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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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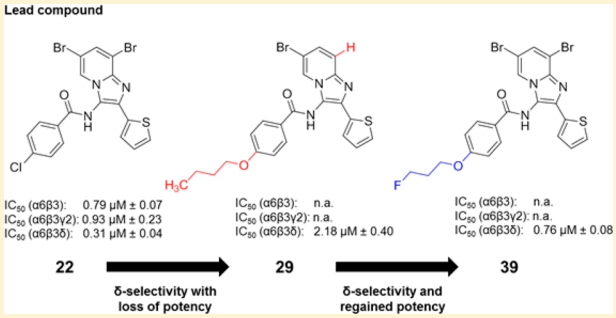
Supporting Information

ABSTRACT: δ-Selective compounds 1 and 2 (DS1, compound **22**; DS2, compound **16**) were introduced as functionally selective modulators of δ-containing GABA type A receptors ($GABA_AR$). In our hands, [^{3}H]EBOB-binding experiments with recombinant $GABA_AR$ and compound **22** showed no proof of δ-selectivity, although there was a minimally higher preference for the α4β3δ and α6β2/3δ receptors with respect to potency. In order to delineate the structural determinants of δ preferences, we synthesized 25 derivatives of DS1 and DS2, and investigated their structure−activity relationships (SAR). Four of our derivatives showed selectivity for α6β3δ receptors (**29**, **38**, **39**, and **41**). For all of them, the major factors that distinguished them from compound **22** were variations at the *para*-positions of their benzamide groups. However, two compounds (**29** and **39**), when tested in the presence of GABA, revealed effects 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 type A receptor ($GABA_AR$) system is a target for several molecules, including benzodiazepines, neurosteroids, barbiturates, propofol, volatile anesthetics, and putatively ethanol.[1−6] Their actions lead to anxiolytic, sedative, memory-modifying, anticonvulsant, and 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 compositions differ with different synaptic localizations as well as with the types 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 sensitivities 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 codistributed 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, for example, in thalamic relay cells, the neostriatum, the 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 α1ββδ receptors.[16] The major fraction of non-δ-containing receptors that are 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 coassembly and compositions of these receptors, native or

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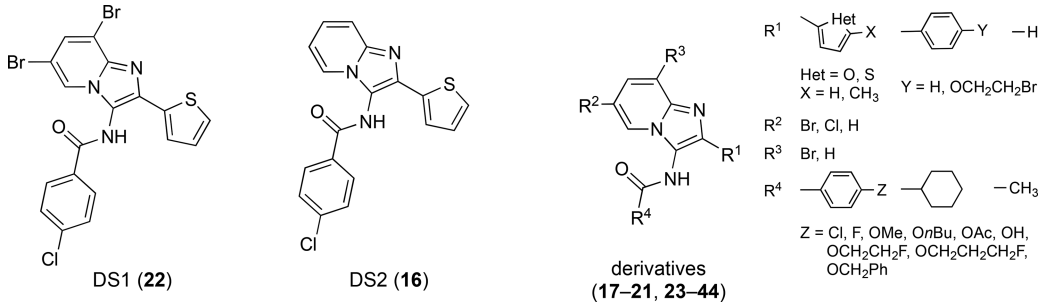
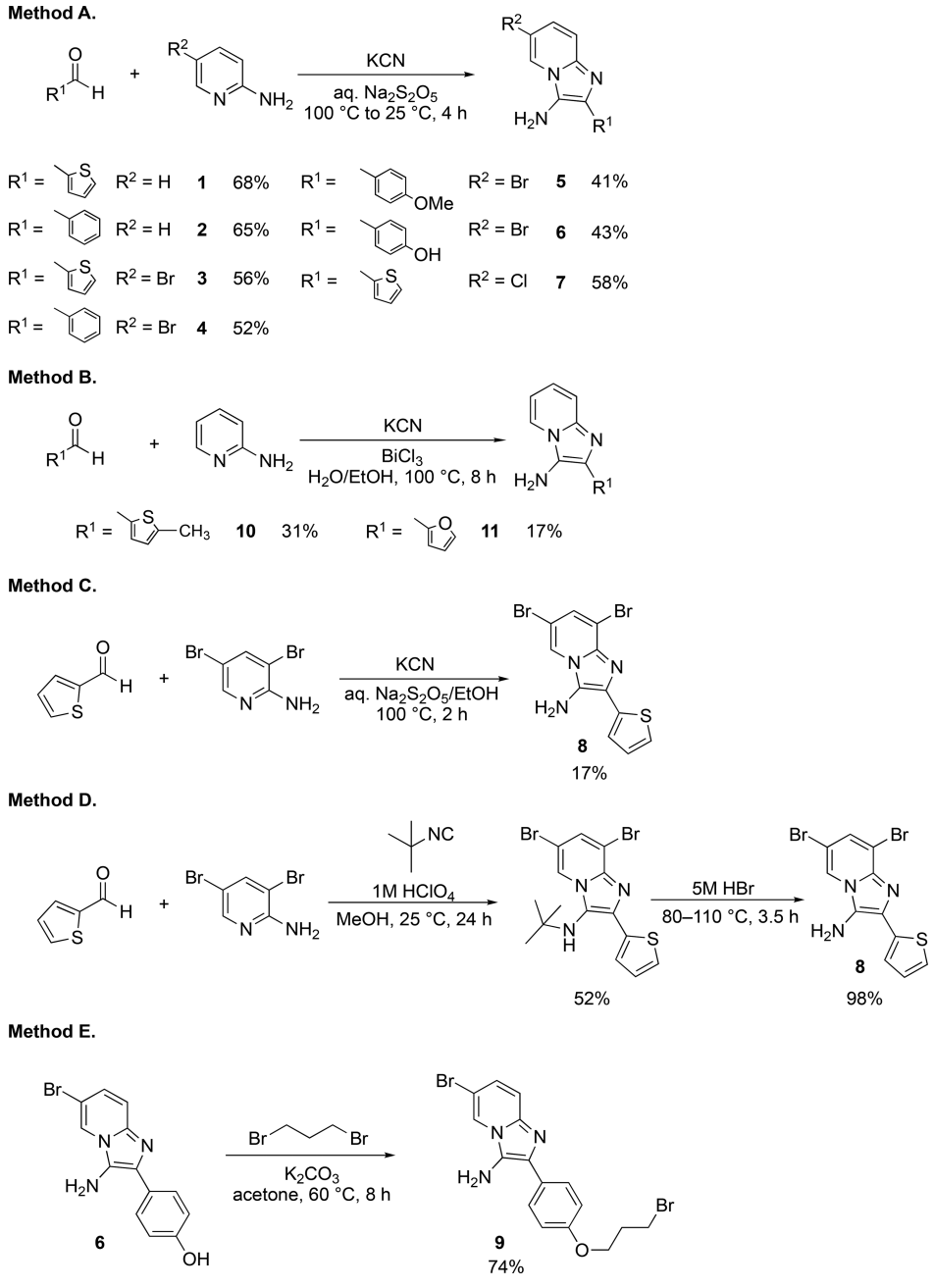


Figure 1. Structures of δ-selective compounds 1 and 2 (DS1 and DS2) and general structure of the derivatives synthesized for the structure–function evaluation.

Scheme 1. Syntheses of the 2-Aryl-imidazo[1,2-*a*]pyridin-3-amines



recombinant, as well as their detailed functions are not completely understood. These receptors may be linked to several disorders, such as alcoholism, stroke, depression, epilepsy, schizophrenia, and traumatic brain injury.[23,24] In

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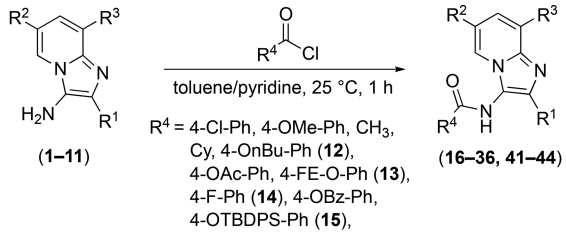
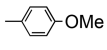
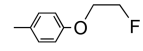
J. Med. Chem. 2018, 61, 1951–1968

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



	R1	R2	R3	R4	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

	R1	R2	R3	R4	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

[a]Compound **16** = DS2, compound **22** = DS1.

addition to the occurrence of δ-containing $GABA_AR$ in the brain, δ-subunit protein and mRNA have been detected in 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 their physiological processes, pharmacological interactions, assembly orders, 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 potencies at δ-containing $GABA_AR$, especially when they contain α4/6 and β3 subunits,[14] their selectivities are insufficient for detailed investigations. In 2009, the imidazopyridines δ-selective compounds 1 and 2 (DS1 and DS2) were introduced by Wafford et al. In electrophysiological experiments using human recombinant $GABA_AR$, the authors showed δ-selectivity for both compounds in the presence of α4 and β3 subunits.[28] Later experiments revealed GABA-enhancing effects of DS2 at additional δ-containing receptors (α1β2δ, α4β1/2/3δ, and α6β2δ), whereas 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 recombinant rat $GABA_AR$ as an assay for the modulatory effects of the studied compounds on $GABA_AR$ function. [^{3}H]EBOB as well as *t*-[^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS), both so-called "cage convulsants", are potent noncompetitive $GABA_AR$ antagonists that bind 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; for example, their binding can be 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 has also been shown in the absence of GABA, indicating that there is a direct effect on the conformation of the receptor.[32,35,38−40]

The correlation between the 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 profiles of allosteric agents for [^{35}S]TBPS- and [^{3}H]EBOB-binding modulation are 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 α6β2δ. In electrophysiological experiments, this was also shown for DS2 (compound **16**, Figure 1), though in both cases with higher effects on the δ-containing

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J. Med. Chem. 2018, 61, 1951−1968

receptors. Thus, we set out to identify the pharmacophore of these δ-preferring candidates. A systematic structure−activity-relationship (SAR study) was performed by the synthesis of 25 derivatives of DS1 and DS2 (Figure 1), followed by their characterization in radioligand-binding assays employing their abilities to modulate [^{3}H]EBOB binding.

