mL) and put under nitrogen atmosphere. Pyridine was added and the solution was stirred for 5 min followed by addition of 4-chlorobenzoyl chloride (0.19 mL, 1.51 mmol), the reaction

was stirred at ambient temperature for 17 h and then at 50 °C for 30 min. After cooling to rt, H_2O (10 mL) was added and the aqueous phase was extracted with EtOAc (1 x 150 mL, 1 x 75 mL). The combined organic phase was washed with H_2O and brine and dried over $MgSO_4$ after which it was concentrated *in vacuo* and purified by flash column chromatography (2:1 EtOAc/heptane). The product was precipitated from EtOAc/heptane to afford a light yellow solid (264 mg, 52%). ^{1}H-NMR (600 MHz; DMSO-d_6): δ [ppm] = 10.67 (s, 1H), 8.15—8.12 (m, 3H), 7.71—7.69 (m, 2H), 7.59 (dt, J = 9.1, 1.1 Hz, 1H), 7.32 (ddd, J = 9.1, 6.7, 1.3 Hz, 1H), 7.28 (d, J = 3.5 Hz, 1H), 6.94 (td, J = 6.8, 1.1 Hz, 1H), 6.81—6.80 (m, 1H), 2.45 (d, J = 0.9 Hz, 3H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 166.1, 142.6, 140.0, 137.7, 134.8, 134.3, 132.3, 130.4, 129.3, 126.6, 125.8, 124.9, 124.2, 117.0, 114.1, 112.7, 15.4. Purity by HPLC: 96%, HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{14}ClN_3OS$: 368.0619; found: 368.0625.

Synthesis of 4-chloro-*N*-(imidazo[1,2-*a*]pyridin-3-yl)benzamide (44)

To a solution of formaldehyde (37% in H_2O, 0.94 mL, 12.6 mmol) in EtOH (10 mL) $BiCl_3$ (394 mg, 1.25 mmol) was added to form a white suspension, which was stirred at ambient temperature for 20 min. To the reaction mixture 2-aminopyridine (1.2 g, 12.7 mmol) was added and the reaction was refluxed for 1.5 h followed by addition of KCN (1.6 g, 24.5 mmol) along with H_2O (10 mL), the heating was continued for 4 h after which the reaction was quenched with NaOH (1 M, 6 mL) and filtered. The aqueous phase was extracted with EtOAc (100 mL) and the organic layer was washed with H_2O and brine and dried over $MgSO_4$ after which it was reduced on celite and purified by flash column chromatography (9:1 DCM/MeOH) to afford a yellow oil which crystallized over time (216 mg, 13%). The yellow residue was taken up in dry THF (20 mL) and pyridine (1.9 mL, 23.8 mmol) was added under nitrogen atmosphere followed by addition of 4-chlorobenzoyl chloride (0.25 mL, 1.90 mmol). The mixture was stirred at ambient temperature until the reaction was shown to be complete after 4 days. At the end of this time, H_2O (5 mL) was added, and the mixture was extracted with EtOAc (3 x 100 mL). The combined organic phase was washed with H_2O and brine and dried over $MgSO_4$. The residue was evaporated on celite and purified by flash column chromatography (9.5:0.5 DCM/MeOH) to afford a mixture of white solid and yellow oil. The product was precipitated from EtOAc/heptane as a white fibrous solid (200 mg, 47%). ^{1}H-NMR (600 MHz; CD_3OD): δ [ppm] = 8.11 (d, J = 6.9 Hz, 1H), 8.06—8.05 (m, 2H), 7.60—7.57 (m, 4H), 7.38 (ddd, J = 9.1, 6.7, 1.2 Hz, 1H), 7.01 (td, J = 6.8, 1.0 Hz, 1H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 165.6, 142.8, 137.5, 132.4, 130.4, 129.1, 128.2, 124.6, 124.5, 120.3, 117.7, 112.3. mp: 141.5—143.7 °C, purity by HPLC: 99%, HRMS (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{10}ClN_3O$: 272.0585; found: 272.0591.

Ligand binding to recombinant receptors. Human embryonic kidney cells [HEK 293 cells; German collection of microorganisms and cell cultures (DSMZ), Braunschweig, Germany] were grown to < 50% confluency on 15-cm tissue plates in 20 mL DMEM supplemented with 10% heat-inactivated fetal calf serum, 1.8 mM glutamine as well as penicillin (1785 units) and streptomycin (1.8 mg). Transfection was carried out with a Ca^{2+}-phosphate precipitation method essentially as previously

described.[55] Briefly, plasmids were diluted in 1 mL/plate of 0.3125 M $CaCl_2$ in H_2O. One mL/plate of 2x HBS (274 mM NaCl; 1.5 mM Na_2HPO_4; 54.6 mM HEPES/ NaOH; pH 7.0) was added to the DNA and incubated for 90 s. Two mL of the mixture were pipetted immediately onto 15-cm plates. These were incubated for 18-24 h before the transfection medium was replaced by fresh medium. Double and triple combinations of rat $GABA_A$ receptor cDNAs in eukaryotic expression vectors [37, 56-57] of the α1, α4, α6, β2, β3, γ2S and δ subunits were employed. For optimal receptor expression, final concentrations (µg vector DNA per 15-cm tissue culture plate) were: α1, 2.5; α4, 12; α6, 2.5; β2, 12; β3, 0.5; γ2S, 0.375 and δ, 2.5.

