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4-Hydroxy-1,2,3-triazole moiety as bioisostere of the carboxylic acid function: a novel scaffold to probe the orthosteric γ-aminobutyric acid receptor binding site

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KEYWORDS
hydroxy-1,2,3-triazole; bioisosterism, scaffold hopping, GABA, receptor.

ABSTRACT

The correct application of bio(iso)stere replacement, a potent tool for the design of optimized compounds, requires the continuous development of new isosters able to respond to specific target requirements. Among carboxylic acid isosters, as the hydroxylated pentatomic heterocyclic systems, the hydroxy-1,2,3-triazole represents one of the most versatile but less
investigated. With the purpose to enlarge its bioisosteric application, we report the results of a study devoted to obtain potential biomimetics of the \(\gamma\)-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS). A series of \(N_1\) - and \(N_2\)-functionalized 4-hydroxy-1,2,3-triazole analogues of the previous reported GABA\(_A\)R ligands, including muscimol, 4-PIOL, and 4-PHP has been synthesized and characterized pharmacologically. Furthermore, this study led to development of straightforward chemical strategies directed to decorate the hydroxytriazole core scaffold, opening for further elaborative studies based on this system. The unsubstituted \(N_1\) - and \(N_2\)-piperidin-4-yl-4-hydroxy-1,2,3-triazole analogues (3a, 4a) of 4-PIOL and 4-PHP showed weak affinity (high to medium micromolar range), whereas substituting the 5-position of the triazole core with a 2-naphthylmethyl or 3,3-diphenylpropyl led to binding affinities in the low micromolar range. Based on electrostatic analysis and docking studies using a \(\alpha_1\beta_2\gamma_2\) GABA\(_A\)R homology model we were able to rationalize the observed divergence in SAR for the series of \(N_1\) - and \(N_2\)-piperidin-4-yl-4-hydroxy-1,2,3-triazole analogues, offering more detailed insight into the orthosteric GABA\(_A\)R binding site.

1. Introduction

A number of clear bioisosteric relationships[1] has been established for the carboxylic acid group, which successfully has been substituted by hydroxylated pentatomic heterocyclic systems such as thiazaoles,[2, 3] 1,2,5-oxadiazaoles,[4] pyrazoles,[5, 6] and isoxazoles.[7] Recently, the 4-hydroxy-1H-1,2,3-triazole acidic system has been successfully used by the authors[8-11] and by others[12] as bioisostere of the carboxylic acid group. In fact, due to its acidic properties (pK\(_a\) ranged from 5 to 7, depending on the nature the substituents), this system is deprotonated to a large extent at physiological pH.[13, 14] These approaches successfully produced promising glutamate analogues,[8] novel Sortilin inhibitors,[12] new anti-cancer
compounds[9-11] and new immunosuppressive agents.[9] Compared to other above mentioned hydroxylated pentatomic heterocyclic systems, the hydroxy-1,2,3-triazole represents one of the most versatile but less investigated heterocycle. In particular, the three nitrogen atoms present in the triazole ring offer the possibility to regio-direct substituents in set directions with advantage to reach additional binding areas and improve properties as potency, as well as target selectivity. As an example of application of this concept, during the design of dihydroorotate dehydrogenase (dHODH) inhibitors by mimicking the benzoic acid present in brequinar,[9, 15] the 1,2,3-triazole ring substitution allowed a fine tuning of the chemical space with the result of reaching optimized candidates.

With the purpose to widen the bioisosteric applications of the hydroxy-1,2,3-triazole system, in this paper we report the results of a work devoted to obtain potential biomimetics of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in CNS. In GABA neurotransmission, GABA activates the GABA\textsubscript{A} receptors (GABA\textsubscript{A}Rs), which belong to the family of ligand-gated ion channels. A high degree of structural heterogeneity of the GABA\textsubscript{A}Rs has been revealed and is reflected in multiple receptor subtypes built up as pentameric assemblies comprised of 19 different GABA\textsubscript{A}R subunits: α\textsubscript{1}-6, β\textsubscript{1}-3, γ\textsubscript{1}-3, δ, ε, θ, π, and ρ\textsubscript{1}-3.[16] A rich and complex pharmacology has been observed based on multiple subtypes, allosteric binding sites, and diverse subcellular and regional localization.[17] More detailed structural insight is emerging for the GABA\textsubscript{A}Rs in terms of full-length crystal structures of related receptors and the more recent publication of the β\textsubscript{3} homopentameric GABA\textsubscript{A}R.[18-21] Furthermore, extensive structure-activity relationship (SAR) studies have been performed over the years.[22, 23] Consequently, a large number of potent and selective ligands for the orthosteric GABA\textsubscript{A}R binding site have been reported. Especially, the conformational restriction of the structure of GABA by bioisosteric replacement of the carboxylic acid moiety with acidic heterocycles has been successful. Besides being carboxylic acid bioisosteres, these
heterocyclic rings allow for introduction of substituents of different shape, size, and electronic properties in well-defined positions useful for mapping the binding site.[24]

The broad range of ligands include muscimol, 5-((piperidin-4-yl)-3-isoxazolol (4-PIOL), 4-((piperidin-4-yl)-1-hydroxyrazole (4-PHP), and 5-((piperidin-4-yl)-3-hydroxyrazol (aza-4-PIOL) analogues (Figure 1), which all have supported the development of solid GABA,R homology models optimized for agonists or antagonist binding and identified specific cavities in the vicinity of the core part of the binding site for GABA.[25, 26]

![Figure 1. Reference compounds GABA, Muscimol, 4-PIOL, 4-PHP, Aza-4-PIOL and compound 1][4]

In the present study, we investigated the orthosteric GABA,R binding site by introducing the 4-hydroxy-1,2,3-triazole as a new bioisostere to the carboxyl group of GABA as described for the 3-hydroxyisoxazole, hydroxy-1,2,5-oxadiazole, and 1- and 3-hydroxyrazole moieties of reported GABA,R ligands. In order to explore the potential of the aminoethyl substituted hydroxy-1,2,5-oxadiazole [4, 27] (Figure 1), a low affinity GABA,R agonist, we designed the corresponding 4-hydroxy-1,2,3-triazole (2a) and hydroxythiadiazole analogues (2b) (Figure 2).
Subsequently, to challenge the above mentioned homology model and verify the structural similarity, binding modes, and bioisosteric potential of the 4-hydroxy-1,2,3-triazole, two regiosomeric series $N_1$- and $N_2$- piperidin-4-yl-4-hydroxy-1,2,3-triazole analogues were synthesized (compounds 3a–c and 4a–c, respectively, Figure 2) corresponding to a selected subgroup of previously reported 4-PIOL, 4-PHP, and aza-4-PIOL analogues. The syntheses and pharmacological properties at native GABA$_A$Rs in rat brain homogenate are reported and SARs are discussed using the above mentioned homology model.