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

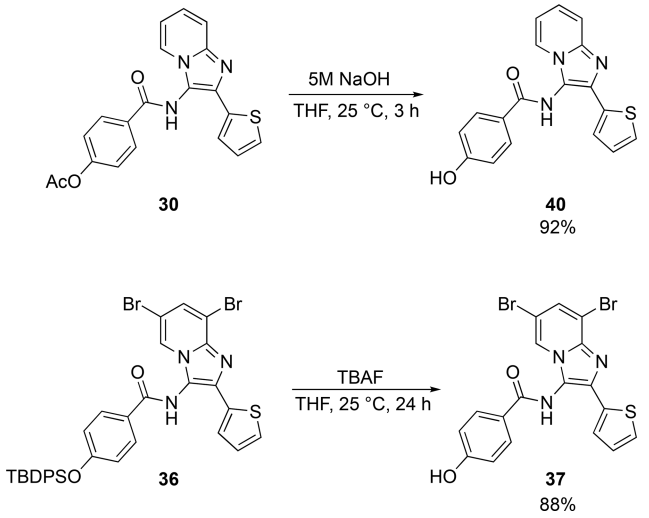
RESULTS AND DISCUSSION

Chemistry. The syntheses of the *N*-[2-arylimidazo[1,2-*a*]pyridin-3-yl]amides (**16**−**36**, **41**−**44**) were typically achieved in two-step procedures. 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 modified versions of the multicomponent reaction reported by van Niel et al. for the preparation of 8-methyl-2-thiophen-2-yl-imidazo[1,2-*a*]pyridin-3-ylamine (Scheme 1, Method A).[44] The aldehyde was converted into a bisulfite adduct with $Na_2S_2O_5$ and then refluxed with the respective 2-amino-pyridine to form the corresponding Schiff base. The addition of KCN to the reflux followed by a slow 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** (Scheme 1, Method B). For those, the reactions were performed in ethanol/water with added $BiCl_3$ to enhance the electrophilicities of the aldehydes. However, amine **8** could not be derived with the above procedures. The double bromination of the 2-amino-pyridine starting material likely resulted in the amine group having reduced nucleophilicity and poor solubility in an aqueous solution. In the first attempt, amine **8** was synthesized in a mixture of water/ethanol (2.25:1) and refluxed for several hours after the addition of KCN (Scheme 1, Method C), but the yields ranged between 9 and 17%. Therefore, the approach by Bienaymé et al. was envisaged for this compound (Scheme 1, Method D).[45] Bienaymé et al. used 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 the acidic removal of the *tert*-butyl group afterward. 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 the base (Scheme 1, Method E).

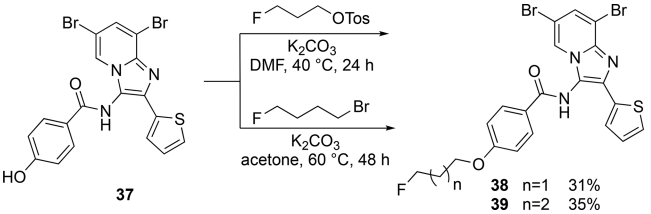
The second step in the reaction sequence was the amide-bond formation (Scheme 2). The amines, **1**−**11**, were reacted with the respective acid chlorides in mixtures of toluene and pyridine at room temperature, giving the desired *N*-[2-arylimidazo[1,2-*a*]pyridin-3-yl]amides (**16**−**36** and **41**−**44**) in good yields (60−80%). Attempts to form the amide bonds via standard coupling protocols (HBTU/TBTU, PPA, and IBCF) failed because of the low nucleophilicities of the aromatic amines. The compounds with free hydroxyl groups (**37** and **40**) were synthesized either from the acetoxy-protected precursor, **30**, or from the TBDPS-protected compound, **36** (Scheme 3).

Scheme 3. Syntheses of Compounds with a Hydroxyl Group in the *p*-Position of the Benzamide Part



The removal of the acetoxy group was accomplished with 5 M NaOH in THF at room temperature. The 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**, which have terminal fluorines, as analogues to compound **29** (Scheme 4).

Scheme 4. Syntheses of Compounds 38 and 39 with Terminally Fluorinated Alkyl Chains



Screening for DS Derivatives Directly Acting on δ Subunits. In order to identify the structural characteristics of a directly acting, δ-selective [^{3}H]EBOB modulator of $GABA_AR$, 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 the 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 on α1-, α4-, and α6-containing receptors, as in vivo δ subunits only occur with these three α subunits in addition to any of the three β variants.[11,13,15,16]

Structure−Activity Relationship. All of the studied compounds possessed imidazopyridine moieties as their core

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Table 1. [^{3}H]EBOB Modulation by the Test Compounds[a]

compd	receptor 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**

[a]Crude membranes derived from transiently transfected HEK 293 cells were incubated with increasing concentrations of the derivatives and 3 nM [^{3}H]EBOB. The highest tested concentrations were 10 μM because of the limited solubilities of the compounds, except for compounds **28** and **29**, which were tested up to 20 μM. The experimental and calculation procedures were as described in the Experimental Section. The IC_{50} values and pseudo-Hill coefficients (η) derived from nonlinear-regression-curve fits are means ± SE from n separate experiments. The maximum-inhibition values are means ± SD from n separate experiments. When n = 2, the maximum-inhibition values, IC_{50} values, and pseudo-Hill coefficients (η) were calculated as means of two experiments; the SD and SEM values were then omitted. The maximum inhibition of compound **28** for the $\alpha6\beta3\gamma2$ receptors is denoted as ≥89 because for the fitting process, the maximum inhibition of one experiment was constrained to 100%. The maximum inhibition describes the percentage decrease of [^{3}H]EBOB relative to the total binding, which is set to 100%. When no modulation of [^{3}H]EBOB binding could be observed at a ligand concentration of 10 μM (20 μM for **29**) in at least two separate experiments, "no effect" was noted. The derivatives that showed no effect on any tested $GABA_AR$ were not listed in this table. For the structures of the compounds, see Schemes 2 and 4. Receptor type refers to the recombinant receptor type.

structures. The lead compounds, **16** and **22**, were modified at three sites, that is, at their thiophene groups, at their chlorinated benzamide groups, and at the bromo substituents on their pyridine moieties, in order to investigate their structure−activity relationships. A broad range of derivatives (**16**−**21**, **23**−**27**, **30**, **32**−**33**, **40**, and **42**−**44**), including the published compound **16** could be dismissed after the first test on $\alpha6\beta3\delta$ $GABA_AR$, as they showed no effect in the absence of GABA.

The compounds that lacked the thiophene group (**44**) or whose thiophene groups had been replaced by phenyl (**17** and **19**), *p*-methoxyphenyl (**24**), bromopropoxyphenyl (**25**), furanyl (**42**), or methylthiophene groups (**43**) showed no [^{3}H]EBOB-binding modulation. This indicated that the thiophene group had an essential role in [^{3}H]EBOB-binding modulation.

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

The third site in the core scaffold of DS1 that was investigated in more detail was the *para*-position of the benzamide group. Compound **26**, which possessed an acetamide group instead of a benzamide group, showed no

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J. Med. Chem. 2018, 61, 1951−1968

effect, indicating that the benzamide group was indeed crucial for action. Interestingly, even with only one bromide substituent, compound **28**, with a cyclohexylcarboxamide group instead of a 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 analogues in which only the *para*-position of the benzamide group was varied, a fluoro atom (**35**) was tolerated, but methoxy (**23**) and fluoroethoxy (**34**) groups were not. However, elongating the carbon chain to a propoxy (**38**) or butoxy (**29** and **39**) group and fluorinating them in two cases (**38** and **39**) seemed to be beneficial for the molecules' activities and selectivities for δ-containing $GABA_AR$ in [^{3}H]EBOB-binding assays (Table 1). Compound **41** bore 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 might simply be due to the absence of bromide atoms. The rest of the active compounds, **31**, **35**, and **22**, did not display any δ-selectivity, but their preferences for most δ-containing $GABA_AR$ were revealed by their differential potencies (Table 1). However, in the case of compound **31**, these effects were not significant.

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

Modulation of [^{3}H]EBOB Binding by Compounds 22 and 16 and Comparisons 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 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 and Figure 2). Comparison of $\alpha4\beta3\delta$ with other $\alpha4$-containing receptors showed increased IC_{50} values of 7-fold for $\alpha4\beta3\gamma2$ receptors, which was significant ($p < 0.005$), and 4-fold for $\alpha4\beta3$ receptors, which nearly reached significance with $p = 0.051$ (Figure 2B). In the case of $\alpha6$- and $\beta2$-containing receptors, the increases were 4- and 7-fold ($p < 0.005$ for $\alpha6\beta2$ and $\alpha6\beta2\gamma2$) compared with the IC_{50} values for $\alpha6\beta2\delta$ $GABA_AR$ (Figure 2D). The smallest difference in potency was observed for the $\alpha6$- and $\beta3$-containing receptors, with only 2.5- to 3-fold shifts compared with the IC_{50} values for $\alpha6\beta3\delta$ receptors ($p < 0.005$ for $\alpha6\beta3$, $p < 0.05$ for $\alpha6\beta3\gamma2$; 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 and Figure 2). A slight modulation of [^{3}H]EBOB binding in $\alpha1$-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 of the tested compounds; however, a 2.5−7-fold preference for δ-containing receptors might be insufficient for investigational use.