Cell membranes were prepared as previously described.[58] Briefly, cells were washed in PBS and harvested from tissue plates 48 h after transfection. Cells were homogenized in an Ultraturrax homogenizer for 15 s. Crude membranes were obtained after two centrifugation steps at 23000 g. Pellets were used immediately or frozen at -20 °C. Membrane pellets were resuspended in 50 mM Tris/citrate buffer, pH 7.3. Resuspended cell membranes (150-200 µg protein per tube) were incubated in a final volume of 0.5 mL of 50 mM Tris/citrate buffer (pH 7.3), supplemented with 0.2 M NaCl, 3 nM [^{3}H]EBOB (*Perkin Elmer*, Waltham, MA, USA) and increasing concentrations of DS compounds in presence (0.1 µM for α6β3γ2/δ and 0.5 µM for α6β3 receptors) or absence of GABA. DS derivatives were diluted in 50 mM Tris/citrate buffer (pH 7.3) from a stock of 10 mM in DMSO (giving a total DMSO concentration of 0.1%). GABA was diluted from a 10 mM solution in 50 mM Tris/citrate buffer (pH 7.3). Non-specific binding of [^{3}H]EBOB was determined by addition of 10 µM of EBOB. The binding assay procedure was essentially performed as described earlier,[58] though in the cited publication binding assays were executed with [^{35}S]TBPS. As the allosteric interaction of tested ligands on the binding properties of both convulsants has proven to be similar,[31, 34-35] we adopted the procedure to assays executed with [^{3}H]EBOB. Briefly, after addition of [^{3}H]EBOB, cell membranes were incubated at room temperature for 90 min. We performed [^{3}H]EBOB binding assays under these pre-equilibrium conditions (90 min incubation at 21 °C). The pre-equilibrium conditions were previously investigated and applied to [^{35}S]TBPS binding studies[32] and adopted to [^{3}H]EBOB binding experiments.[40] Assay mixtures were then rapidly diluted to 5 mL with ice-cold Tris/HCl, pH 7.5, and filtered through glass mircofibre filters, grade GF/C (*GE Healthcare*, Buckinghamshire, UK). The procedure was repeated once. Filters were incubated in 3.5 mL of Aquasafe 300 Plus scintillation fluid (*Zinsser Analytic*, Frankfurt, Germany). The radioactivity was determined in a Beckman liquid scintillation counter using external standardization. Protein quantification was performed according to the Bradford method[59] using Roti-Quant 5x concentrate (*Roth*, Karlsruhe, Germany).

Electrophysiology. HEK 293 cells were grown on glass cover slips and transiently transfected with final concentrations (µg vector DNA/94 mm tissue culture plate) of α1: 1, α4: 5, α6: 1, β3: 0.2, γ2: 0.4, δ: 1.5 and a Green Fluorescent protein (eGFP; *Clontech*, Heidelberg, Germany): 0.5. 24 h after transfection the medium was replaced by fresh solution and 48 h after transfection cover slips were transferred to a recording chamber under an upright microscope (*Zeiss Axioskop FS*, Jena, Germany). Cells were perfused at 3 mL/min with an extracellular solution (135 mM NaCl, 5.3 mM KCl, 2 mM $CaCl_2$, 2 mM

$MgSO_4$, 10 mM HEPES, pH 7.4, adjusted to 320 mOsm with sucrose). Recordings of single, isolated and fluorescent cells were obtained in the whole cell configuration of the patch-clamp technique. The pipette solution contained 10 mM NaCl, 80 mM KCl, 50 mM KOH, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 3.1 mM ATP, 0.4 mM GTP, 10 mM EGTA and 10 mM HEPES, pH 7.2. Drugs were applied using a fast perfusion stepper system (SF-77B, *Warner Instruments, Inc.*, Midwest, USA) enabling solution exchange times of <20 ms (controlled by junction potential measurements). DS compounds were applied alone or in test solutions containing GABA at the receptor specific EC_{20} of GABA for 4 s with 60 s between successive applications. Data were amplified, filtered at 1 kHz (4-pole Bessel) and recorded on a standard personal computer at a sampling rate of 3-5 kHz using EPC-9 patch-clamp amplifier and Pulse 8.11 (*HEKA electronics*, Lambrecht, Germany).

Calculation. [^{3}H]EBOB binding analysis: Nonlinear regression was performed in Prism, version 6.05, on the four-parametric logarithmic dose-response equation formula ($y = min + (max – min) / (1+10^{((LogIC50 – x) * \eta)})$) with min and max being the minimum and maximum value, x the concentration of the derivative in µM and η Hill coefficient. The term "Hill coefficient" should be read as "pseudo-Hill coefficient" when dealing with allosteric as contrasting to direct effects. 100% was defined as the value in the absence of any modulator; 0% was defined by the blank value, i.e., in the presence of an excess of unlabelled EBOB. The maximum was set constant to 100%, the minimum was fitted. Data are given as the mean ± S.E.M. for the IC_{50} curves and mean ± S.D. for effects of single concentration. For statistical comparison Student's t-test was used. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Electrophysiology: Data analysis was performed using ClampFit 8.1 (Axon Instruments) and Origin 8.5 (*Microcal*, Northampton, MA, USA). Direct activation by compounds **16** and **22** were expressed by the ratio $I_{1\mu M\ X}/I_{1mM\ GABA}$. To calculate concentrations eliciting 50% of the maximal modulation of GABA induced currents (EC_{50}), peak currents (I_{max}) were plotted against the ligand concentration (LIG) and fitted with the logistic function $y = I_{min} + (I_{max}-I_{min}) / (1 + ([LIG]/EC_{50})^{Hill}$ using a least square fitting routine, where I_{min} represents the current induced by the receptor specific EC_{20} for GABA, I_{max} the maximal modulation, and Hill the Hill coefficient. The term "Hill coefficient" should be read as "pseudo-Hill coefficient" when dealing with allosteric as contrasting to direct effects. Responses of compounds **16** and **22** were expressed as percentage of control responses.

It should be explicitly mentioned that in this manuscript all shown Hill coefficients should be read as pseudo-Hill coefficients. True Hill coefficients reflect the number of binding sites of a ligand inducing the measured effect, i.e., the effect of GABA on $GABA_AR$ has to be seen in this context. In contrast, a modulatory or indirect effect can assume any positive or negative figure, i.e., independent of the number of binding sites. It has to be called pseudo-Hill coefficient as the mathematics behind its determination are identical to that for the true Hill coefficient.

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ABBREVIATIONS USED

DS1, delta selective compound 1; DS2, delta selective compound 2; [^{3}H]EBOB, [^{3}H]ethynylbicycloorthobenzoate; GABA, γ-aminobutyric acid; $GABA_AR$, γ-aminobutyric acid receptors type A; HEK, Human embryonic kidney; [^{35}S]TBPS, *tert*-[^{35}S]butylbicyclophosphorothionate

ASSOCIATED CONTENT

Supporting Information

Molecular Formula Strings: Data are available free of charge via the Internet at http://pubs.acs.org.