2. Result and discussion

2.1 Chemistry.

The target compounds 2a and 2b were synthesized as described in Scheme 1. The alcohol 6 was obtained from compound 5[8] by reduction of the ethyl ester using LiAlH$_4$. Treatment of 6 with N-bromosuccinimide and triphenylphosphine afforded 7, which, due to its instability, was converted into 8 using sodium cyanide immediately upon purification. Following a one-pot procedure, previously described by Petersen et al.[29, 30] compound 8 was converted into 9 by reduction of the nitrile group followed by benzyloxy carbonyl (Cbz) protection of the
formed amine. This latter protection of the amino group was performed to optimize the purification procedure of 9. Deprotection of 9 under acidic conditions afforded target compound 2a. Compound 2b was synthesized starting from 10 (Scheme 1), a compound previously described by Treder et al. in high yields.[31] Because, in our hands, the published synthetic scheme was not reproduced in satisfactory yields, we developed an alternative method starting from glutamic acid for the synthesis of 10 (please refer to Supplementary Information for synthetic details), which was obtained in an overall yield of 8% (four steps). Annulation of 10 and sulphur monochloride, a method previously described by Weinstock et al.[32] and subsequent deprotection under acidic condition afforded target compound 2b.

Scheme 1. Reagents and conditions: (a) LiAlH₄, THF, 0 °C to rt, (b) PPh₃, NBS, CH₂Cl₂, −10 °C, (c) NaCN, EtOH/H₂O, rt, (d) BrOOCCl, NaBH₄, NiCl₂, MeOH, 0 °C to rt, (e) 2M HCl, reflux, (f) S₂Cl₂, DMF, rt.

Target compounds 3a–c and 4a–c were synthesized (Schemes 2 to 4) starting from 12, which was prepared as previously reported.[8] Compound 14 (Scheme 2) was obtained from 12 in two steps starting by hydrolysis of the ethoxycarbonyl moiety followed by decarboxylation of the formed acid (13) at elevated temperatures. As for 12, also compound 14 represent a valuable intermediate for the synthesis of regiosubstituted hydroxytriazoles. In analogy to
12[8] also 14 follows an alkylation scheme directed toward the $N_2$- and $N_1$- position of the triazole ring. Alkylation of 14 using tert-butyl 4-bromopiperidine-1-carboxylate (19) afforded a mixture of the $N_2$- (15) and $N_1$- (16) regioisomers, which were isolated using standard column chromatography in 60% and 10% yields, respectively. The substitution pattern between the $N_2$- and $N_1$- position was determined by 2D NMR analyses (please refer to Supplementary Information). Subsequent deprotection of compounds 15 and 16 under acidic conditions afforded compounds 3a and 4a, respectively.

Scheme 2. Reagents and conditions: (a) 6M NaOH, EtOH, 50 °C, (b) DMF, 130 °C, 6h, (c) tert-butyl 4-bromopiperidine-1-carboxylate (19), K$_2$CO$_3$, CH$_3$CN, reflux, (d) ethyl 4-bromopiperidine-1-carboxylate (20), Cs$_2$CO$_3$, 1,4-dioxane, reflux, (e) 6M HCl, reflux.

Target compounds 3b–c and 4b–c (Scheme 3 and 4) were obtained from intermediates 17 and 18, which were synthesized as described for 15 and 16 (Scheme 2), using ethyl 4-bromopiperidine-1-carboxylate (20). Analogously to 15 and 16, the $N_2$- (17) and $N_1$- (18) regioisomers were obtained in 63% and 17% yield, respectively. In order to obtain a higher yield of the $N_1$- isomer, different alkylation conditions were attempted. Interestingly, caesium carbonate in anhydrous 1,4-dioxane at reflux improved the ratio between the $N_1$- and $N_2$- regioisomers and a 1:1 mixture of 18 and 17 was obtained (isolated yields of 41% and 39%).
respectively). Iodination of 17 and 18 (Scheme 3) using iodine monochloride afforded compounds 21 and 22, which were converted into the corresponding Grignard reagents using isopropylmagnesium chloride. Quenching of the Grignard reagents in situ with either 2-naphthaldehyde or 3,3-diphenylpropanal afforded the corresponding alcohol derivatives 23b–c and 24b–c. Ionic hydrogenation of the formed alcohol using triethylsilane and trifluoroacetic acid[33] followed by deprotection under acidic conditions afforded target compounds 3b–c and 4b.

Scheme 3. Reagents and conditions: (a) ICl, AcOH, H2O, 80 °C, (b) iPrMgCl, THF, –10 °C, (c) 2-naphthaldehyde or 3,3-diphenylpropanal (27), THF, 0 °C to rt, (d) Et3SiH, TFA, CH2Cl2, 0 °C to rt, (e) 35% HCl v/v, EtOH, reflux.

In contrast to compounds 23b–c and 24b, the ionic hydrogenation of 24c (Scheme 4) afforded a mixture of saturated and unsaturated products (determined using LC-MS analysis), which could not be separated using conventional purification methods. However, the crude mixture
was hydrogenated using palladium on carbon, which afforded compound 28c. Subsequent deprotection under acidic conditions afforded target compound 4c.

Scheme 4. Reagents and conditions: (a) Et₃SiH, TFA, CH₂Cl₂, 50 °C, sealed tube, (b) H₂, Pd/C, MeOH, rt, (c) 35% HCl, EtOH, reflux.

2.2 Structure–activity relationship and electrostatic properties

The synthesized compounds 2a–b, 3a–c, and 4a–c were characterized in receptor binding studies using rat brain membrane preparations, where the binding affinities of the compounds at native GABA₄Rs were measured by displacement of [³H]muscimol (Table 1). As previously reported for the corresponding 3-hydroxyisoxazole[34] the monocyclic analogues 2a and 2b showed no or low affinity for native GABA₄Rs. Since these carboxylic acid isosteres show pKₐ values in a range (pKₐ 3.12–5.92) comparable to muscimol, a potent GABA₄R agonist, the lack of affinity might reflect a suboptimal conformation of the pharmacophoric elements of the compounds.
Also, the $N_2$- and $N_1$-piperidin-4-yl-4-hydroxy-1,2,3-triazole analogues of 4-PIOL (3a and 4a, respectively) displayed low GABA$_A$R affinities in the high to medium micromolar range comparable to aza-4-PIOL and more than 5-fold lower than 4-PIOL and 4-PHP. Introduction of 2-naphthylmethyl in the 5-position of the $N_1$-piperidin-4-yl-4-hydroxy-1,2,3-triazole analogue (4b) led to a 20-fold increase in affinity compared to the non-substituted analogue. Similar receptor affinity was observed by introduction of the 2-naphthylmethyl substituent in the 5-position of the $N_2$-piperidin-4-yl-4-hydroxy-1,2,3-triazole analogue 3b. Replacing the naphthylmethyl to a more flexible 3,3-biphenylpropyl moiety did not change the receptor affinity for the 5-substituted $N_1$-piperidin-4-yl-4-hydroxy-1,2,3-triazole analogue 4c. In contrast, the corresponding structural change for the $N_2$-piperidin-4-yl-4-hydroxy-1,2,3-triazole analogue 3c was detrimental for affinity and led to a compound with complete loss of GABA$_A$R affinity.