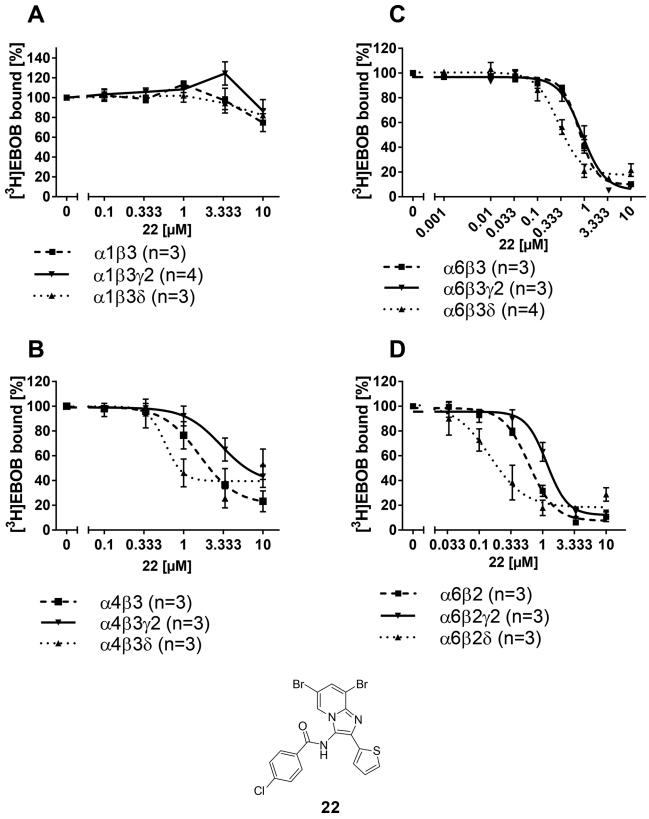

Figure 2. [^{3}H]EBOB-binding modulation by compound **22** at recombinant receptors consisting of $\alpha1\beta3 \pm \gamma2/\delta$ (A), $\alpha4\beta3 \pm \gamma2/\delta$ (B), $\alpha6\beta3 \pm \gamma2/\delta$ (C), and $\alpha6\beta2 \pm \gamma2/\delta$ (D). Crude membranes derived from transiently transfected HEK 293 cells were incubated with increasing concentrations of compound **22** and 3 nM [^{3}H]EBOB, the ligand. The experimental and calculation procedures were as described in the Experimental Section. The curves are depicted as the mean modulations ± SE of at least three separate experiments. The modulated-[^{3}H]EBOB-binding values refer to the total specific binding, which is set to 100%. The IC_{50} and maximum-inhibition values are listed in Table 1.

The results of [^{3}H]EBOB-binding modulation by **22** contradict the δ-selectivity of this compound reported 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 $\alpha4\beta2\gamma2$ receptors in the presence of GABA, as shown electrophysiologically.[46] In order to allow better comparisons between the published electrophysiological data and our findings from the [^{3}H]EBOB-binding assays, we also performed patch-clamp experiments with the lead compounds on recombinant rat $GABA_AR$. 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 data 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 on [^{3}H]EBOB-binding modulation. This approach supported our results from the [^{3}H]EBOB-binding-modulation experiments, as compound

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J. Med. Chem. 2018, 61, 1951−1968

16 was unable to sufficiently activate recombinant $GABA_AR$ in the absence of GABA (Table 2 and Figure 3A). Even at a concentration of 10 μM, **16** only slightly activated $\alpha4/6\beta3$ and $\alpha4/6\beta3\delta$ $GABA_AR$. In the presence of GABA at its $EC_{20,\ GABA}$, compound **16** modulated $\alpha1/4/6\beta3\delta$ receptors as well as $\alpha6\beta3$ ones (Table 3). These results not only confirm the assumption that compound **16** is solely a positive allosteric modulator[28] but also challenge the compound's 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 and Figure 3B). However, no δ-selectivity was observed as the compound was highly efficient with $\alpha4/6\beta\delta$ receptors as well as with $\alpha6\beta3$ and $\alpha6\beta3\gamma2$ receptors. The only discrepancy with the [^{3}H]EBOB-binding-modulation data is the lack of activation of the $\alpha4\beta3$ and $\alpha4\beta3\gamma2$ receptors in the electrophysiological experiments. In the presence of GABA at its $EC_{20,\ GABA}$, the effect of compound **22** on non-δ-containing receptors was even more pronounced (Table 3). The binary receptors $\alpha1/4/6\beta3$ especially exhibited EC_{50} values similar to those of the corresponding δ-containing ones.

Table 2. Direct Activation of Recombinant Receptors by Compounds 16 and 22[a]

compd	receptor type	$I_0/I_{maxGABA}$	n
16	$\alpha1\beta3$	0.042 ± 0.016	3
	$\alpha1\beta3\gamma2$	0.004 ± 0.002	3
	α1β3δ	**0.053 ± 0.072**	**4**
	$\alpha4\beta3$	0.093 ± 0.074	3
	$\alpha4\beta3\gamma2$	0.020 ± 0.019	3
	α4β3δ	**0.158 ± 0.162**	**4**
	$\alpha6\beta3$	0.124 ± 0.078	3
	$\alpha6\beta3\gamma2$	0.004 ± 0.002	3
	α6β3δ	**0.414 ± 0.069**	**3**
22	$\alpha1\beta3$	0.074 ± 0.029	3
	$\alpha1\beta3\gamma2$	0.058 ± 0.042	3
	α1β3δ	**0.323 ± 0.073**	**3**
	$\alpha4\beta3$	0.124 ± 0.021	3
	$\alpha4\beta3\gamma2$	0.163 ± 0.123	3
	α4β3δ	**1.615 ± 0.562**	**3**
	$\alpha6\beta3$	1.479 ± 0.318	6
	$\alpha6\beta3\gamma2$	1.119 ± 0.273	3
	α6β3δ	**1.043 ± 0.341**	**4**

[a]Concentrations of 10 μM of compounds **16** and **22** were tested with different recombinant $GABA_AR$ in the absence of GABA. The measured currents were correlated to the effect of 1 mM GABA on the equivalent receptor type ($I_0/I_{maxGABA}$). The experimental and calculation procedures were as described in the Experimental Section. The $I_0/I_{maxGABA}$ values are means ± SD from n separate experiments. The graphs are depicted in Figure 3. The structures of compounds **16** and **22** are also shown in Figure 3. Receptor type refers to the recombinant receptor type.

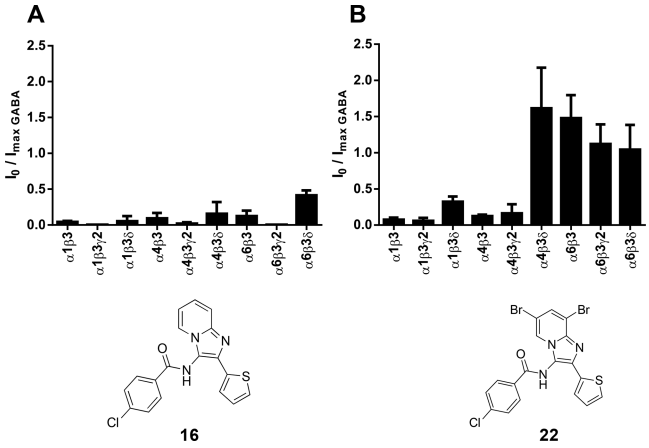

Figure 3. Direct activation of $GABA_AR$ by compounds **16** (A) and **22** (B) in whole-cell patch-clamp experiments. Both compounds were measured at concentrations of 10 μM with different recombinant receptors. The measured currents were correlated to the effect of 1 mM GABA on the equivalent receptor ($I_0/I_{maxGABA}$). The experimental and calculation procedures were as described in the Experimental Section. The $I_0/I_{maxGABA}$ values are means ± SD from n separate experiments and are listed in Table 2.

Table 3. Modulation of GABA $EC_{20,\ GABA}$ Currents by Compounds 16 and 22[a]

compd	receptor type	EC_{50} value [μM]	η	modulation [%]	n
16	$\alpha1\beta3$		no effect ≤10 μM		3
	$\alpha1\beta3\gamma2$		no effect ≤1 μM		3
	α1β3δ	**0.86 ± 0.10**	**1.0 ± 0.1**	**213 ± 8**	**3**
	$\alpha4\beta3$		no effect ≤1 μM		2
	$\alpha4\beta3\gamma2$		no effect ≤1 μM		3
	α4β3δ	**0.97 ± 0.35**	**1.2 ± 0.4**	**231 ± 76**	**3**
	$\alpha6\beta3$	2.44 ± 0.82	3.1 ± 3.3	588 ± 254	3
	$\alpha6\beta3\gamma2$		no effect ≤1 μM		5
	α6β3δ	**2.29 ± 0.67**	**0.9 ± 0.1**	**993 ± 519**	**5**
22	$\alpha1\beta3$	0.10 ± 0.01	1.9 ± 0.3	35 ± 2	4
	$\alpha1\beta3\gamma2$	2.01 ± 1.02	0.9 ± 0.2	139 ± 23	3
	α1β3δ	**0.10 ± 0.02**	**1.0 ± 0.1**	**201 ± 12**	**6**
	$\alpha4\beta3$	0.06 ± 0.02	1.7 ± 0.7	168 ± 29	4
	$\alpha4\beta3\gamma2$		no effect ≤1 μM		4
	α4β3δ	**0.15 ± 0.08**	**0.8 ± 0.2**	**297 ± 14**	**3**
	$\alpha6\beta3$	0.17 ± 0.02	1.2 ± 0.1	1144 ± 29	7
	$\alpha6\beta3\gamma2$	0.87 ± 0.15	1.3 ± 0.3	157 ± 10	7
	α6β3δ	**0.13 ± 0.03**	**1.5 ± 0.4**	**802 ± 68**	**7**

[a]Increasing concentrations of compounds **16** and **22** were tested with different recombinant $GABA_AR$ in the presence of the respective GABA $EC_{20,\ GABA}$ concentration. The highest tested concentration of each compound was 10 μM. The EC_{50} values, pseudo-Hill coefficients (η) and percentage-modulation values, derived from the nonlinear-regression-curve fits, are means ± SE from n separate experiments. The experimental and calculation procedures were as described in the Experimental Section. The structures of compounds **16** and **22** are depicted in Figure 1. Receptor type refers to the recombinant receptor type.

δ-Selectivity of Compound 29 and Effects of Bromo Substituents on Potency and Efficacy. Compound **29**, with its butoxy moiety attached to the benzamide group, showed selectivity for $\alpha6\beta2/3\delta$ receptors with IC_{50} values of 2.18 ± 0.40 μM and 2.43 ± 0.78 μM, respectively, with no measurable potency on $\alpha6\beta2/3$ or $\alpha6\beta2/3\gamma2$ receptors (Figure 4A,B). However, a marginal modulation of $\alpha6\beta3\gamma2$ receptors could be observed at a concentration of 10 μM (10 ± 4%). [^{3}H]EBOB binding in $GABA_AR$ containing $\alpha1$ and $\alpha4$ subunits was unaffected (Table 1).