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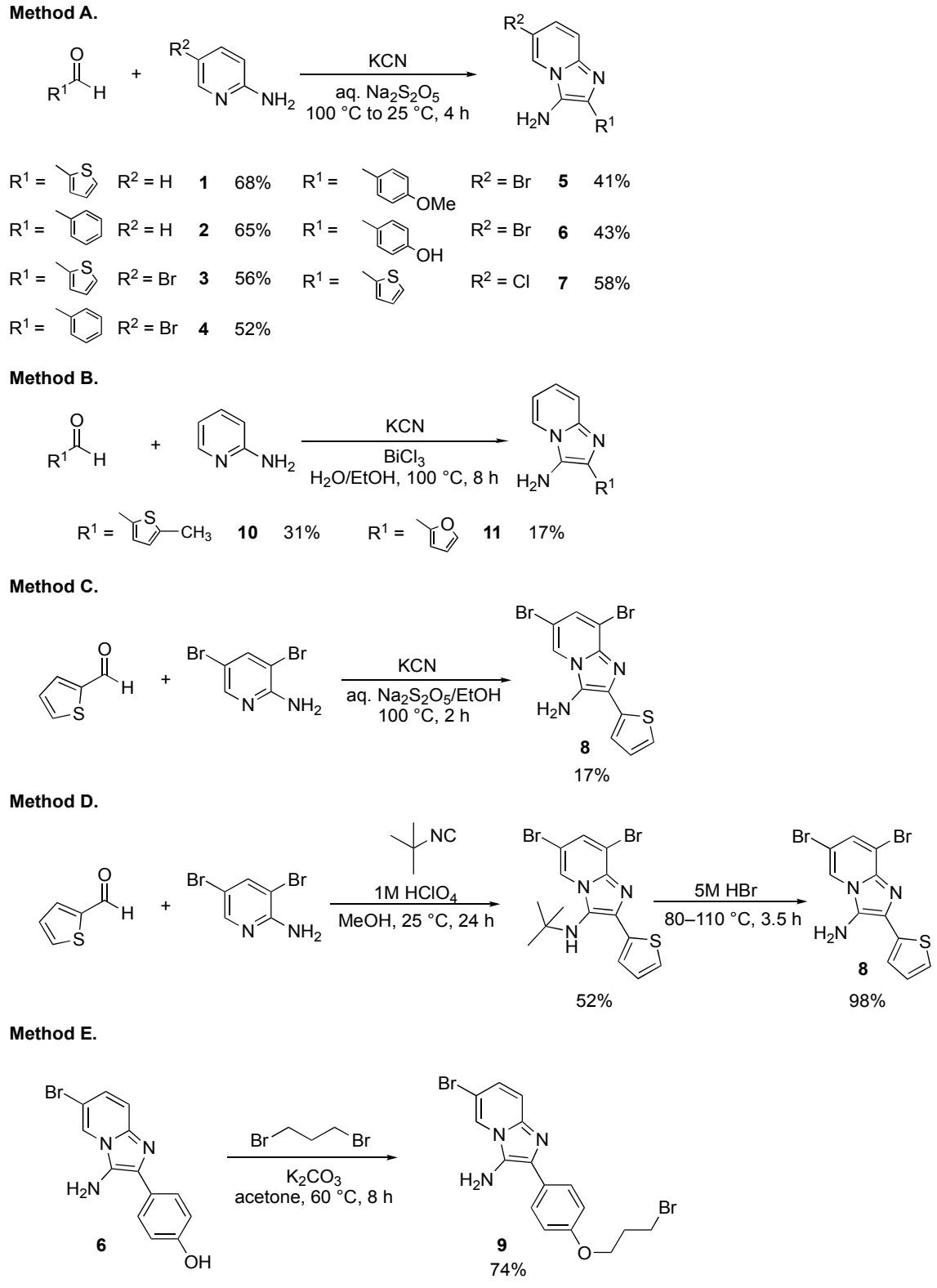
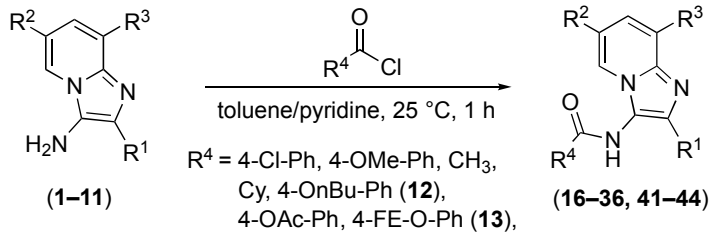
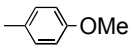
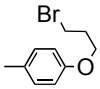
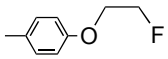
Scheme 1. Syntheses of 2-aryl-imidazo[1,2-a]pyridin-3-amines



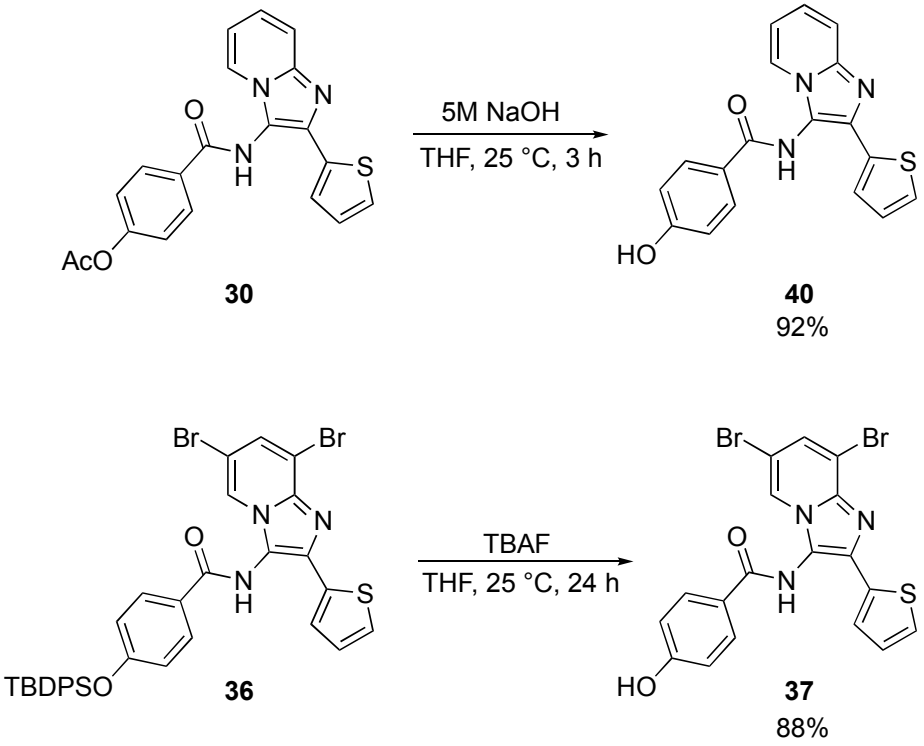
Scheme 2. Syntheses of *N*-[2-arylimidazo[1,2-*a*]pyridin-3-yl]amides. Compound **16** = DS2, compound **22** = DS1.