Considering the overall structural similarity of the core scaffolds of 4-PIOL, 4-PHP, 3a and 4a, and because the substituted analogues of 3a and 4a to an extent showed affinity, a high desolvation energy of the non-substituted analogues 3a and 4a could be the reason for the lack of receptor affinity observed in the binding study. A similar case was previously reported for the corresponding 3-hydroxypyrazol analogue of 4-PIOL (aza-4-PIOL).[6] Using the program Jaguar,[35] the free energies of solvation for the zwitterionic forms of 4-PHP (~77.9 kcal/mol), aza-4-PIOL (~101.2 kcal/mol), 3a (~88.5 kcal/mol), and 4a (~97.2 kcal/mol) were calculated, indicating a significantly higher desolvation energy penalty for compounds 3a and 4a than for 4-PHP.
Table 1. Pharmacological data and ionization constants for reference compounds GABA, 4-PIOL, 4-PHP, Aza-4-PIOL, and compounds 1, 2a–b, 3a–c and 4a–c.

<table>
<thead>
<tr>
<th></th>
<th>[3H]muscimol binding</th>
<th>pKᵢ⁺</th>
<th>[pKᵢ±SEM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>0.049</td>
<td>4.04 ±0.02</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>3.12 ±0.02</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>&gt;100</td>
<td>5.92 ±0.02</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>75 [4.13±0.04]</td>
<td>4.54 ±0.03</td>
<td></td>
</tr>
<tr>
<td>4-PIOL</td>
<td>9</td>
<td>5.3 ±0.3</td>
<td></td>
</tr>
<tr>
<td>4-PHP</td>
<td>10</td>
<td>5.4 ±0.3</td>
<td></td>
</tr>
<tr>
<td>Aza-4-PIOL</td>
<td>&gt;100</td>
<td>6.7 ±0.3</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>&gt;100</td>
<td>6.36 ±0.01</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>55 [4.26±0.05]</td>
<td>6.51 ±0.03</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>3.3 [5.49±0.04]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>2.4 [5.62±0.04]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3c</td>
<td>&gt;100</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4c</td>
<td>1.6 [5.80±0.03]</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*GABAₐ receptor binding affinities at rat synaptic membranes: IC₅₀ values were calculated from inhibition curves and converted to Kᵢ values. Data is given as the mean [mean pKᵢ±SEM] of three to five independent experiments. *The ionization constants of compounds 2a–b, 3a, and 4a were determined by potentiometric titration using a GLpKᵢ apparatus (Sirius Analytical Instruments Ltd., Forest Row, East Sussex, UK). *Data from Lolli et al.[4] *Data from Krall et al.[22]

The heterocyclic carboxylic acid bioisosteres interacting with the GABAₐR in general resembles the electrostatic properties of the carboxylic acid in GABA.[23] As shown for 4-PHP and aza-4-PIOL (Figure 3A,B), the electronegative charge is centred in the area around the hydroxy group and the neighbouring nitrogen allowing the ligands to interact in a bidentate manner with the conserved α₁-Arg66 in the GABA binding site. In contrast, the electrostatic profile shows a slightly different charge distribution for compounds 3a and 4a (Figure 3C,D) which could indicate that this bidentate interaction could be compromised leading to reduced
binding affinity. The higher $pK_a$ values observed for the hydroxytriazoles ($pK_a$, 6.36–6.51) compared to that of 4-PHP ($pK_a$, 5.4), and 4-PIOL ($pK_a$, 5.3) (Table 1) might also add to lower binding. The $N_1$- and $N_2$-piperidin-4-yl hydroxytriazoles, as the 3-hydroxypyrazole aza-4-PIOL, are thus less acidic than 4-PHP and protonated to a greater extent under physiological pH, which in turn might lead to a weaker interaction in the orthosteric GABA$_A$R binding site.

![Figure 3](image)

**Figure 3.** Electrostatic potential mapped on the surface of the molecular density for the anionic form of (A) 4-methyl-1-hydroxypyrazole, (B) 5-methyl-3-hydroxypyrazole, (C) 1-methyl-4-hydroxy-1,2,3-triazole, and (D) 2-methyl-4-hydroxy-1,2,3-triazole ring systems. Increasing negative potential coloured from purple/blue over green to red. Calculations were carried out with Jaguar[35] using the cc-PVDZS basis set and the B3LYP hybrid potential. Au, atomic units.

2.3. Molecular modelling.

To further assess the obtained pharmacological data, the binding modes of the synthesized compounds were evaluated using the reported homology model of the $\alpha_1\beta_2\gamma_2$ GABA$_A$R in the antagonist bound state.[26] The obtained docking poses for the ligands match the binding mode previously reported,[26] with the amine moiety forming hydrogen bonds with $\beta_2$-Glu155 and
the backbone carbonyl of β2-Tyr157 and the hydroxytriazole moiety forming a bidentate interaction to α1-Arg66 mimicking the binding interactions of GABA. As reported for 4-PIOL and 4-PHP, two different orientations of the triazole-piperidine core scaffold of 4a are possible while still maintaining the bidentate interactions described above (Figure 4B,C). The naphthylmethyl substituted analogue 4b is able to bind in either of the two orientations (Figure 4B,C), whereas the diphenylpropyl substituted analogue 4c adopts a binding pose where the triazole moiety is found in the aforementioned alternative orientation (180° flip), thus the substituent is accommodated in the more spacious cavity below the core scaffold (Figure 4C). The binding site optimized for 3a shows a marked difference in the conformation of α1-Arg66, with the side chain moving to a position further towards the membrane, thus allowing it to form a bidentate interaction with 3a and 3b, and with the hydrophobic substituent reaching out into the previously reported cavity above the core scaffold (Figure 4A). The more bulky diphenylpropyl substituted analogue, 3c, is not able to interact with α1-Arg66 in this conformation, likely due to limited space in the aforementioned cavity. Unlike 4c, the suggested 180° flip as described for 4a and analogues is not optimal for this series of compounds (3a–c).

Figure 4. Compounds 3a (A, green), 3b (A, pink), 4a (B and C, cyan), 4b (B and C, yellow) and 4c (C, salmon) docked into the α1β2γ2 GABA<sub>2</sub>R homology model. Residues surrounding
the ligand binding site from the principal side (light-teal carbons) and complementary side (olive-green carbons) are shown. Hydrogen bonds are depicted with yellow dashes.