Compound **31** represents the doubly brominated derivative of **29**. Compared with compound **29**, it showed increased potency and efficacy on $\alpha6\beta3\delta$ 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 $\alpha6$- and $\beta3$-containing receptors (Table 1). Compound **33**, as the

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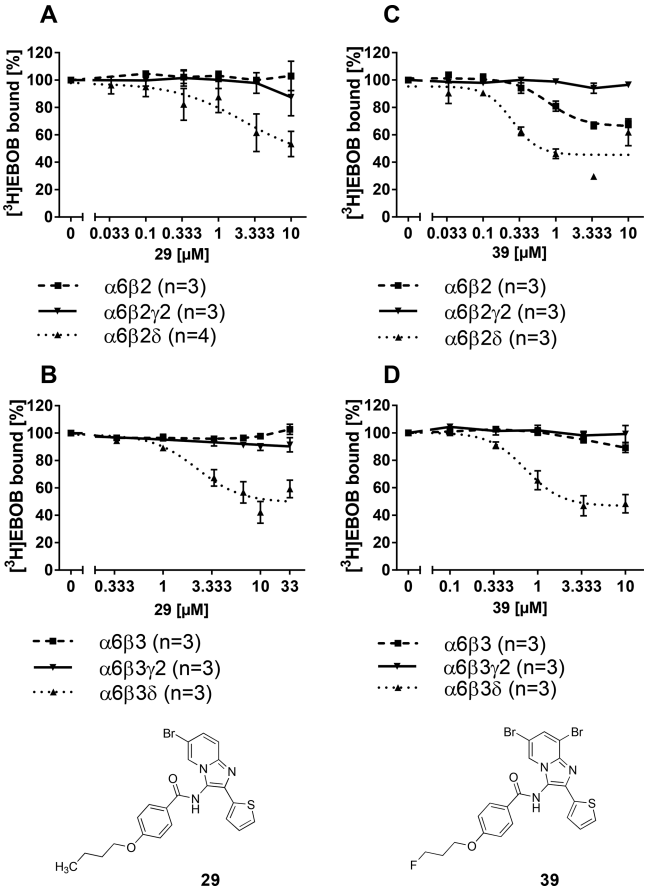

Figure 4. Modulation of [^{3}H]EBOB binding by compounds **29** and **39**. (A,B) [^{3}H]EBOB-binding modulation with recombinant receptors consisting of $\alpha6\beta2 \pm \gamma2/\delta$ (A) and $\alpha6\beta3 \pm \gamma2/\delta$ (B) by compound **29**. (C,D) Modulation of [^{3}H]EBOB binding with recombinant receptors consisting of $\alpha6\beta2 \pm \gamma2/\delta$ (C) and $\alpha6\beta3 \pm \gamma2/\delta$ (D) by compound **39**. The experimental procedures were as those described for Figure 2. The IC_{50} and maximum-inhibition values are listed in Table 1.

nonbrominated derivative of **29**, was devoid of any activity. The dissimilar results for these three compounds, only differing in the bromination state, underline the crucial role of bromination in a compound's action on $GABA_AR$.

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

Effect of GABA on the 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**, and **39**). Surprisingly, the potent effect of compound **22** on [^{3}H]EBOB binding to nearly all of the $\alpha6$- and $\beta3$-containing receptors tested was independent of GABA in the test solution (Table 4). Similar findings have previously been described in electrophysiological experiments of **22** with $\alpha4\beta3\delta$ receptors.[28]

Compounds **29** and **39** were both selective for $\alpha6\beta3\delta$ receptors in comparison with $\alpha6\beta3$ and $\alpha6\beta3\gamma2$ ones in the absence of GABA. However, both lost their selectivity in the presence of GABA (Table 4). Although the modulatory effects on $\alpha6\beta3$ and $\alpha6\beta3\gamma2$ receptors were enhanced in the presence of GABA, no significant effect on $\alpha6\beta3\delta$ receptor modulation was observed in either potency or efficacy. In the presence of GABA, compound **29** modulated $\alpha6\beta3$ receptors to a maximum effect of 26 ± 6% and $\alpha6\beta3\gamma2$ receptors to 40 ± 5%, and compound **39** modulated $\alpha6\beta3$ receptors to a maximal effect of 57 ± 11% and $\alpha6\beta3\gamma2$ receptors to 44 ± 7%. The effects of GABA on the efficacies of both compounds were highly significant (Table 4).

The fact that both compounds modulate additional receptors in the presence of GABA may be explained in several ways. One possibility is that GABA activates receptors leading to IC_{50} shifts and thus visualizes an effect that is obscured in its absence. That is a reasonable possibility for low-affinity ligands like **29** for which further shifts in potency between the $\alpha6\beta3\delta$ receptors and other $\alpha6$- and $\beta3$-containing receptors would result in IC_{50} values approaching the solubility limit. Alternatively or additionally, GABA could change the receptor conformation in such a way that it enables the compounds to enter the now available binding site. As a third option, the selective compounds may bind to $\alpha6\beta3$ and $\alpha6\beta3\gamma2$ 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

Table 4. [^{3}H]EBOB Modulation of Several Derivatives in the Presence of GABA[a]

compd	receptor type	IC_{50} value [μM]	p value	max inhibition [%]	p value	η	p value	n
22	$\alpha6\beta3$	0.96 ± 0.36	0.660	96 ± 3	0.039	−1.88 ± 0.12	0.006	3
	$\alpha6\beta3\gamma2$	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	$\alpha6\beta3$	4.07 ± 0.36	n.a.	26 ± 6	0.002	−7.27 ± 4.63	n.a.	3
	$\alpha6\beta3\gamma2$	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	$\alpha6\beta3$	1.29 ± 0.02	n.a.	57 ± 11	0.003	−3.61 ± 1.08	n.a.	3
	$\alpha6\beta3\gamma2$	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**

[a]The experimental and calculation procedures were as those described for Table 1 and in the Experimental Section. The maximum-inhibition values, IC_{50} values, and pseudo-Hill coefficients (η) were compared with the values derived from the experiments performed in the absence of GABA (Table 1). For the statistical comparisons, unpaired Student's t tests were used. If the compound had no effect in the absence of GABA, n.a. was used to indicate not applicable. For the structures of the derivatives, see Schemes 2 and 4. Receptor type refers to the recombinant receptor type.

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these possibilities was beyond the scope of this series of experiments.

Binding Sites and Binding Properties. The modulatory effects of the DS derivatives are clearly dependent on the α variant of $GABA_AR$. Receptors containing $\alpha6$ subunits are preferred by compound **22** over $\alpha4$-containing ones with regard to potency (Table 1). However, this effect was only significant for $\alpha4/6\beta3\gamma2$ receptors ($p = 0.008$). The δ-selective compounds **29** and **39** exclusively modulated $\alpha6$-containing receptors (Table 1). None of the tested compounds were able to modulate [^{3}H]EBOB binding to $\alpha1$-containing receptors. The β subunit seems to play a minor role, although for compound **39**, selectivity for δ-containing receptors was only observed in combination with the $\beta3$ variant (Table 1 and Figure 4C,D). For this compound, the potency for $\alpha6\beta2\delta$ receptors (0.27 ± 0.02 μM) was increased 3-fold ($p < 0.005$) compared with that for $\alpha6\beta3\delta$ 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 for $\alpha6\beta2\delta$ receptors. No significant difference could be measured for the $\alpha6\beta2/3$ ($p = 0.185$) and $\alpha6\beta2/3\gamma2$ ($p = 0.486$) receptor pairs. Further, compound **22** did not differentiate between $\beta2/3$-containing receptors regarding its efficacy (Table 1). Compound **29** did not distinguish between $\alpha6\beta2/3\delta$ receptors either in potency ($p = 0.812$) or in efficacy ($p = 0.615$). The significantly ($p < 0.05$) increased efficacy of compound **39** for $\alpha6\beta2$ receptors (33 ± 4%) in comparison with that for $\alpha6\beta3$ receptors (11 ± 6%), and the shifts in potency for the δ-containing receptors by compounds **22** and **39** indicate a slight influence of the β subunit on the modulatory properties of the mentioned compounds.

Compounds **22**, **28**, **35**, and **31** also modulated [^{3}H]EBOB binding in the absence of δ subunits but had preferences for most δ-containing receptors (Table 1); however, these effects were not significant for compound **31**. In the absence of GABA, compounds **29**, **38**, **39**, and **41** showed selectivity for $\alpha6\beta3\delta$ receptors, and compound **29** showed selectivity for $\alpha6\beta2\delta$ receptors as well (Table 1 and Figure 4). These results led to the question of whether the nonselective and δ-selective compounds address the same, additional, or completely different binding sites. 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 also extend the three possibilities to explain the differences in the selectivities in the absence and presence of GABA; that is, one binding site would be GABA-mimetic and thus enable access to the modulatory site.

Regarding the high efficacy (84 ± 8%) and potency (0.31 ± 0.04 μM) of compound **22** in comparison with those of compound **29** (46 ± 9%; 2.18 ± 0.40 μM) for $\alpha6\beta3\delta$ receptors, the question of a correlation between potency and efficacy arises. After comparing measurements of several compounds at $\alpha6\beta3\delta$ 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**, whose potency is especially low (10.1 ± 2.8 μM) but whose efficacy is quite high (85 ± 14%). In contrast, compound **39** showed a high potency (0.76 ± 0.08 μM) and only a modest efficacy (53 ± 12%). Thus, potencies and efficacies of the compounds in modulating $GABA_AR$ seem to be independently influenced by the structural compositions 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 instead showed 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 [^{3}H]EBOB binding to δ-containing $GABA_AR$. These compounds have comparable alkyl groups in the *para*-positions of their benzamide groups, which are the major differences between them and compound **22**, which possesses a chloro substituent at this position. Two of these compounds (**29** and **39**) were further investigated because of their promising modulatory effects on [^{3}H]EBOB binding. Both compounds showed selectivity in the absence of GABA for $\alpha6\beta3\delta$ receptors, and compound **29** also showed selectivity for $\alpha6\beta2\delta$ receptors. Besides the δ-subunit selectivity, both compounds also showed $\alpha6$ selectivity. In the presence of GABA, compounds **29** and **39** lost their δ-selectivity and modulated other $\alpha6$- and $\beta3$-containing receptors.