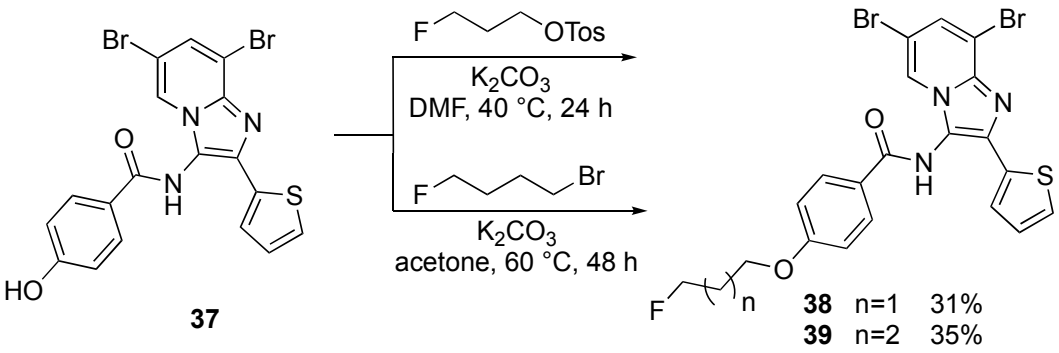
	R^1	R^2	R^3	R^4	Yield (%)
16		H	H		71
17		H	H		67
18		Br	H		79
19		Br	H		61
20		H	H		66
21		Br	H		75
22		Br	Br		59
23		Br	Br		62
24		Br	H		78
25		Br	H		71
26		Br	H	—CH_3	78
27		Cl	H		73
28		Br	H		67

	R^1	R^2	R^3	R^4	Yield (%)
29		Br	H		57
30		H	H		65
31		Br	Br		62
32		Cl	H		66
33		H	H		60
34		Br	Br		70
35		Br	Br		91
36		Br	Br		75
41		H	H		63
42		H	H		40
43		H	H		52
44	H	H	H		47

Scheme 3. Syntheses of compounds with a hydroxyl group in *p*-position of the benzamide part



Scheme 4. Syntheses of compounds **38** and **39** with terminally fluorinated alkyl chains

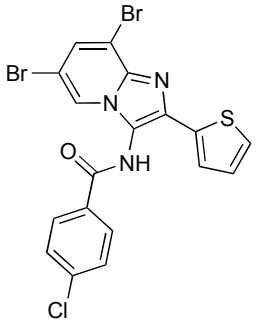


37

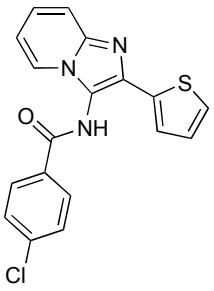
38 n=1 31%
39 n=2 35%

Figure 1. Structure of δ-selective compounds 1 and 2 (DS1 and DS2) and general structure of the derivatives synthesized for structure-function evaluation.

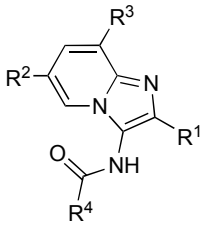
Figure 1



DS1 (**22**)



DS2 (**16**)



derivatives
(**17**–**21**, **23**–**44**)