3. Conclusions

In this study we show that the 4-hydroxy-1,2,3-triazole ring system is a valid bioisostere for previous identified five membered heterocyclic carboxylic acid bioisosteres as ligands for the GABA<sub>₅</sub>Rs. A series of 4-hydroxy-1,2,3-triazole analogues were synthesized and characterized pharmacologically at native rat GABA<sub>₅</sub>Rs. In general, the synthesized N<sub>₁</sub>- and N<sub>₂</sub>- piperidin-4-yl analogues displayed affinities in the medium to low micromolar range (Ki values of 1.6–55 µM). Despite previous identified cavities in the vicinity of the core of the orthosteric binding site, the two structural closely related series of substituted analogues (3b–c and 4b–c) displayed slightly different SAR indicating different binding modes. These results were rationalized by using a homology model for the orthosteric binding site of the α<sub>₁</sub>β<sub>₂</sub>γ<sub>₂</sub> GABA<sub>₅</sub>R implying a 180° flip of the core scaffold of the N<sub>₁</sub>- piperidin-4-yl analogues 4b and 4c enabling accommodation of the larger substituent of 4c in the more spacious cavity below the core scaffold. This binding mode is not optimal for the corresponding N<sub>₂</sub>- piperidin-4-yl analogue 3c.

The synthesis strategy applied in this study included directed alkylation of the triazole ring system useful for future application of this heterocyclic moiety, which, in the present study, has offered a more detailed insight into the architecture and flexibility of the orthosteric binding site in the GABA<sub>₅</sub>R.

4. Experimental section

4.1 Chemistry
4.1.1 General methods

Compounds 10, 19, 20 and 27 were synthesized as reported in Supplementary Information, while compounds 5 and 12 were prepared as described in the literature.[8] All chemical reagents and solvents (analytical grade) were obtained from commercial sources (Sigma Aldrich, Alfa Aesar, or TCI) and used without further purification. Air- and/or moisture sensitive reactions were performed under a nitrogen atmosphere using syringe-septum techniques and dried glassware. Anhydrous solvents were dried over 4 Å molecular sieves or by distillation (THF) prior to use from Na and benzophenone under nitrogen atmosphere. iPrMgCl (in THF) was titrated prior to use as described elsewhere.[36] Thin layer chromatography (TLC) on silica gel was carried out using 5 × 20 cm plates with a silica layer of 0.25 mm in thickness. Purification of synthesized compounds were performed using flash column chromatography on silica gel (Merck Kieselgel 60, 230–400 mesh ASTM) or by the use of a CombiFlash RF 200 apparatus (Teledyne Isco) with 5–200 mL/min, 200 psi (with automatic injection valve) using RediSep RF Silica columns (Teledyne Isco). Melting points (mp) were measured on a Büchi 540 apparatus in open capillary tubes and are uncorrected. Analytical high performance liquid chromatography (HPLC) analyses were performed on a Perkin Elmer Flexar UHPLC system equipped with an UHPLC Acquity BEH C18 column (1.7 μm, 2.1 × 50 mm, Waters) and a 20 μL loop. Elution of analysed samples were performed using mixtures of eluent A (H₂O/TFA, 100/0.1) and eluent B (CH₃CN/TFA, 100/0.1) at a flow rate of 0.5 mL/min. For HPLC control, data collection, and data handling Chromera Software ver. 4.1.0 was used. Alternatively, analytical HPLC analyses were performed on an Ultimate 3000 HPLC system (Thermo Scientific) with an LPG-3400A pump, a WPS-3000SL autosampler, and a DAD-3000D detector using a Gemini® NX-C18 column (3 μm, 110 Å, 4.6 × 250 mm) and eluents A (H₂O/TFA, 100/0.1) and B (CH₃CN/H₂O/TFA, 90/10/0.1) at a flow rate of 1 mL/min. For HPLC control, data collection, and data handling, Chromeleon Software
Preparative reversed phase HPLC was carried out on an Ultimate 3000 HPLC system (Thermo Scientific) with a LPG-3200BX pump, a Rheodyne 7125i injector, a 10 mL loop, and a MWD-3000SD detector (200, 210, 225, and 254 nm) using a preparative Phenomenex Gemini NX-C18 column (5 µm, 21.2 × 250 mm) and eluents A (H₂O/TFA, 100/0.1) and B (CH₃CN/H₂O/TFA, 90/10/0.1) at a flow rate of 20 mL/min. For HPLC control, data collection, and data handling, Chromeleon Software ver. 6.80 was used.

Elementary analyses were performed by Mr. J. Theiner, Department of Physical Chemistry, University of Vienna, Austria. HPLC-HRMS analyses were performed on a system comprised of an Agilent 1200 HPLC system comprising of a quaternary pump with a built-in degasser, a thermostated column compartment, an autosampler, and a photodiode array detector, coupled with a Bruker microOTOF-QII mass spectrometer equipped with an electrospray ionization (ESI) source and operated via a 1:99 flow splitter. Mass spectra were acquired in positive ionization mode, using drying temperature of 200 °C, a capillary voltage of −4100V, nebulizer pressure of 2.0 bar, and drying gas flow of 7 L/min. A solution of sodium formate clusters was injected in the beginning of each run to enable internal mass calibration. Chromatographic separation was acquired on a Phenomenex Luna C18(2) column (150 mm × 4.6 mm, 3 µm, 100 Å) maintained at 40 °C, using a flow rate of 0.8 mL/min and a linear gradient of the binary solvent system water-acetonitrile-formic acid (eluent A: 95/5/0.1, and eluent B: 5/95/0.1) rising from 0% to 100% of eluent B over 20 minutes. Data was acquired using Compass HyStar Ver.
3.2 (Bruker Daltonic GmbH, Germany) and processed using Compass DataAnalysis Ver. 4.0 (Bruker Daltonic GmbH, Germany).

4.1.2. (5-(Benzyloxy)-2-methyl-2H-1,2,3-triazol-4-yl)methanol (6). LiAlH₄ (0.36 g, 9.6 mmol) was added to a cooled (0 °C) solution of compound 5[8] (2.50 g, 9.6 mmol) in anhydrous THF (125 mL). The reaction mixture was stirred for 2 h at 0 °C before it was quenched by adding in sequence water (0.37 mL), 15% w/w NaOH (0.37 mL) and then water (0.37 mL). The volatiles were evaporated in vacuo and the residue was taken up in water. The resulting mixture was extracted with Et₂O (3 × 100 mL) and the combined organic phase was washed with brine (150 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo to afford compound 6 as colourless oil (1.82 g, 87%). ¹H NMR (300 MHz, DMSO-d₆): δ 7.50–7.28 (m, 5H), 5.22 (s, 2H), 5.05 (t, J = 5.5 Hz, 1H), 4.36 (d, J = 5.5 Hz, 2H), 3.95 (s, 3H). ¹³C NMR (75 MHz, DMSO-d₆): δ 157.4, 136.5, 131.8, 128.3, 128.0, 127.8, 71.4, 52.2, 41.3. HRMS (ESI-TOF): m/z calculated for C₁₁H₁₂N₃O [M+H₂O+H]+, 202.0975. Found, 202.0974 (ΔM=0.3 ppm).