Because no correlations between the potencies and efficacies of the compounds for $\alpha6\beta3\delta$ 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 in further investigations on the assembly of $\alpha6\beta2/3\delta$ receptors.

EXPERIMENTAL SECTION

Syntheses. *General.* All reagents and solvents were of analytical-grade quality and purchased from Sigma-Aldrich, Alfa Aesar, Acros, or TCI. The commercial chemicals were used without further purification, unless otherwise noted. The 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, 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 the internal standard. DMSO-d_6, $CDCl_3$, or CD_3OD was used as the solvent. The chemical shifts (δ) are given in parts per million (ppm) using the residual proton peaks of the solvent as references (DMSO: 2.50 and 39.52 ppm, $CHCl_3$: 7.26 and 77.16 ppm, and MeOH: 3.31 and 49.00 ppm, each for ^{1}H and ^{13}C, respectively). 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) equipped with an AP-SMALDI 10 ion source (TransmitMIT) and operated at mass-resolving power of 140 000@m/z200 in positive-ion mode with MALDI ionization. FD-MS was measured on a Finnigan MAT 95 instrument. The purities of the compounds were determined using analytical reverse-phase HPLC and were >95% for all of the compounds. Analytical HPLC was executed with a 250 × 4.6 mm Kromasil 100 C18 7 μm column from MZ-Analysentechnik. The pump was a PK-2080 from Jasco. The detector was a UV-2075 Plus from Jasco. The detection wavelength was $\lambda = 280$ 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 a Chromolith SpeedROD RP-18 column (4.6 × 50 mm) with an elution flow rate of 4.0 mL/min. A linear-gradient elution was performed with a solution of eluent A (H_2O/TFA 100:0.1) containing 0% solvent B (MeCN/H_2O/TFA, 90:10:0.01) that rose to 100% B over 5 min. Column chromatography was performed with silica gel

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(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.* $Na_2S_2O_5$ (0.015 mol, 0.5 equiv) was dissolved in 80 mL of distilled water. Then, 0.03 mol (1.0 equiv) of the respective aldehyde was added, and the mixture was stirred for 30 min at room temperature (rt). After the addition of 0.03 mol (1.0 equiv) 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 equiv) KCN was added in one portion. After being stirred for 1 h at 80 °C, the mixture was cooled to rt and stirred for an additional 3 h. Then, 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 of cooled (−18 °C) ethyl acetate. Recrystallization of the crude product with 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**).* Compound **1** was synthesized following the general procedure using 2.85 g of Na_2S_2O5, 3.36 g of thiophen-2-carbaldehyde, 2.82 g of 2-amino-pyridine, and 1.95 g of 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**).* Compound **2**[47] was synthesized following the general procedure using 2.85 g of $Na_2S_2O_5$, 3.18 g of benzaldehyde, 2.82 g of 2-amino-pyridine, and 1.95 g of 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**).* Compound **3** was synthesized following the general procedure using 2.85 g of $Na_2S_2O_5$, 3.36 g of thiophen-2-carbaldehyde, 5.19 g of 2-amino-5-bromo-pyridine, and 1.95 g of 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**).* Compound **4**[48] was synthesized following the general procedure using 2.85 g of $Na_2S_2O_5$, 3.18 g of benzaldehyde, 5.19 g of 2-amino-5-bromo-pyridine, and 1.95 g of 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**).* Compound **5** was synthesized following the general procedure using 2.85 g of $Na_2S_2O_5$, 4.08 g of 4-methoxy-benzaldehyde, 5.19 g of 2-amino-5-bromo-pyridine, and 1.95 g of 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**).* Compound **6** was synthesized following the general procedure using 2.85 g of $Na_2S_2O_5$, 3.66 g of 4-hydroxybenzaldehyde, 5.19 g of 2-amino-5-bromo-pyridine, and 1.95 g of 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**).* Compound **7** was synthesized following the general procedure using 2.85 g of $Na_2S_2O_5$, 3.36 g of thiophen-2-carbaldehyde, 3.86 g of 2-amino-5-chloro-pyridine, and 1.95 g of 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.* $Na_2S_2O_5$ (0.01 mol, 0.5 equiv) was dissolved in 90 mL of distilled water, and 0.02 mol (1.0 equiv) of thiophen-2-carbaldehyde was added. The resulting solution was stirred at rt for 30 min. Then, 36 mL of EtOH and 0.02 mol (1.0 equiv) of 2-amino-3,5-dibromopyridine were added, and the mixture was refluxed for 2 h. KCN (0.02 mol, 1.0 equiv) was added in small portions to the refluxing mixture. After being heated at reflux for 2 h, the mixture was allowed to cool to rt and stirred for an 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 a 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). Thiophen-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-(thiophen-2-yl)imidazo[1,2-*a*]-pyridin-3-amine as yellow crystals (888 mg, 2.07 mmol) with a yield of 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 and then at 80 °C for 1.5 h. After alkalization with a 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 a yellow solid (610 mg, 1.64 mmol) with a yield of 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**).* Compound **6** (0.3 g, 1 mmol, 1.0 equiv), 0.4 g (0.2 mL, 2 mmol, 2.0 equiv) of 1,3-dibromopropane, and 0.17 g (1.2 mmol, 1.2 equiv) of K_2CO_3 were refluxed in 10 mL of acetone for 8 h. The acetone was removed in vacuo, and the residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate, 1:1) yielding **9** as a 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,

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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**).* 2-Aminopyridine (229 mg, 2.43 mmol), KCN (164 mg, 2.52 mmol), and $BiCl_3$ (37 mg, 0.117 mmol) were added 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). 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 (EtOAc/heptane, 7:3) yielding the product as an orange semisolid (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-Methylthiophen-2-yl)imidazo[1,2-a]pyridin-3-amine (**11**).* $BiCl_3$ (250 mg, 0.792 mmol) was added to a solution of 5-methylthiophen-2-carbaldehyde (0.87 mL, 7.93 mmol) in EtOH (10 mL) to form a suspension, which was stirred at ambient temperature for 20 min. This was followed by the 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 × 200 mL). The combined organic phase was dried over $MgSO_4$ and purified by flash column chromatography (EtOAc/heptane, 8:2). 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**).* Methyl-4-hydroxybenzoate (5.0 g, 0.033 mol, 1.0 equiv), 6.75 g (0.05 mol, 1.5 equiv) of 4-bromobutane, and 5.5 g (0.04 mol, 1.2 equiv) of K_2CO_3 were refluxed in 65 mL of acetone for 10 h. The solution was cooled to rt, and the precipitated inorganic salts were filtered off before the 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 a 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. Methyl-4-butoxybenzoate (1.0 g, 5 mmol, 1.0 equiv) and 1.0 g (25 mmol, 5 equiv) of NaOH in 10 mL of distilled water were refluxed for 2 h. After being cooled to rt, the solution was acidified to pH 1 by the 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 a 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. 4-Butoxybenzoic acid (0.7 g) was dissolved in 3 mL of $SOCl_2$ and refluxed for 2 h. Then, the $SOCl_2$ was removed in vacuo yielding **12**[49,50] as a light-yellow oil, which was used directly in the next step without further purification or characterization.

*4-(2-Fluoroethoxy)benzoyl Chloride (**13**).* 4-Hydroxybenzoic acid (200 mg, 1.45 mmol) and a 40% aqueous n-Bu_4POH solution (2.02 mL, 2.896 mmol) were 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 a 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) with a yield of 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). The spectral data matched that reported in the 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 an inert atmosphere. After being heated at 85 °C for 4 h, the excess of the thionyl chloride was removed under a high vacuum to give **13**[51] as a light-yellow oil. It was used without further purification or characterization.

*4-Fluorobenzoyl Chloride (**14**).* Thionyl chloride (77.7 μL, 1.07 mmol) was added dropwise to 4-fluorobenzoic acid (50.0 mg, 0.357 mmol) under an inert atmosphere. After being heated at 85 °C for 1.5 h, the excess of the thionyl chloride was removed under a high vacuum to give the desired product, **14**,[52] as colorless oil at room temperature that sets under cooling. It was used without further purification or characterization in the next step.

*4-((tert-Butyldiphenylsilyl)oxy)benzoyl Chloride (**15**).* *tert*-Butylchlorodiphenylsilane (2.77 mL, 10.7 mmol) was added to a solution of 4-hydroxybenzaldehyde (1.00 g, 8.20 mmol) and imidazole (1.45 g, 31.3 mmol) in DMF (15 mL), and the mixture was stirred for 42 h at room temperature. After repeated additions 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 the solution was 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 (cyclohexane/ethyl acetate, 30:1) gave 4-((*tert*-butyldiphenylsilyl)oxy)benzaldehyde as colorless crystals (2.13 g, 5.92 mmol) with a yield of 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, 1:1) before tetrabutylammonium hydrogen sulfate (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 the extraction of the aqueous phase with dichloromethane (2 × 20 mL), the organic layers were dried with Na_2SO_4, and the solvent was removed in vacuo. Purification by column chromatography (cyclohexane/ethyl acetate, 9:1 + 3% formic acid) gave 4-((*tert*-butyldiphenylsilyl)oxy)benzoic acid as a colorless solid (74 mg, 0.197 mmol) with a yield of 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 an inert atmosphere and stirred for 3 h at room temperature. Excess oxalyl chloride was removed under a high vacuum to yield **15**[53] as a 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.* The respective 2-aryl-imidazo[1,2-*a*]pyridin-3-amines (**1**−**9**, 1.7 mmol, 1.0 equiv) were dissolved in 8 mL of dry toluene and 4 mL of dry pyridine. Then, 1.9 mmol (1.1 equiv) of the specified acid chloride was added in one portion, and the resulting mixture was stirred for 1 h at rt. Distilled water (6 mL) was added, and the solution was stirred for an additional 15 min. The solution was cooled in an ice bath, and the precipitate was collected by vacuum filtration and 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-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl]-benzamide (**16**).* Compound **16**[44] was synthesized following the

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J. Med. Chem. 2018, 61, 1951−1968

general procedure using 0.37 g of **1** and 0.33 g of 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%. The spectral data matched that reported in the literature.[44]

*4-Chloro-N-(2-phenylimidazo[1,2-a]pyridin-3-yl)benzamide (**17**).* Compound **17** was synthesized following the general procedure using 0.36 g of **2** and 0.33 g of 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-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl]-4-chlorobenzamide (**18**).* Compound **18** was synthesized following the general procedure using 0.50 g of **3** and 0.33 g of 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 $_2$O, 1:1) = 18.07 min, purity: 98%.