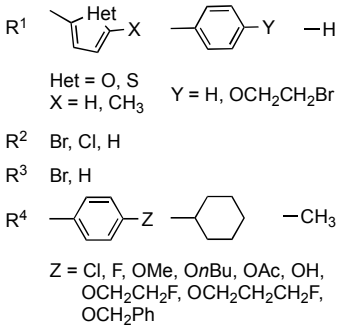
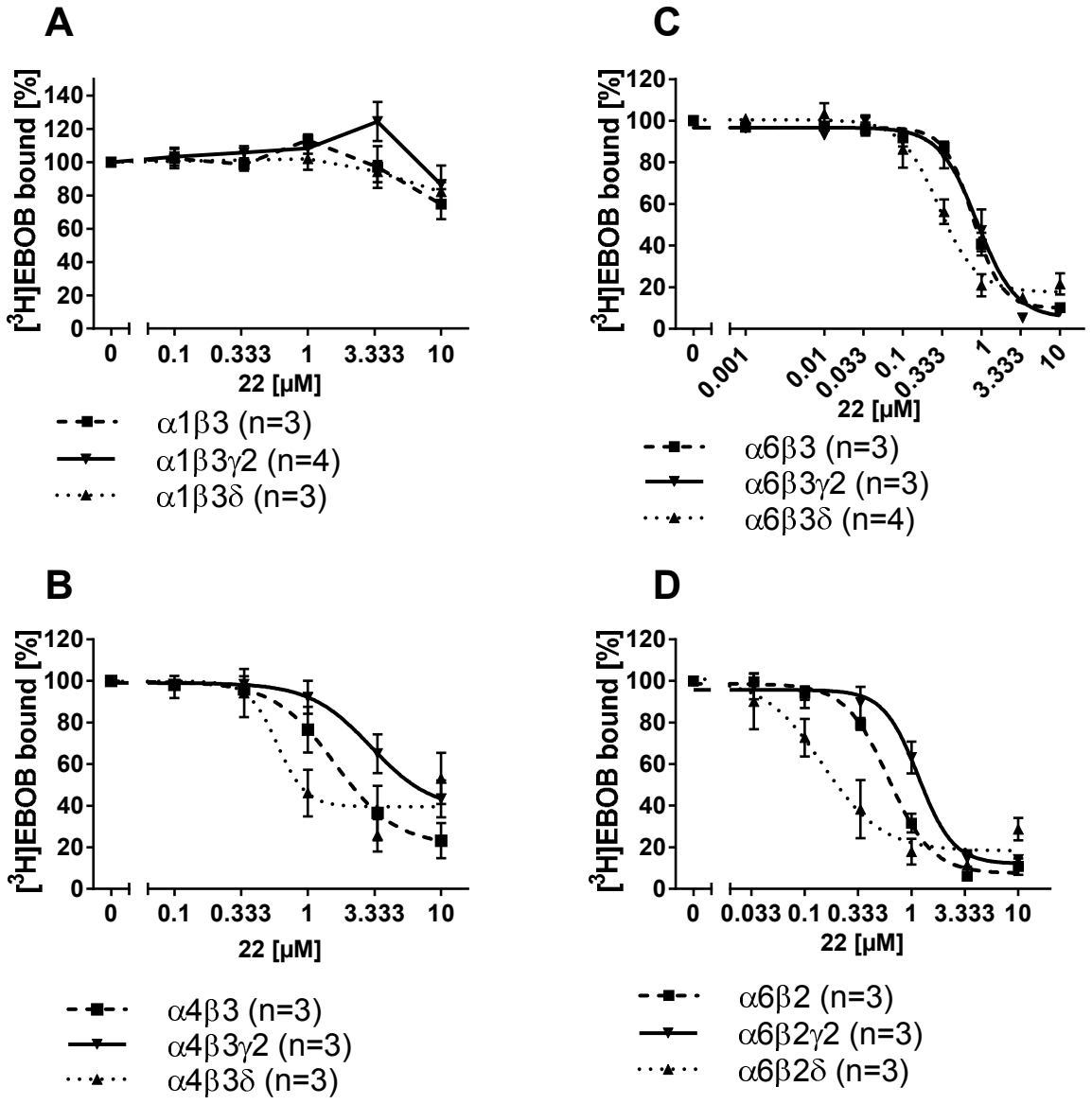
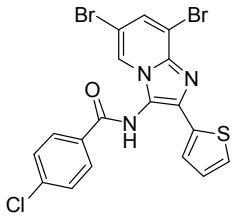


Figure 2. [^{3}H]EBOB binding modulation by compound **22**. [^{3}H]EBOB binding modulation at recombinant receptors consisting of α1β3 ± γ2/δ (A), α4β3 ± γ2/δ (B), α6β3 ± γ2/δ (C) and α6β2 ± γ2/δ (D). Crude membranes derived from transiently transfected HEK 293 cells were incubated with increasing concentrations of compound **22** and 3 nM of the ligand [^{3}H]EBOB. Experimental and calculation procedures were as described under "Experimental section". Curves are depicted as mean modulation ± S.E. of at least three separate experiments. Modulated [^{3}H]EBOB binding values refer to total specific binding, set to 100%. IC_{50} and maximal inhibition values are listed in Table 1.

Figure 2


A
[3H]EBOB bound [%]
0 20 40 60 80 100 120 140
0 0.1 0.333 1 3.333 10
22 [μM]
α1β3 (n=3)
α1β3γ2 (n=4)
α1β3δ (n=3)
C
[3H]EBOB bound [%]
0 20 40 60 80 100 120
0 0.001 0.01 0.033 0.1 0.333 1 3.333 10
22 [μM]
α6β3 (n=3)
α6β3γ2 (n=3)
α6β3δ (n=4)
B
[3H]EBOB bound [%]
0 20 40 60 80 100 120
0 0.1 0.333 1 3.333 10
22 [μM]
α4β3 (n=3)
α4β3γ2 (n=3)
α4β3δ (n=3)
D
[3H]EBOB bound [%]
0 20 40 60 80 100 120
0 0.033 0.1 0.333 1 3.333 10
22 [μM]
α6β2 (n=3)
α6β2γ2 (n=3)
α6β2δ (n=3)


Br
Br
N
N
O
N
H
S
Cl

22

Figure 3. Direct activation of $GABA_AR$ by compounds **16** (A) and **22** (B) in whole-cell patch-clamp experiments. Both compounds were measured at a concentration of 10 μM at different recombinant receptors. Measured currents were set in correlation to the effect of 1 mM GABA at the equivalent receptor ($I_0/I_{max\ GABA}$). Experimental and calculation procedures were as described under "Experimental section". $I_0/I_{max\ GABA}$ values are means ± S.D. from n separate experiments and listed in Table 2.