4.1.3. (4-Benzyloxy)-5-(bromomethyl)-2-methyl-2H-1,2,3-triazole (7). PPh₃ (1.58 g, 6.0 mmol) was added to a stirred solution of 6 (1.10 g, 5.0 mmol) in anhydrous CH₂Cl₂ (30 mL) at –10 °C. To the resulting mixture, NBS (1.07 g, 6.03 mmol) was added in small portions over 30 min. The reaction mixture was stirred for 1 h at –10 °C before the solvent was evaporated in vacuo. Purification of the resulting residue by flash chromatography (CH₂Cl₂) afforded compound 7 as colourless oil (1.13 g, 81%), which was used immediately upon purification in the synthesis of compound 8 due to stability issues of 7. ¹H NMR (300 MHz, CDCl₃): δ 7.43–7.22 (m, 5H), 5.18 (s, 2H), 4.38 (s, 2H), 3.93 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 158.0, 136.2, 128.7, 128.6, 128.4, 128.0, 72.2, 42.0, 20.4.
4.1.4. 2-(5-(Benzyloxy)-2-methyl-1,2,3-triazol-4-y1)acetonitrile (8). A solution of 7 (1.13 g, 4.0 mmol) in EtOH (20 mL) was added dropwise to a solution of NaCN (0.39 g, 8.0 mmol) in EtOH/water (9:1 v/v, 25 mL). The reaction mixture was stirred for 48 h at rt before the volatiles were evaporated in vacuo. The resulting residue was taken up in water and extracted with EtOAc (3 × 100 mL). The combined organic phase was washed with water (1 × 100 mL), brine (1 × 100 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 0%–20% EtOAc) afforded 8 as colourless oil (0.63 g, 69%). ¹H NMR (300 MHz, DMSO-d₆): δ 7.50–7.30 (m, 5H), 5.24 (s, 2H), 4.05–3.90 (m, 5H).

13C NMR (75 MHz, DMSO-d₆): δ 156.8, 136.1, 128.3, 128.1, 127.8, 121.8, 117.0, 71.7, 41.7, 12.3. HRMS (ESI-TOF): m/z calculated for C₁₂H₁₃N₄O [M+H]⁺, 229.1084. Found, 229.1085 (ΔM=0.5 ppm).

4.1.5. Benzyl (2-(5-(benzyloxy)-2-methyl-1,2,3-triazol-4-y1)ethyl)carbamate (9). Benzyl chloroformate (0.65 mL, 4.6 mmol) and NiCl₂·6H₂O (54 mg, 0.23 mmol) were added to a stirred solution of 8 (0.52 g, 2.3 mmol) in MeOH (20 mL) at 0 °C. NaBH₄ (0.69 g, 18 mmol) was then added in small portions over 1 h while keeping the temperature at 0 °C, whereupon the reaction mixture was allowed to reach rt and stirred for 24 h before water was added (300 mL). The resulting mixture was extracted with CH₂Cl₂ (6 × 100 mL) and the combined organic phase was washed with brine (200 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by column chromatography (petroleum ether 40–60 °C/EtOAc, gradient 0%–70% EtOAc) afforded 9 (0.28 g, 35%) as white solid: mp 44–46 °C. ¹H NMR (300 MHz, DMSO-d₆): δ 7.51–7.23 (m, 10H), 5.19 (s, 2H), 4.99 (s, 2H), 3.92 (s, 3H), 3.21 (q, J = 6.9 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H). ¹³C NMR (75 MHz, DMSO-d₆): δ 157.4, 155.9, 137.1, 136.5, 128.9, 128.3, 128.2, 127.9, 127.7, 127.6, 71.4, 65.1, 41.2, 39.2, 23.8. HRMS (ESI-TOF): m/z calculated for C₂₀H₂₃N₄O₃ [M+H]⁺, 367.1765. Found, 367.1760 (ΔM=1.3 ppm).
4.1.6. 5-(2-Aminoethyl)-2-methyl-2H-1,2,3-triazol-4-ol hydrochloride (2a). A solution of 9 (0.18 g, 0.50 mmol) in MeOH/2M HCl (1:4 v/v, 25 mL) was refluxed for 72 hours. The resulting solution was washed with EtOAc (3 × 15 mL) and evaporated in vacuo. Recrystallization from PrOH/Et₂O afforded 2a (40 mg, 45%) as white solid: mp 191–192 °C. ¹H NMR (300 MHz, D₂O): δ 3.94 (s, 3H), 3.29 (t, J = 7.0 Hz, 2H), 2.96 (t, J = 7.0 Hz, 2H). ¹³C NMR (75 MHz, D₂O, int. std. MeOH): δ 156.1, 128.2, 41.5, 38.8, 21.7. HRMS (ESI-TOF): m/z calculated for C₅H₁₁N₄O [M+H]⁺, 143.0927. Found, 143.0927 (∆M=0.5 ppm).

4.1.7. Benzyl (2-(4-hydroxy-1,2,5-thiadiazol-3-yl)ethyl)carbamate (11). A solution of S₂Cl₂ (0.45 mL, 5.7 mmol) in anhydrous DMF (20 mL) was added dropwise to a solution of 10 (0.47 g, 1.9 mmol) in anhydrous DMF (10 mL). The reaction mixture was stirred for 12 hours at rt before poured into 200 mL of iced water. The mixture was filtered, the filtrate was acidified to pH 1 and extracted with Et₂O (4 × 100 mL). The combined organic phase was washed with brine (1 × 100 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by flash chromatography (CH₂Cl₂/MeOH 95:5 v/v) afforded 11 (0.090 g, 17%) as white solid: mp 86–87 °C. ¹H NMR (300 MHz, DMSO-d₆): δ 12.59 (br s, 1H), 7.42–7.26 (m, 5H), 4.99 (s, 2H), 3.37 (t, J = 7.1 Hz, 2H), 2.83 (t, J = 7.1 Hz, 2H). ¹³C NMR (75 MHz, DMSO-d₆): δ 162.5, 155.9, 149.9, 137.1, 128.2, 127.6, 127.5, 65.0, 38.1, 29.0. HRMS (ESI-TOF): m/z calculated for C₁₂H₁₄N₃O₃S [M+H]⁺, 280.0750. Found, 280.0744 (∆M=2.4 ppm).

4.1.8. 4-(2-Aminoethyl)-1,2,5-thiadiazol-3-ol hydrochloride (2b). A solution of 11 (81 mg, 0.29 mmol) in MeOH/2M HCl (1:3 v/v, 16 mL) was refluxed for 72 h. The resulting solution was washed with EtOAc (3 × 10 mL) and evaporated in vacuo. Tritration of the resulting residue with Pr₂O afforded 2b (34 mg, 64%) as white solid: mp 215–217 °C. ¹H NMR (600 MHz, D₂O): δ 3.49 (t, J = 6.8 Hz, 2H), 3.18 (t, J = 6.8 Hz, 2H). ¹³C NMR (150 MHz, D₂O): δ
HRMS (ESI-TOF): m/z calculated for C₄H₈N₃OS [M+H]^+, 146.0383. Found, 146.0385 (ΔM=1.8 ppm).