*N-(6-Bromo-2-phenylimidazo[1,2-a]pyridin-3-yl)-4-chlorobenzamide (**19**).* Compound **19** was synthesized following the general procedure using 0.49 g of **4** and 0.33 g of 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-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl]-benzamide (**20**).* Compound **20**[44] was synthesized following the general procedure using 0.37 g of **1** and 0.32 g of 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%. The spectral data matched that reported in the literature.[44]

*N-[6-Bromo-2-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl]-4-methoxybenzamide (**21**).* Compound **21** was synthesized following the general procedure using 0.50 g of **3** and 0.32 g of 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-(thiophen-2-yl)imidazo[1,2-a]-pyridin-3-yl]benzamide (**22**).* Compound **22**[44] was synthesized following the general procedure using 0.63 g of **8** and 0.33 g of 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%. The spectral data matched that reported in the literature.[44]

*N-[6,8-Dibromo-2-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl]-4-methoxybenzamide (**23**).* Compound **23**[44] was synthesized following the general procedure using 0.63 g of **8** and 0.32 g of 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%. The spectral data matched that reported in the literature.[44]

*N-[6-Bromo-2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl]-4-chlorobenzamide (**24**).* Compound **24** was synthesized following the general procedure using 0.54 g of **5** and 0.33 g of 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**).* Compound **25** was synthesized following the general procedure using 0.72 g of **9** and 0.33 g of 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-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl]-acetamide (**26**).* Compound **26** was synthesized following the general procedure using 0.50 g of **3** and 0.15 g of 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,

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J. Med. Chem. 2018, 61, 1951−1968

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-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl]benzamide (**27**).* Compound **27**[44] was synthesized following the general procedure using 0.42 g of **7** and 0.33 g of 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. ^1H 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-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl]-cyclohexancarboxamide (**28**).* Compound **28** was synthesized following the general procedure using 0.50 g of **3** and 0.28 g of 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. ^1H 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-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl]-4-butoxybenzamide (**29**).* Compound **29** was synthesized following the general procedure using 0.50 g of **3** and 0.28 g of **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. ^1H 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-(Thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl)carbamoyl)-phenyl Acetate (**30**).* Compound **30** was synthesized following the general procedure using 197 mg of **1** and 200 mg of 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. ^1H 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-(thiophen-2-yl)imidazo[1,2-a]-pyridin-3-yl)benzamide (**31**).* Compound **31** was synthesized following the general procedure using 200 mg of **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. ^1H 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-(thiophen-2-yl)-imidazol[1,2-α]pyridin-3-yl)benzamide (**32**).* Compound **32** was synthesized following the general procedure using 0.50 g of **7** and 0.64 g of **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. ^1H 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-(thiophen-2-yl)-imidazol[1,2-α]pyridin-3-yl)-benzamide (**33**).* Compound **33** was synthesized following the general procedure using 0.30 g of **1** and 0.45 g of **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. ^1H 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-(thiophen-2-yl)imidazo[1,2-a]-pyridin-3-yl)-4-(2-fluoroethoxy)benzamide (**34**).* Compound **13** (93.4 mg, 0.461 mmol) was added 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 an inert atmosphere, 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 being cooled in an ice bath, the precipitate was filtered off and washed with cold water. Purification of the crude product by column chromatography (gradient of hexane/ethyl acetate from 1:1 to 0:1) yielded **34** as colorless solid (132.6 mg, 0.25 mmol) with a yield of 70%. ^1H 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-(thiophen2-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 an inert atmosphere before **14** (60.0 μL, 0.508 mmol) was added. The mixture was stirred for 69 h, and then dichloromethane (10 mL) was added. The organic phase was washed with equal volumes 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 of cyclohexane/ethyl acetate from 6:1 + 3% triethylamine to 1:1 + 3% triethylamine) to yield **35** as a colorless solid (89.8 mg, 0.181 mmol) with a yield of 91%. ^1H 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-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl)benzamide (**36**).* Com-

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pound **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 an 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 a colorless solid (271 mg, 0.370 mmol) with a yield of 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-(thiophen-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 the removal of the solvent under reduced pressure, the crude product was obtained. Purification by column chromatography (gradient from 4:1 cyclohexane/ethyl acetate + 3% formic acid to 100% ethyl acetate + 3% formic acid) yielded product **37** as a colorless solid (76.6 mg, 0.155 mmol) with a yield of 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-(thiophen-2-yl)imidazo[1,2-a]-pyridin-3-yl)-4-(3-fluorpropoxy)benzamide (**38**).* 4-Methylbenzenesulfonyl chloride (839 mg, 4.40 mmol) was added portion-wise and pyridine (360 μL, 4.40 mmol, 1.10 equiv) was added dropwise to a solution of 3-fluoropropan-1-ol (324 μL, 4.00 mmol) in dichloromethane (4 mL) under ice cooling. The reaction mixture was then stirred for 7 h at room temperature. After the addition of water (20 mL) and a 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 the purification of the crude product by column chromatography (cyclohexane/ethyl acetate, 7:1), which yielded 3-fluoropropyl 4-methylbenzenesulfonate as a highly viscous, colorless oil (684 mg, 2.95 mmol) with a yield of 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 an 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 a colorless solid (17.5 mg, 0.0316 mmol) with a yield of 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-(thiophen-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 a 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 a colorless solid (15.3 mg, 0.0270 mmol) with a yield of 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-(thiophen-2-yl)imidazo[1,2-a]-pyridin-3-yl)benzamide (**40**).* Compound **30** (0.15 g) was dissolved in 3 mL of THF and 3 mL of 5 M NaOH was 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 of H_2O. The solution was acidified to pH 1 by the addition of 3 M HCl. The precipitate formed was collected by vacuum filtration and dried in vacuo. The product was obtained as a 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-(thiophen-2-yl)imidazo[1,2-a]pyridin-3-yl)-benzamide (**41**).* Compound **1** was reacted with 4-benzyloxybenzoyl chloride 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 a nitrogen atmosphere, and the reaction mixture was stirred for 5 min, followed by the 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 × 100 mL). The combined organic phase was washed with H_2O and brine, and 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 (EtOAc/heptane, 8:2 + 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,

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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-methylthiophen-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 a nitrogen atmosphere. Pyridine was added and the solution was stirred for 5 min. This was followed by the addition of 4-chlorobenzoyl chloride (0.19 mL, 1.51 mmol), and then the reaction was stirred at ambient temperature for 17 h and then at 50 °C for 30 min. After the solution was cooled to rt, H_2O (10 mL) was added, and the aqueous phase was extracted with EtOAc (1 × 150 mL, 1 × 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 (EtOAc/heptane, 2:1). 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**).* $BiCl_3$ (394 mg, 1.25 mmol) was added to a solution of formaldehyde (37% in H_2O, 0.94 mL, 12.6 mmol) in EtOH (10 mL) to form a white suspension, which was stirred at ambient temperature for 20 min. 2-Aminopyridine (1.2 g, 12.7 mmol) was added to the reaction mixture, and the reaction was refluxed for 1.5 h, followed by the 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 (DCM/MeOH, 9:1) to afford a yellow oil that 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 a nitrogen atmosphere. This was followed by the 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 × 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 (DCM/MeOH, 9.5:0.5) to afford a mixture of a white solid and a 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) were grown to <50% confluency on 15 cm tissue plates in 20 mL of DMEM supplemented with 10% heat-inactivated fetal calf serum and 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, the plasmids were diluted in 1 mL of 0.3125 M $CaCl_2$ in H_2O per plate. HBS (2×; 274 mM NaCl, 1.5 mM Na_2HPO_4, 54.6 mM HEPES/NaOH, pH 7.0; 1 mL/plate) was added to the DNA and incubated for 90 s. Each mixture (2 mL) was pipetted immediately onto 15 cm plates. These were incubated for 18−24 h before the transfection medium was replaced with 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 the optimal receptor expressions, the final concentrations were 2.5, 12, 2.5, 12, 0.5, 0.375, and 2.5 μg of vector DNA per 15 cm tissue-culture plate for α1, α4, α6, β2, β3, γ2S, and δ, respectively.

The cell membranes were prepared as previously described.[58] Briefly, the cells were washed in PBS and harvested from the tissue plates 48 h after transfection. The cells were homogenized in an Ultraturrax homogenizer for 15 s. Crude membranes were obtained after two centrifugation steps at 23 000g. The pellets were used immediately or frozen at −20 °C. The membrane pellets were resuspended in 50 mM Tris/citrate buffer (pH 7.3). The resuspended cell membranes (150−200 μg of protein per tube) were incubated in final volumes of 0.5 mL of 50 mM Tris/citrate buffer (pH 7.3) supplemented with 0.2 M NaCl, 3 nM [^{3}H]EBOB (PerkinElmer), and increasing concentrations of DS compounds in presence (0.1 μM for $\alpha6\beta3\gamma2/\delta$ and 0.5 μM for the $\alpha6\beta3$ receptors) or absence of GABA. The 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). Nonspecific binding of [^{3}H]EBOB was determined by the addition of 10 μM of EBOB. The binding assay procedure was essentially performed as described earlier,[58] though in the cited publication, the binding assays were executed with [^{35}S]TBPS. As the allosteric interactions of tested ligands on the binding properties of both of the convulsants have proven to be similar,[31,34,35] we adopted the procedure for our assays with [^{3}H]EBOB. Briefly, after addition of [^{3}H]EBOB, the cell membranes were incubated at room temperature for 90 min. We performed [^{3}H]EBOB-binding assays under these pre-equilibrium conditions (90 min incubations at 21 °C). The pre-equilibrium conditions were previously investigated, applied to [^{35}S]TBPS binding studies,[32] and adapted to [^{3}H]EBOB-binding experiments.[40] The assay mixtures were then rapidly diluted to 5 mL with ice-cold 10 mM Tris/HCl (pH 7.5) and filtered through glass microfiber filters of grade GF/C (GE Healthcare). The procedure was repeated once. The filters were incubated in 3.5 mL of Aquasafe 300 Plus scintillation fluid (Zinsser Analytic). The radioactivity was determined in a Beckman liquid scintillation counter using external standardization. Protein quantification was performed according to the Bradford method[59] using a Roti-Quant 5× concentrate (Roth).