Figure 3

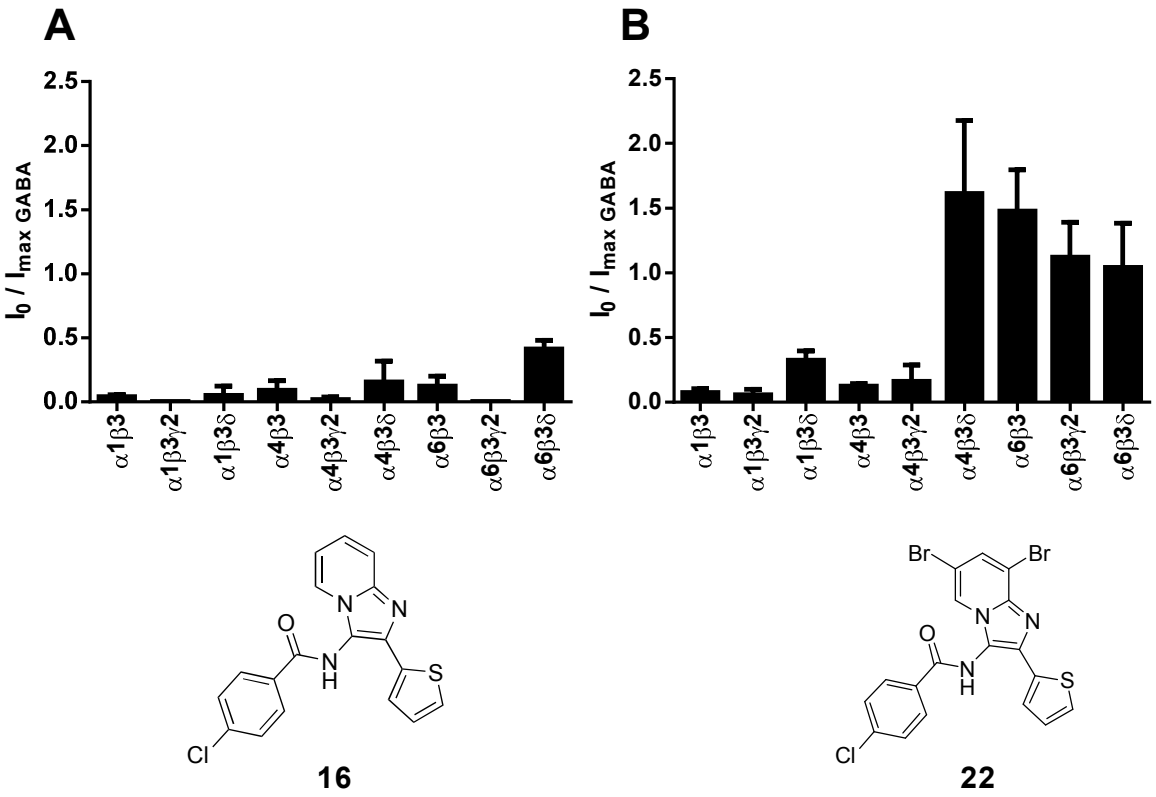

A
B
I_0 / I_{max} GABA
2.5
2.0
1.5
1.0
0.5
0.0
α1β3
α1β3γ2
α1β3δ
α4β3
α4β3γ2
α4β3δ
α6β3
α6β3γ2
α6β3δ
16
22

Figure 4. Modulation of [^{3}H]EBOB binding by compounds **29** and **39**. (A,B) [^{3}H]EBOB binding modulation at recombinant receptors consisting of α6β2 ± γ2/δ (A) and α6β3 ± γ2/δ (B) by compound **29**. (C,D) Modulation of [^{3}H]EBOB binding at recombinant receptors consisting of α6β2 ± γ2/δ (C) and α6β3 ± γ2/δ (D) by compound **39**. Experimental procedures as described for Figure 2. IC_{50} and maximal inhibition values are listed in Table 1.

Figure 4

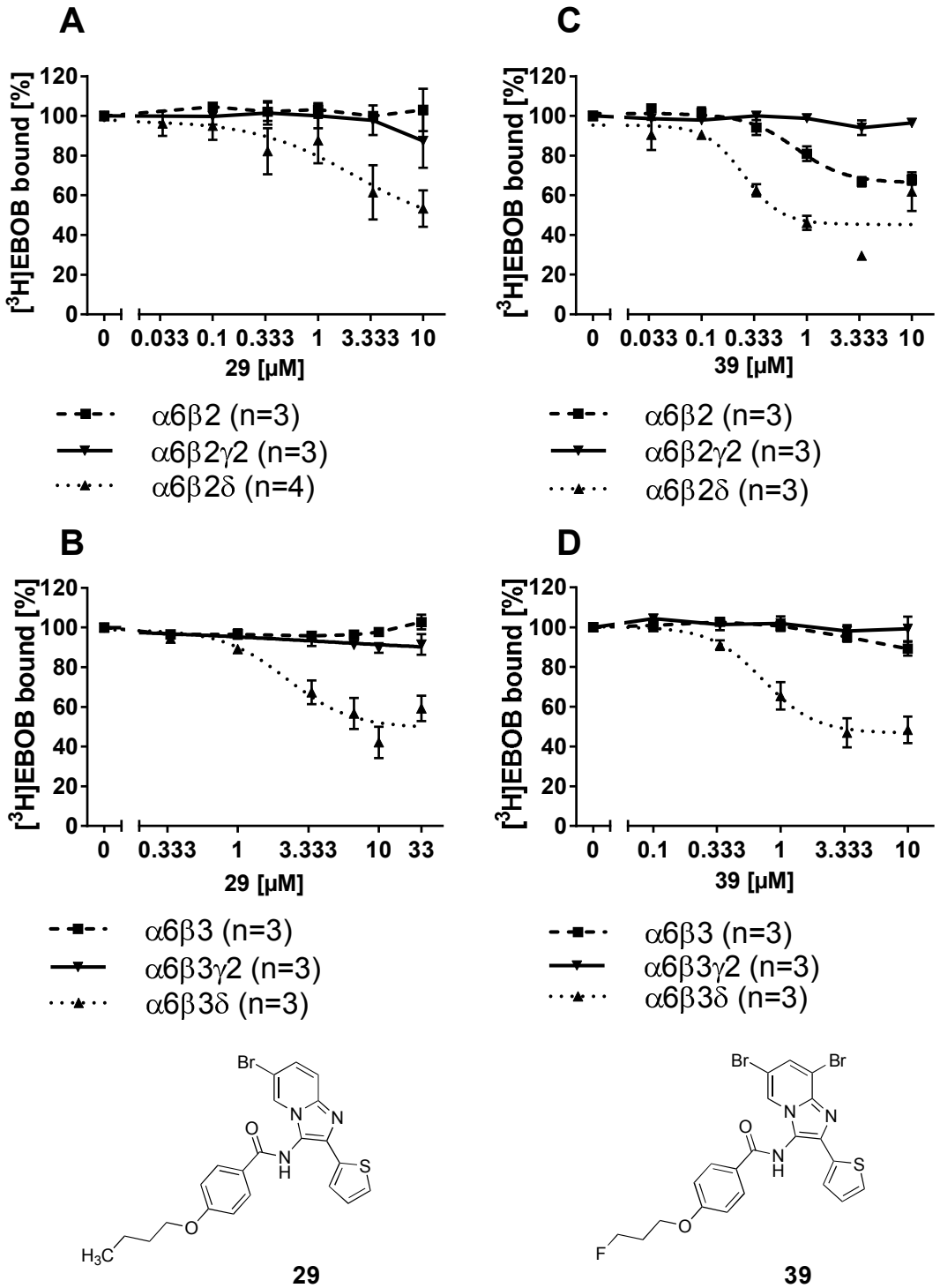

A
C
B
D
[3H]EBOB bound [%]
29 [μM]
39 [μM]
α6β2 (n=3)
α6β2γ2 (n=3)
α6β2δ (n=4)
α6β2δ (n=3)
α6β3 (n=3)
α6β3γ2 (n=3)
α6β3δ (n=3)
29
39