### 4.1.9. 5-(Benzyloxy)-2H-1,2,3-triazole-4-carboxylic acid (I3)

6M NaOH (14.2 mL, 85.0 mmol) was added to a solution of 12[8] (3.5 g, 14.2 mmol) in EtOH (100 mL). The reaction mixture was heated at 50 °C for 24 h. Upon cooling to rt, the reaction mixture was neutralized with 6M HCl and the solvents were evaporated. The residue was taken up in water and 1M HCl was added until pH 1. The resulting suspension was filtered and the solid was washed with hexane to give 13 (3.1 g, quant.) as white solid: mp 172 °C (dec.). ¹H NMR (600 MHz, DMSO-d₆): δ 14.05 (br s, 1H), 7.54–7.27 (m, 5H), 5.32 (s, 2H). ¹³C NMR (75 MHz, DMSO-d₆): δ 161.2, 159.8, 136.5, 128.4, 128.1, 128.0, 121.6, 71.5. HRMS (ESI-TOF): m/z calculated for C₁₀H₁₀N₃O₃ [M+H]^+, 220.0717. Found, 220.0712 (ΔM=2.3 ppm).

### 4.1.10. 4-(Benzyloxy)-2H-1,2,3-triazole (I4)

13 (3.5 g, 16.0 mmol) was dissolved in anhydrous DMF (50 mL) and the resulting solution heated at 130 °C for 6 h. Upon cooling to rt, water (500 mL) was added and the mixture was extracted with Et₂O (5 × 100 mL). The combined organic phase was washed with water (2 × 100 mL), brine (2 × 100 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by flash chromatography (CH₂Cl₂/EtOAc, 95:5 v/v) afforded 14 (2.25 g, 70%) as white solid: mp 100–102 °C. ¹H NMR (600 MHz, DMSO-d₆): δ 14.1 (br s, 1H), 7.29–7.49 (m, 6H), 5.19 (s, 2H). ¹³C NMR (75 MHz, DMSO-d₆): δ 160.5, 136.6, 128.4, 128.1, 128.0, 118.7, 71.4. HRMS (ESI-TOF): m/z calculated for C₉H₁₀N₃O [M+H]^+, 176.0812. Found, 176.0812 (ΔM=3.6 ppm).

### 4.1.11. tert-Butyl 4-(4-(benzyloxy)-2H-1,2,3-triazol-2-yl)piperidine-1-carboxylate (I5) and tert-butyl 4-(4-(benzyloxy)-1H-1,2,3-triazol-1-yl)piperidine-1-carboxylate (I6)

K₂CO₃ (1.7 g, 12.6 mmol) was added to a solution of 14 (1.1 g, 6.3 mmol) in CH₃CN (35 mL). The reaction
mixture was heated at reflux and tert-butyl 4-bromopiperidine-1-carboxylate (19, 2.2 g, 8.2 mmol) was added in portions over 48 h. The reaction mixture was cooled at rt and the solvent was evaporated in vacuo. The resulting residue was taken up in water (200 mL) and extracted with EtOAc (3 × 100 mL). The combined organic phase was washed with brine (50 mL), dried over anhydrous Na$_2$SO$_4$, and evaporated in vacuo. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 10%–40% EtOAc) afforded 15 (first eluting, N$_2$-isomer) and 16 (second eluting, N$_1$-isomer) as white solids. 15 (1.36 g, 60%): mp 87–88 °C.

1H NMR (300 MHz, DMSO-d$_6$): δ 7.48–7.30 (m, 6H), 5.17 (s, 2H), 4.52 (tt, J = 10.8, 4.0 Hz, 1H), 3.94 (d, J = 13.3 Hz, 2H), 3.08–2.86 (m, 2H), 2.09–1.96 (m, 2H), 1.78 (qd, J = 4.3, 11.6 Hz, 2H), 1.41 (s, 9H).

13C NMR (75 MHz, DMSO-d$_6$): δ 160.1, 153.9, 136.3, 128.4, 128.2, 128.1, 118.6, 78.9, 71.5, 60.6, 41.8, 31.0, 28.0. HRMS (ESI-TOF): m/z calculated for C$_{19}$H$_{26}$N$_4$O$_3$Na [M+Na]$^+$, 381.1897. Found, 381.1895 (ΔM=0.6 ppm).

16 (0.21 g, 10%): mp 106–107 °C.

1H NMR (300 MHz, DMSO-d$_6$): δ 7.85 (s, 1H), 7.49–7.29 (m, 5H), 5.15 (s, 2H), 4.59 (tt, J = 11.3, 3.8 Hz, 1H), 4.03 (d, J = 12.9 Hz, 2H), 3.05–2.80 (m, 2H), 2.08–1.96 (m, 2H), 1.79 (qd, J = 12.2, 4.3 Hz, 2H), 1.41 (s, 9H).

13C NMR (75 MHz, DMSO-d$_6$): δ 160.1, 153.7, 136.5, 128.1, 128.0, 105.6, 78.9, 71.5, 57.7, 42.1, 31.7, 28.1. HRMS (ESI-TOF): m/z calculated for C$_{19}$H$_{27}$N$_4$O$_3$ [M+H]$^+$, 359.2078. Found, 359.2068 (ΔM=2.7 ppm).

4.11.2. 2-(Piperidin-4-yl)-2H-1,2,3-triazol-4-ol hydrochloride (3a). 15 (0.25 g, 0.70 mmol) was suspended in 6M HCl (10 mL) and the suspension was heated at reflux for 48 h. Upon cooling to rt, the reaction mixture was washed with EtOAc (2 × 10 mL) and the aqueous phase evaporated in vacuo. Recrystallization from EtOH/Et$_2$O afforded 3a (90 mg, 63%) as white crystals: mp 259–263 °C.

1H NMR (300 MHz, D$_2$O): δ 7.17 (s, 1H), 4.65 (tt, J = 10.3, 4.3 Hz, 1H), 3.52 (dt, J = 13.3, 3.9 Hz, 2H), 3.28–3.13 (m, 2H), 2.42–2.14 (m, 4H).

13C NMR (75 MHz, D$_2$O): δ 158.4, 120.1, 58.1, 42.7, 27.9. HRMS (ESI-TOF): m/z calculated for C$_{10}$H$_{13}$N$_3$O [M+H]$^+$, 169.1084. Found, 169.1083 (ΔM=0.6 ppm).
4.1.13. 1-(Piperidin-4-yl)-1H-1,2,3-triazol-4-ol hydrochloride (4a). 16 (0.17 g, 0.47 mmol) was suspended in 6M HCl (10 mL) and the suspension heated at reflux for 48 h. Upon cooling to rt, the reaction mixture was washed with EtOAc (2 × 10 mL) and the aqueous phase evaporated in vacuo. Recrystallization from EtOH/Et₂O afforded 4a (20 mg, 21%) as white crystals: mp 243 °C (dec.).

1H NMR (300 MHz, D₂O): δ 7.36 (s, 1H), 4.73 (m, 1H), 3.57 (dt, J = 6.9, 3.2 Hz, 2H), 3.22 (td, J = 13.2, 3.2 Hz, 2H), 2.49–2.36 (m, 2H), 2.34–2.16 (m, 2H).

13C NMR (75 MHz, D₂O): δ 157.5, 107.8, 56.3, 42.9, 28.3. HRMS (ESI-TOF): m/z calculated for C₇H₁₃N₄O [M+H]+, 169.1084. Found, 169.1085 (ΔM=0.9 ppm).