Electrophysiology. HEK 293 cells were grown on glass coverslips and transiently transfected with final concentrations of 1, 5, 1, 0.2, 0.4, and 1.5 μg of vector DNA per 94 mm tissue-culture plate for α1, α4, α6, β3, γ2, and δ, respectively, and a green fluorescent protein (eGFP, Clontech) at a concentration of 0.5. Twenty-four hours after the transfection, the medium was replaced with fresh solution, and 48 h after the transfection, the coverslips were transferred to a recording chamber under an upright microscope (Zeiss Axioskop FS). The 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 pipet 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. The drugs were applied using a fast-perfusion stepper system (SF-77B, Warner Instruments, Inc.) enabling solution-exchange times of <20 ms (controlled by junction-potential measurements). The DS compounds were applied alone or in test solutions containing GABA at the receptor specific EC_{20} of GABA ($EC_{20,\ GABA}$) for 4 s with 60 s between successive applications. The 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 an EPC-9 patch-clamp amplifier and a Pulse 8.11 (HEKA electronics).

Calculation. *[^{3}H]EBOB-Binding Analysis.* Nonlinear regression was performed in Prism, version 6.05, with the four-parametric logarithmic dose−response equation formula (y = min + (max − min)/(1 + $10^{((\mathrm{LogIC}_{50}-x)\times\eta)}$)) with min and max being the minimum and maximum values, x being the concentration of the derivative in μM, and η being the Hill coefficient. The term "Hill coefficient" should be read as "pseudo-Hill coefficient" when dealing with allosteric as

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J. Med. Chem. 2018, 61, 1951−1968

opposed to direct effects. The value in the absence of any modulator was defined as 100%; the blank value, that is, the value in the presence of an excess of unlabeled EBOB, was set as 0%. The maximum was set constant to 100%, and the minimum was fitted. The data are given as the means ± SEM for the IC_{50} curves and as the means ± SD for the effects of single concentrations. For the statistical comparison, Student's t test was used; * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$.

Electrophysiology. Data analysis was performed using ClampFit 8.1 (Axon Instruments) and Origin 8.5 (Microcal). Direct activation by compounds **16** and **22** was expressed by the ratio $I_{X(1\mu M)}/I_{GABA(1mM)}$. To calculate the concentrations eliciting 50% of the maximal modulation of the GABA-induced currents (EC_{50}), the peak currents (I_{max}) were plotted against the ligand concentrations (LIG) and fitted with the logistic function $y = I_{min} + (I_{max} - I_{min})/(1 + ([LIG]/EC_{50})^{\eta})$ using a least-squares fitting routine, where I_{min} represents the current induced by the receptor-specific $EC_{20,\ GABA}$, I_{max} represents the maximal modulation, and η represents the Hill coefficient. As above, the term "Hill coefficient" should be read as "pseudo-Hill coefficient" when dealing with allosteric as opposed to direct effects. The responses of compounds **16** and **22** were expressed as percentages of the control responses.

It should be explicitly mentioned that in this manuscript, all of the shown Hill coefficients should be read as pseudo-Hill coefficients. True Hill coefficients reflect the number of binding sites of a ligand that induces the measured effect; 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; it is independent of the number of binding sites. It has to be called a pseudo-Hill coefficient as the mathematics behind its determination are identical to that of the true Hill coefficient.

■ ASSOCIATED CONTENT

Supporting Information

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Molecular-formula strings (CSV)

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Notes

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■ ABBREVIATIONS USED

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

■ REFERENCES

(1) Rudolph, U.; Antkowiak, B. Molecular and neuronal substrates for general anaesthetics. *Nat. Rev. Neurosci.* **2004**, *5* (9), 709−720.

(2) Rudolph, U.; Knoflach, F. Beyond classical benzodiazepines: novel therapeutic potential of $GABA_A$ receptor subtypes. *Nat. Rev. Drug Discovery* **2011**, *10* (9), 685−697.

(3) Korpi, E. R.; Gründer, G.; Lüddens, H. Drug interactions in $GABA_A$ receptors. *Prog. Neurobiol.* **2002**, *67* (2), 113−159.

(4) Roberto, M.; Varodayan, F. P. Synaptic targets: chronic alcohol actions. *Neuropharmacology* **2017**, *122*, 85−99.

(5) Wang, M. Neurosteroids and GABA-A receptor function. *Front. Endocrinol. (Lausanne, Switz.)* **2011**, *2*, 44.

(6) Zurek, A. A.; Yu, J.; Wang, D. S.; Haffey, S. C.; Bridgwater, E. M.; Penna, A.; Lecker, I.; Lei, G.; Chang, T.; Salter, E. W.; Orser, B. A. Sustained increase in α5$GABA_A$ receptor function impairs memory after anesthesia. *J. Clin. Invest.* **2014**, *124* (12), 5437−5441.

(7) Farrant, M.; Nusser, Z. Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. *Nat. Rev. Neurosci.* **2005**, *6* (3), 215−229.

(8) Pirker, S.; Schwarzer, C.; Wieselthaler, A.; Sieghart, W.; Sperk, G. $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* **2000**, *101* (4), 815−850.

(9) Sieghart, W.; Sperk, G. Subunit composition, distribution and function of $GABA_A$ receptor subtypes. *Curr. Top. Med. Chem.* **2002**, *2* (8), 795−816.

(10) Wisden, W.; Korpi, E. R.; Bahn, S. The cerebellum: a model system for studying $GABA_A$ receptor diversity. *Neuropharmacology* **1996**, *35* (9−10), 1139−1160.

(11) Jones, A.; Korpi, E. R.; McKernan, R. M.; Pelz, R.; Nusser, Z.; Makela, R.; Mellor, J. R.; Pollard, S.; Bahn, S.; Stephenson, F. A.; Randall, A. D.; Sieghart, W.; Somogyi, P.; Smith, A. J.; Wisden, W. Ligand-gated ion channel subunit partnerships: $GABA_A$ receptor α6 subunit gene inactivation inhibits δ subunit expression. *J. Neurosci.* **1997**, *17* (4), 1350−1362.

(12) Chandra, D.; Jia, F.; Liang, J.; Peng, Z.; Suryanarayanan, A.; Werner, D. F.; Spigelman, I.; Houser, C. R.; Olsen, R. W.; Harrison, N. L.; Homanics, G. E. $GABA_A$ receptor α4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (41), 15230−15235.

(13) Sur, C.; Farrar, S. J.; Kerby, J.; Whiting, P. J.; Atack, J. R.; McKernan, R. M. Preferential coassembly of α4 and δ subunits of the γ-aminobutyric $acid_A$ receptor in rat thalamus. *Mol. Pharmacol.* **1999**, *56* (1), 110−115.

(14) Olsen, R. W.; Sieghart, W. International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric $acid_A$ receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol. Rev.* **2008**, *60* (3), 243−260.

(15) Villumsen, I. S.; Wellendorph, P.; Smart, T. G. Pharmacological characterisation of murine α4β1δ $GABA_A$ receptors expressed in Xenopus oocytes. *BMC Neurosci.* **2015**, *16*, 8.

(16) Glykys, J.; Peng, Z.; Chandra, D.; Homanics, G. E.; Houser, C. R.; Mody, I. A new naturally occurring $GABA_A$ receptor subunit partnership with high sensitivity to ethanol. *Nat. Neurosci.* **2007**, *10* (1), 40−48.