Table 1. [^{3}H]EBOB modulation by test compounds

Cpd.	Rec. type	IC_{50} values [μM]	Max. inhibition [%]	η	n
16	**α6β3δ**	**-**	**20 ± 10 (10 μM)**	**-**	**3**
22	α1β3	-	25 ± 18 (10 μM)	-	4
	α1β3γ2	-	13 ± 23 (10 μM)	-	4
	α1β3δ	**-**	**18 ± 10 (10 μM)**	**-**	**3**
	α4β3	1.86 ± 0.43	80 ± 16	-2.41 ± 0.51	3
	α4β3γ2	3.29 ± 0.42	67 ± 28	-5.29 ± 3.85	3
	α4β3δ	**0.51 ± 0.23**	**61 ± 16**	**-10.91 ± 4.61**	**3**
	α6β3	0.79 ± 0.07	90 ± 2	-2.41 ± 0.13	3
	α6β3γ2	0.93 ± 0.23	94 ± 5	-1.87 ± 0.28	3
	α6β3δ	**0.31 ± 0.04**	**84 ± 8**	**-1.56 ± 0.33**	**4**
	α6β2	0.63 ± 0.08	93 ± 6	-2.22 ± 0.20	3
	α6β2γ2	1.13 ± 0.11	89 ± 6	-2.53 ± 0.53	3
	α6β2δ	**0.15 ± 0.03**	**82 ± 6**	**-1.63 ± 0.29**	**3**
28	α6β3	16 (12.6, 19.5)	21 (24, 17)	-	2
	α6β3γ2	25 (21.5, 29)	≥ 89	-3.2 (-2.2, -4.1)	2
	α6β3δ	**10.1 ± 2.8**	**85 ± 14**	**-1.92 ± 0.19**	**3**
29	α1β3γ2	-	no effect	-	2
	α1β3δ	**-**	**no effect**	**-**	**2**
	α4β3	-	no effect	-	4
	α4β3γ2	-	no effect	-	4
	α4β3δ	**-**	**no effect**	**-**	**7**
	α6β3	-	no effect	-	3
	α6β3γ2	-	10 ± 4 (10 μM)	-	3
	α6β3δ	**2.18 ± 0.40**	**46 ± 9**	**-2.60 ± 0.64**	**3**
	α6β2	-	no effect	-	3
	α6β2γ2	-	13 ± 23 (10 μM)	-	3
	α6β2δ	**2.43 ± 0.78**	**54 ± 24**	**-1.37 ± 0.45**	**4**
31	α6β3	2.50 ± 0.89	46 ± 10	-1.31 ± 0.04	3
	α6β3γ2	5.51 ± 1.65	24 ± 7	-2.19 ± 0.85	3
	α6β3δ	**0.86 ± 0.30**	**71 ± 7**	**-1.18 ± 0.35**	**3**
34	α6β3	-	no effect	-	3
	α6β3γ2	-	15 ± 16 (10 μM)	-	3
	α6β3δ	**-**	**37 ± 31 (10 μM)**	**-**	**3**
35	α6β3	3.06 ± 0.25	91 ± 2	-9.69 ± 4.09	3
	α6β3γ2	3.39 ± 0.25	103 ± 4	-6.32 ± 2.94	3
	α6β3δ	**1.20 ± 0.24**	**87 ± 9**	**-6.00 ± 3.75**	**3**
38	α6β3	-	no effect	-	3
	α6β3γ2	-	no effect	-	3
	α6β3δ	**1 (1.1, 0.9)**	**38 (38, 38)**	**-6.6 (-11.3, -1.8)**	**2**
39	α1β3	-	no effect	-	2
	α1β3γ2	-	no effect	-	2
	α1β3δ	**-**	**no effect**	**-**	**2**
	α4β3	-	no effect	-	3
	α4β3γ2	-	no effect	-	3
	α4β3δ	**-**	**no effect**	**-**	**4**
	α6β3	-	11 ± 6 (10 μM)	-	3
	α6β3γ2	-	no effect	-	3
	α6β3δ	**0.76 ± 0.08**	**53 ± 12**	**-2.28 ± 0.16**	**3**
	α6β2	0.74 ± 0.16	33 ± 4	-5.20 ± 3.62	3
	α6β2γ2	-	no effect	-	3
	α6β2δ	**0.27 ± 0.02**	**55 ± 7**	**-4.82 ± 2.59**	**3**
41	α6β3	-	no effect	-	2
	α6β3γ2	-	no effect	-	2
	α6β3δ	**-**	**54 ± 11 (10 μM)**	**-**	**3**

Crude membranes derived from transiently transfected HEK 293 cells were incubated with increasing concentrations of the derivatives and 3 nM of [^{3}H]EBOB. Highest tested concentrations were 10 μM due to the limited solubility of the compounds, except for compounds **28** and **29**, which were tested up to 20 μM. Experimental and calculation procedures were as described under "Experimental section". IC_{50} values and pseudo-Hill coefficients η derived from non-linear regression curve fits are means ± S.E. from n separate experiments. Maximum inhibition values are means ± S.D. from n separate experiments. When n = 2 was measured, maximum inhibition, IC_{50}, values and pseudo-Hill coefficients η were calculated as means of two experiments; S.D. and S.E.M. values were then omitted. Maximal inhibition of compound **28** at α6β3γ2 receptors is denoted with a "≥" sign as for the fitting process the maximal inhibition of one experiment was constrained to 100%. Maximal inhibition describes the percentage decrease of [^{3}H]EBOB relative to total binding, set to 100%. When no modulation of [^{3}H]EBOB binding at a ligand concentration of 10 μM (20 μM for **29**) could be observed in at least two separate experiments "no effect" was noted. Derivatives which showed no effect at any tested $GABA_AR$ are not listed in this table. For the structures of the compounds see Schemes 2 and 4. Rec. type is the abbreviation for recombinant receptor type.