4.1.14. Ethyl 4-(4-(benzyloxy)-2H-1,2,3-triazol-2-yl)piperidine-1-carboxylate (17) and ethyl 4-(4-(benzyloxy)-1H-1,2,3-triazol-1-yl)piperidine-1-carboxylate (18). Cs₂CO₃ (17.3 g, 53 mmol) was added to a solution of 14 (4.6 g, 26.5 mmol) in anhydrous 1,4-dioxane (100 mL). The reaction mixture was heated at reflux and ethyl 4-bromopiperidine-1-carboxylate (20, 18.8 g, 80 mmol) was added in small portions over 72 h. The reaction mixture was cooled to rt, neutralized by adding 1M HCl, and the volatiles were removed in vacuo. The resulting residue was taken up in water (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic phase was washed with brine (50 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 15%–40% EtOAc) afforded 17 (first eluting, N₂- isomer) and 18 (second eluting, N₁- isomer) as colourless oil and white solid, respectively. 17 (3.45 g, 39%). 1H NMR (300 MHz, DMSO-d₆): δ 7.50–7.30 (m, 6H), 5.17 (s, 2H), 4.54 (t, J = 10.7, 4.0 Hz, 1H), 4.05 (q, J = 7.1 Hz, 2H), 4.03–3.89 (m, 2H), 3.18–2.90 (m, 2H), 2.05 (dd, J = 12.8, 3.0 Hz, 2H), 1.79 (ddd, J = 15.9, 12.1, 4.3 Hz, 2H), 1.19 (t, J = 7.1 Hz, 3H). 13C NMR (75 MHz, DMSO-d₆) δ 160.1, 154.6, 136.3, 128.4, 128.2, 128.1, 118.6, 71.5, 60.8, 60.4, 41.9, 31.0, 14.6. HRMS (ESI-TOF): m/z calculated for C₃₅H₃₇N₄O₃ [M+H]+, 331.1765. Found, 331.1760 (ΔM=1.5 ppm). 18 (3.6 g, 41%): mp 98–100
°C. ¹H NMR (600 MHz, DMSO-d₆): δ 7.85 (s, 1H), 7.48–7.31 (m, 5H), 5.15 (s, 2H), 4.62 (tt, J = 11.3, 4.0 Hz, 1H), 4.14–4.01 (m, 4H), 3.09–2.89 (m, 2H), 2.06–1.99 (m, 2H), 1.82 (qd, J = 12.3, 4.4 Hz, 2H), 1.19 (t, J = 7.1 Hz, 3H). ¹³C NMR (150 MHz, DMSO-d₆) δ 160.1, 154.5, 136.5, 128.4, 128.1, 128.0, 105.6, 71.5, 60.9, 57.6, 42.2, 31.6, 14.6. HRMS (ESI-TOF): m/z calculated for C₁₇H₂₃N₄O₃ [M+H]+, 331.1765. Found, 331.1761 (ΔM=1.0 ppm).

4.1.15. Ethyl 4-(4-(benzyloxy)-5-ido-1H-1,2,3-triazol-2-yl)piperidine-1-carboxylate (21). A solution of ICl (0.12 g, 0.73 mmol) in AcOH (2 mL) were added to a solution of 17 (0.20 g, 0.61 mmol) in AcOH (3 mL). Water (7 mL) was added and the resulting mixture was heated at 80 °C for 24 h. A solution of sodium thiosulfate 15–20% w/w was added and the reaction mixture was concentrated in vacuo. Water (50 mL) was added and the mixture was extracted with Et₂O (3 × 50 mL). The combined organic phase was washed with brine (50 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 0%–25% EtOAc) afforded 21 as colourless oil (0.21 g, 76%). ¹H NMR (300 MHz, DMSO-d₆): δ 7.54–7.28 (m, 5H), 5.23 (s, 2H), 4.67–4.50 (m, 1H), 4.05 (q, J = 7.1 Hz, 2H), 4.02–3.88 (m, 2H), 3.14–2.90 (m, 2H), 2.12–1.97 (m, 2H), 1.78 (qd, J = 12.2, 4.1 Hz, 2H), 1.19 (t, J = 7.1 Hz, 3H). ¹³C NMR (75 MHz, DMSO-d₆) δ 161.3, 154.6, 136.0, 128.5, 128.3, 128.2, 77.2, 72.0, 61.3, 60.8, 41.8, 30.9, 14.6. HRMS (ESI-TOF): m/z calculated for C₁₇H₂₂N₄O₃I [M+H]+, 457.0731. Found, 457.0732 (ΔM=0.3 ppm).

4.1.16. Ethyl 4-(4-(benzyloxy)-5-ido-1H-1,2,3-triazol-1-yl)piperidine-1-carboxylate (22). A solution of ICl (0.14 g, 0.88 mmol) in AcOH (4 mL) were added to a solution of 18 (0.22 g, 0.68 mmol) in AcOH (6 mL). Water (14 mL) was added and the resulting mixture was heated at 80 °C for 24 h. A solution of sodium thiosulfate 15–20% w/w was added and the reaction mixture was concentrated in vacuo. Water (50 mL) was added and the mixture was extracted with Et₂O (3 × 50 mL). The combined organic phase was washed with brine (50 mL), dried
over anhydrous Na$_2$SO$_4$, and evaporated in vacuo. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 0%–30% EtOAc) afforded 22 as white solid (0.19 g, 60%): mp 103–107 °C. $^1$H NMR (600 MHz, DMSO-$d_6$): δ 7.48–7.31 (m, 5H), 5.31 (s, 2H), 4.54 (tt, $J$ = 11.4, 4.1 Hz, 1H), 4.14–4.07 (m, 2H), 4.06 (q, $J$ = 7.1 Hz, 2H), 3.15–2.94 (m, 2H), 2.04–1.96 (m, 2H), 1.89 (qd, $J$ = 12.1, 4.4 Hz, 2H), 1.19 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ 161.9, 154.6, 136.6, 128.5, 128.2, 128.1, 71.4, 65.0, 60.9, 57.9, 42.2, 31.1, 14.6. HRMS (ESI-TOF): m/z calculated for C$_{17}$H$_{21}$N$_4$O$_3$INa [M+Na]+, 479.0551. Found, 479.0556 ($\Delta M$=1.2 ppm).