DOI: 10.1021/acs.jmedchem.7b01484
J. Med. Chem. 2018, 61, 1951−1968

(17) Fritschy, J. M.; Johnson, D. K.; Mohler, H.; Rudolph, U. Independent assembly and subcellular targeting of $GABA_A$-receptor subtypes demonstrated in mouse hippocampal and olfactory neurons in vivo. *Neurosci. Lett.* **1998**, *249* (2−3), 99−102.
(18) Baumann, S. W.; Baur, R.; Sigel, E. Forced subunit assembly in $\alpha1\beta2\gamma2$ $GABA_A$ receptors. Insight into the absolute arrangement. *J. Biol. Chem.* **2002**, *277* (48), 46020−46025.
(19) Baumann, S. W.; Baur, R.; Sigel, E. Subunit arrangement of γ-aminobutyric acid type A receptors. *J. Biol. Chem.* **2001**, *276* (39), 36275−36280.
(20) Baur, R.; Kaur, K. H.; Sigel, E. Structure of $\alpha6\beta3\delta$ $GABA_A$ receptors and their lack of ethanol sensitivity. *J. Neurochem.* **2009**, *111* (5), 1172−1181.
(21) Baur, R.; Kaur, K. H.; Sigel, E. Diversity of structure and function of $\alpha1\alpha6\beta3\delta$ $GABA_A$ receptors: comparison with $\alpha1\beta3\delta$ and $\alpha6\beta3\delta$ receptors. *J. Biol. Chem.* **2010**, *285* (23), 17398−17405.
(22) Kaur, K. H.; Baur, R.; Sigel, E. Unanticipated structural and functional properties of δ-subunit-containing $GABA_A$ receptors. *J. Biol. Chem.* **2009**, *284* (12), 7889−7896.
(23) Egawa, K.; Fukuda, A. Pathophysiological power of improper tonic $GABA_A$ conductances in mature and immature models. *Front. Neural Circuits* **2013**, *7*, 170.
(24) Whissell, P. D.; Lecker, I.; Wang, D. S.; Yu, J.; Orser, B. A. Altered expression of $\delta GABA_A$ receptors in health and disease. *Neuropharmacology* **2015**, *88*, 24−35.
(25) Bonin, R. P.; Labrakakis, C.; Eng, D. G.; Whissell, P. D.; De Koninck, Y.; Orser, B. A. Pharmacological enhancement of δ-subunit-containing $GABA_A$ receptors that generate a tonic inhibitory conductance in spinal neurons attenuates acute nociception in mice. *Pain* **2011**, *152* (6), 1317−1326.
(26) Takahashi, A.; Mashimo, T.; Uchida, I. GABAergic tonic inhibition of substantia gelatinosa neurons in mouse spinal cord. *NeuroReport* **2006**, *17* (12), 1331−1335.
(27) Peng, H. Y.; Chen, G. D.; Lee, S. D.; Lai, C. Y.; Chiu, C. H.; Cheng, C. L.; Chang, Y. S.; Hsieh, M. C.; Tung, K. C.; Lin, T. B. Neuroactive steroids inhibit spinal reflex potentiation by selectively enhancing specific spinal $GABA_A$ receptor subtypes. *Pain* **2009**, *143* (1−2), 12−20.
(28) Wafford, K. A.; van Niel, M. B.; Ma, Q. P.; Horridge, E.; Herd, M. B.; Peden, D. R.; Belelli, D.; Lambert, J. J. Novel compounds selectively enhance δ subunit containing $GABA_A$ receptors and increase tonic currents in thalamus. *Neuropharmacology* **2009**, *56* (1), 182−189.
(29) Jensen, M. L.; Wafford, K. A.; Brown, A. R.; Belelli, D.; Lambert, J. J.; Mirza, N. R. A study of subunit selectivity, mechanism and site of action of the delta selective compound 2 (DS2) at human recombinant and rodent native $GABA_A$ receptors. *Br. J. Pharmacol.* **2013**, *168* (5), 1118−1132.
(30) Squires, R. F.; Casida, J. E.; Richardson, M.; Saederup, E. [^{35}S]*t*-butylbicyclophosphorothionate binds with high affinity to brain-specific sites coupled to γ-aminobutyric acid-A and ion recognition sites. *Mol. Pharmacol.* **1983**, *23* (2), 326−336.
(31) Cole, L. M.; Casida, J. E. GABA-gated chloride channel: binding-site for 4′-ethynyl-4-normal-[2,3-H-3(2)]-propylbicycloorthobenzoate ([^{3}H]EBOB) in vertebrate brain and insect head. *Pestic. Biochem. Physiol.* **1992**, *44* (1), 1−8.
(32) Maksay, G.; Simonyi, M. Kinetic regulation of convulsant (TBPS) binding by GABAergic agents. *Mol. Pharmacol.* **1986**, *30* (4), 321−328.
(33) Maksay, G.; Biro, T. High affinity, heterogeneous displacement of [^{3}H]EBOB binding to cerebellar $GABA_A$ receptors by neurosteroids and GABA agonists. *Neuropharmacology* **2005**, *49* (4), 431−438.
(34) Yagle, M. A.; Martin, M. W.; de Fiebre, C. M.; de Fiebre, N. C.; Drewe, J. A.; Dillon, G. H. [^{3}H]Ethynylbicycloorthobenzoate ([^{3}H]EBOB) binding in recombinant $GABA_A$ receptors. *Neuro-Toxicology* **2003**, *24* (6), 817−824.
(35) Uusi-Oukari, M.; Maksay, G. Allosteric modulation of [^{3}H]EBOB binding to $GABA_A$ receptors by diflunisal analogues. *Neurochem. Int.* **2006**, *49* (7), 676−682.
(36) Maksay, G.; Korpi, E. R.; Uusi-Oukari, M. Bimodal action of furosemide on convulsant [^{3}H]EBOB binding to cerebellar and cortical $GABA_A$ receptors. *Neurochem. Int.* **1998**, *33* (4), 353−358.
(37) Korpi, E. R.; Lüddens, H. Furosemide interactions with brain $GABA_A$ receptors. *Br. J. Pharmacol.* **1997**, *120* (5), 741−748.
(38) Supavilai, P.; Karobath, M. Differential modulation of [^{35}S]TBPS binding by the occupancy of benzodiazepine receptors with its ligands. *Eur. J. Pharmacol.* **1983**, *91* (1), 145−146.
(39) Evers, A. S.; Chen, Z. W.; Manion, B. D.; Han, M.; Jiang, X.; Darbandi-Tonkabon, R.; Kable, T.; Bracamontes, J.; Zorumski, C. F.; Mennerick, S.; Steinbach, J. H.; Covey, D. F. A synthetic 18-norsteroid distinguishes between two neuroactive steroid binding sites on $GABA_A$ receptors. *J. Pharmacol. Exp. Ther.* **2010**, *333* (2), 404−413.
(40) Maksay, G.; Fodor, L. Differential effects of two major neurosteroids on cerebellar and cortical $GABA_A$ receptor binding and function. *Eur. J. Pharmacol.* **2011**, *650* (1), 94−101.
(41) Korpi, E. R.; Lüddens, H. Regional γ-aminobutyric acid sensitivity of *t*-butylbicyclophosphoro[^{35}S]thionate binding depends on γ-aminobutyric $acid_A$ receptor α subunit. *Mol. Pharmacol.* **1993**, *44* (1), 87−92.
(42) Hawkinson, J. E.; Kimbrough, C. L.; Belelli, D.; Lambert, J. J.; Purdy, R. H.; Lan, N. C. Correlation of neuroactive steroid modulation of [^{35}S]*t*-butylbicyclophosphorothionate and [^{3}H]flunitrazepam binding and γ-aminobutyric $acid_A$ receptor function. *Mol. Pharmacol.* **1994**, *46* (5), 977−985.
(43) Maksay, G. From kinetics and thermodynamics of $GABA_A$ receptor binding to ionophore function. *Neurochem. Int.* **1996**, *29* (4), 361−370.
(44) Van Niel, M. B.; Miah, A. Substituted Imidazo[1,2-a]pyridines and their Use as Agonists at $GABA_A$ Receptors for Treating and Preventing Neurological or Psychiatric Disorders. U.K. Patent GB2448808A, April 21, 2008.
(45) Bienaymé, H.; Bouzid, K. A new heterocyclic multicomponent reaction for the combinatorial synthesis of fused 3-aminoimidazoles. *Angew. Chem., Int. Ed.* **1998**, *37* (16), 2234−2237.
(46) Ahring, P. K.; Bang, L. H.; Jensen, M. L.; Strobaek, D.; Hartiadi, L. Y.; Chebib, M.; Absalom, N. A pharmacological assessment of agonists and modulators at $\alpha4\beta2\gamma2$ and $\alpha4\beta2\delta$ $GABA_A$ receptors: The challenge in comparing apples with oranges. *Pharmacol. Res.* **2016**, *111*, 563−576.
(47) Guchhait, S. K.; Chaudhary, V.; Madaan, C. A chemoselective Ugi-type reaction in water using TMSCN as a functional isonitrile equivalent: generation of heteroaromatic molecular diversity. *Org. Biomol. Chem.* **2012**, *10* (46), 9271−9277.
(48) Shaabani, A.; Maleki, A. Ionic liquid promoted one-pot three-component reaction: synthesis of annulated imidazo[1,2-a]azines using trimethylsilylcyanide. *Monatsh. Chem.* **2007**, *138* (1), 51−56.
(49) Jaseer, E. A.; Prasad, D. J. C.; Sekar, G. Domino synthesis of 2-arylbenzo[b]furans by copper(II)-catalyzed coupling of o-iodophenols and aryl acetylenes. *Tetrahedron* **2010**, *66* (11), 2077−2082.
(50) Reddy, M. K.; Reddy, K. S.; Prakash, M.; Narasimhaswamy, T. Synthesis and characterization of two phenyl ring core-based thiophene mesogens. *Mol. Cryst. Liq. Cryst.* **2013**, *582* (1), 1−14.
(51) Rosenberg, A. J.; Liu, H.; Jin, H.; Yue, X.; Riley, S.; Brown, S. J.; Tu, Z. Design, synthesis, and in vitro and in vivo evaluation of an ^{18}F-labeled sphingosine 1-phosphate receptor 1 (S1P1) PET tracer. *J. Med. Chem.* **2016**, *59* (13), 6201−6220.
(52) Hattori, T.; Satoh, T.; Miyano, M. Convenient synthesis of triarlamines via ester-mediated nucleophilic aromatic substitution. *Synthesis* **1996**, *1996* (4), 514−518.
(53) Manse, Y.; Ninomiya, K.; Nishi, R.; Kamei, I.; Katsuyama, Y.; Imagawa, T.; Chaipech, S.; Muraoka, O.; Morikawa, T. Melanogenesis inhibitory activity of a 7-O-9′-linked neolignan from Alpinia galanga fruit. *Bioorg. Med. Chem.* **2016**, *24* (23), 6215−6224.
(54) Neubauer, T.; Kammerer-Pentier, C.; Bach, T. Total synthesis of (+)-bretonin B: access to the (E,Z,E)-triene core by a late-stage Peterson elimination of a convergently assembled silyl ether. *Chem. Commun. (Cambridge, U. K.)* **2012**, *48* (95), 11629−11631.

DOI: 10.1021/acs.jmedchem.7b01484
J. Med. Chem. 2018, 61, 1951−1968

(55) Jordan, M.; Schallhorn, A.; Wurm, F. M. Transfecting mammalian cells: optimization of critical parameters affecting calcium-phosphate precipitate formation. *Nucleic Acids Res.* **1996**, *24* (4), 596−601.
(56) Hevers, W.; Korpi, E. R.; Lüddens, H. Assembly of functional $\alpha6\beta3\gamma2\delta$ $GABA_A$ receptors *in vitro*. *NeuroReport* **2000**, *11* (18), 4103−4106.
(57) Lüddens, H.; Korpi, E. R. GABA antagonists differentiate between recombinant $GABA_A$/benzodiazepine receptor subtypes. *J. Neurosci.* **1995**, *15* (10), 6957−6962.
(58) Lüddens, H.; Korpi, E. R. Methods for Transient Expression of Hetero-Oligomeric Ligand-Gated Ion Channels. In *Receptor Signal Transduction Protocols*; Challiss, R. A. J., Ed.; Humana Press: Totowa, NJ, 1997; Vol. 83, pp 55−63.
(59) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

DOI: 10.1021/acs.jmedchem.7b01484
J. Med. Chem. 2018, 61, 1951−1968