Table 2. Direct activation of recombinant receptors by compounds 16 and 22

Cpd.	Rec. type	$I_0/I_{max\ GABA}$	n
16	α1β3	0.042 ± 0.016	3
	α1β3γ2	0.004 ± 0.002	3
	α1β3δ	**0.053 ± 0.072**	**4**
	α4β3	0.093 ± 0.074	3
	α4β3γ2	0.020 ± 0,019	3
	α4β3δ	**0.158 ± 0,162**	**4**
	α6β3	0.124 ± 0.078	3
	α6β3γ2	0.004 ± 0.002	3
	α6β3δ	**0.414 ± 0.069**	**3**
22	α1β3	0.074 ± 0.029	3
	α1β3γ2	0.058 ± 0.042	3
	α1β3δ	**0.323 ± 0.073**	**3**
	α4β3	0.124 ± 0.021	3
	α4β3γ2	0.163 ± 0.123	3
	α4β3δ	**1.615 ± 0.562**	**3**
	α6β3	1.479 ± 0.318	6
	α6β3γ2	1.119 ± 0.273	3
	α6β3δ	**1.043 ± 0.341**	**4**

A concentration of 10 μM of compounds **16** and **22** was tested at different recombinant $GABA_AR$ in the absence of GABA. Measured currents were set in correlation to the effect of 1 mM GABA at the equivalent receptor type ($I_0/I_{max\ GABA}$). Experimental and calculation procedures were as described under "Experimental section". $I_0/I_{max\ GABA}$ values are means ± S.D. from n separate experiments. Graphs are depicted in Figure 3. Structures of compounds **16** and **22** are also shown in Figure 3. Rec. type is the abbreviation for recombinant receptor type.

Table 3. Modulation of GABA EC_{20} currents by compounds 16 and 22

Cpd.	Rec. type	EC_{50} value [μM]	η	Modulation [%]	n
16	α1β3	no effect ≤ 10 μM			3
	α1β3γ2	no effect ≤ 1 μM			3
	α1β3δ	**0.86 ± 0.10**	**1.0 ± 0.1**	**213 ± 8**	**3**
	α4β3	no effect ≤ 1 μM			2
	α4β3γ2	no effect ≤ 1 μM			3
	α4β3δ	**0.97 ± 0.35**	**1.2 ± 0.4**	**231 ± 76**	**3**
	α6β3	2.44 ± 0.82	3.1 ± 3.3	588 ± 254	3
	α6β3γ2	no effect ≤ 1 μM			5
	α6β3δ	**2.29 ± 0.67**	**0.9 ± 0.1**	**993 ± 519**	**5**
22	α1β3	0.10 ± 0.01	1.9 ± 0.3	35 ± 2	4
	α1β3γ2	2.01 ± 1.02	0.9 ± 0.2	139 ± 23	3
	α1β3δ	**0.10 ± 0.02**	**1.0 ± 0.1**	**201 ± 12**	**6**
	α4β3	0.06 ± 0.02	1.7 ± 0.7	168 ± 29	4
	α4β3γ2	no effect ≤ 1 μM			4
	α4β3δ	**0.15 ± 0.08**	**0.8 ± 0.2**	**297 ± 14**	**3**
	α6β3	0.17 ± 0.02	1.2 ± 0.1	1144 ± 29	7
	α6β3γ2	0.87 ± 0.15	1.3 ± 0.3	157 ± 10	7
	α6β3δ	**0.13 ± 0.03**	**1.5 ± 0.4**	**802 ± 68**	**7**

Increasing concentrations of compounds **16** and **22** were tested at different recombinant $GABA_AR$ in the presence of the respective GABA EC_{20} concentration. Highest tested concentration of each compound was 10 μM. EC_{50} values, pseudo-Hill coefficients η and percentage modulation values, derived from non-linear regression curve fits, are means ± S.E. from n separate experiments. Experimental and calculation procedures were as described under "Experimental section". Structures of compounds **16** and **22** are depicted in Figure 1. Rec. type is the abbreviation for recombinant receptor type.

Table 4. [^{3}H]EBOB modulation of several derivatives in the presence of GABA

Cpd.	Rec. type	IC_{50} value [μM]	p value	Max. inhibition [%]	p value	η	p value	n
22	α6β3	0.96 ± 0.36	0.660	96 ± 3	0.039	-1.88 ± 0.12	0.006	3
	α6β3γ2	1.09 ± 0.42	0.765	93 ± 3	0.760	-1.58 ± 0.03	0.151	3
	α6β3δ	**0.37 ± 0.17**	**0.688**	**88 ± 4**	**0.516**	**-0.93 ± 0.08**	**0.031**	**3**
29	α6β3	4.07 ± 0.36	n.a.	26 ±6	0.002	-7.27 ± 4.63	n.a.	3
	α6β3γ2	4.34 ± 1.18	n.a.	40 ± 5	0.006	-9.19 ± 7.12	n.a.	3
	α6β3δ	**0.87 ± 0.29**	**0.058**	**51 ± 1**	**0.484**	**-7.28 ± 5.76**	**0.465**	**3**
39	α6β3	1.29 ± 0.02	n.a.	57 ± 11	0.003	-3.61 ± 1.08	n.a.	3
	α6β3γ2	1.28 ± 0.10	n.a.	44 ± 7	0.001	-2.28 ± 0.70	n.a.	3
	α6β3δ	**0.49 ± 0.11**	**0.112**	**55 ± 8**	**0.795**	**-1.14 ± 0.20**	**0.012**	**3**

Experimental and calculation procedures were as described for Table 1 and under "Experimental section". Maximum inhibition, IC_{50} values and pseudo-Hill coefficients η were compared with values derived from experiments in the absence of GABA (Table 1). For statistical comparison unpaired Student's t test was used. n.a.: not applicable due to no effect of the compound in the absence of GABA. For the structures of the derivatives see Schemes 2 and 4. Rec. type is the abbreviation for recombinant receptor type.

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