4.1.17. Ethyl 4-(4-(benzylloxy)-5-(1-hydroxy-3,3-diphenylpropyl)-1H-1,2,3-triazol-1-yl)piperidine-1-carboxylate (24c). A 1.7M solution of iPrMgCl in THF (0.67 mL, 1.1 mmol) was added dropwise to a cooled (–10 °C) solution of 22 (0.48 g, 1.0 mmol) in anhydrous THF (7 mL). The resulting mixture was stirred at the same temperature for 2 h. A solution of 3,3-diphenylpropanal (27, 0.24 g, 1.1 mmol) in anhydrous THF (3 mL) was added and the mixture was allowed to reach rt. After 48 h, saturated aqueous NH$_4$Cl (7 mL) was added and the mixture stirred for 30 min before it was evaporated in vacuo. The residue was taken up in water (50 mL) and extracted with Et$_2$O (3 × 50 mL). The combined organic phase was washed with brine (50 mL), dried over anhydrous Na$_2$SO$_4$, and evaporated in vacuo. Purification by flash chromatography (CH$_2$Cl$_2$/EtOAc, 85:15 v/v) afforded 24c (0.26 g, 46%) as white solid: mp 62–66 °C. $^1$H NMR (600 MHz, DMSO-$d_6$): δ 7.38–7.31 (m, 5H), 7.29–7.21 (m, 8H), 7.20–7.14 (m, 2H), 5.62 (d, $J$ = 5.3 Hz, 1H), 5.27 (s, 2H), 4.47 (dt, $J$ = 8.2, 5.9 Hz, 1H), 4.38 (tt, $J$ = 10.6, 4.6 Hz, 1H), 4.05 (q, $J$ = 7.1 Hz, 2H), 4.01 (t, $J$ = 8.0 Hz, 1H), 4.01–3.95 (m, 2H), 2.96–2.71 (m, 2H), 2.64–2.53 (m, 2H), 1.96–1.87 (m, 2H), 1.80–1.67 (m, 2H), 1.19 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ 155.8, 154.5, 144.5, 144.1, 136.9, 128.5, 128.4, 128.2, 127.8, 127.7, 127.65, 127.6, 126.2, 126.1, 120.8, 71.1, 60.8, 60.3, 55.7, 47.0, 42.4, 42.3, 31.9, 14.6. HRMS (ESI-TOF): m/z calculated for C$_{37}$H$_{39}$N$_4$O$_3$INa $[M+Na]^{+}$, 726.2221. Found, 726.2213 ($\Delta M$=0.8 ppm).
31.3, 14.6. HRMS (ESI-TOF): m/z calculated for C_{25}H_{37}N_{4}O_{4} [M+H]^+; 541.2809. Found, 541.2804 (ΔM=1.0 ppm).

4.1.18. Ethyl 4-(5-(3,3-diphenylpropyl)-4-hydroxy-1H-1,2,3-triazol-1-yl)piperidine-1-carboxylate (28c). Et_{3}SiH (0.3 mL, 1.8 mmol) and TFA (0.67 mL, 8.7 mmol) were added to a solution of 24c (0.17 g, 0.31 mmol) in CH_{2}Cl_{2} (6 mL). The reaction mixture was heated at 50 °C in a sealed tube for 48 h. After cooling, CH_{2}Cl_{2} was added up to 50 mL and the resulting mixture washed with 2M NaOH (50 mL). The aqueous phase was extracted with CH_{2}Cl_{2} (2 × 50 mL). The combined organic phases were washed with brine (50 mL), dried over anhydrous MgSO_{4}, and evaporated. The crude product was dissolved in MeOH (20 mL) and added Pd/C (15 mg). The reaction mixture was put under a hydrogen atmosphere and stirred for 16 h. The reaction mixture was filtered through a PVDF filter (0.45 µm) and the volatiles were evaporated in vacuo. Purification by preparative HPLC (gradient 50%–70% solvent B over 10 min) afforded 28c (0.11 g, 81%) as colourless oil. ^1{H} NMR (600 MHz, DMSO-d_{6}): δ 7.37–7.25 (m, 8H), 7.21–7.15 (m, 2H), 4.05 (q, J = 7.0 Hz, 2H), 4.05–3.98 (m, 2H), 3.95 (t, J = 7.8 Hz, 1H), 2.90–2.72 (m, 2H), 2.49–2.44 (m, 2H), 2.30–2.23 (m, 2H), 1.84–1.74 (m, 4H), 1.19 (t, J = 7.1 Hz, 3H). ^1{C} NMR (150 MHz, DMSO-d_{6}) δ 155.4, 154.5, 144.5, 128.5, 127.6, 126.2, 117.0, 60.8, 54.6, 50.0, 42.4, 33.1, 31.5, 19.7, 14.6. HRMS (ESI-TOF): m/z calculated for C_{25}H_{37}N_{4}O_{4} [M+H]^+; 435.2391. Found, 435.2395 (ΔM=1.0 ppm).

4.1.19. Ethyl 4-(4-(benzyl)oxy)-5-(naphthalen-2-ylmethyl)-1H-1,2,3-triazol-1-yl)piperidine-1-carboxylate (26b). A solution of PrMgCl in THF (1.7M, 0.69 mL, 1.2 mmol) was added dropwise to a cooled solution (~10 °C) of 22 (0.50 g, 1.1 mmol) in anhydrous THF (7 mL). The mixture was stirred 1 h before a solution of 2-naphthaldehyde (0.19 g, 1.2 mmol) in anhydrous THF (3 mL) was added. The resulting mixture was allowed to reach rt. After 48 h, saturated aqueous NH_{4}Cl (5 mL) was added and the mixture stirred for 30 min before it was
evaporated *in vacuo*. The residue was taken up in water (50 mL) and extracted with EtO (3 × 50 mL). The combined organic phase was washed with brine (50 mL), dried over anhydrous Na$_2$SO$_4$, and evaporated *in vacuo*. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 0%–40% EtOAc) afforded the alcohol intermediate 24b (0.42 g, 80%).

HRMS (ESI-TOF): m/z calculated for C$_{28}$H$_{31}$N$_4$O$_4$ [M+H]$^+$, 487.2340. Found, 487.2338 (∆M=0.4 ppm).

24b (0.40 g, 0.83 mmol) was dissolved in CH$_2$Cl$_2$ (30 mL) and Et$_3$SiH (0.21 mL, 1.3 mmol) was added. The solution was cooled at 0 °C, TFA (1.8 mL, 23 mmol) was added and the resulting mixture was allowed to reach rt and stirred for 20 h. CH$_2$Cl$_2$ was added up to 50 mL and the resulting mixture was washed with 2M NaOH (50 mL). The aqueous phase was extracted with CH$_2$Cl$_2$ (2 × 50 mL) and the combined organic phase was washed with brine (50 mL), dried over anhydrous Na$_2$SO$_4$, and evaporated *in vacuo*. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 10%–35% EtOAc) afforded 26b (0.30 g, 77%) as colourless oil.

$^1$H NMR (600 MHz, DMSO-$d_6$): δ 7.90–7.78 (m, 3H), 7.66 (s, 1H), 7.52–7.45 (m, 2H), 7.37–7.25 (m, 6H), 5.31 (s, 2H), 4.58 (tt, J = 11.5, 4.0 Hz, 1H), 4.20 (s, 2H), 4.02 (q, J = 7.1 Hz, 2H), 4.00–3.89 (m, 2H), 2.97–2.77 (m, 2H), 1.81 (qd, J = 12.3, 4.5 Hz, 2H), 1.73–1.62 (m, 2H), 1.15 (t, J = 7.1 Hz, 3H). $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ 156.8, 154.5, 137.0, 135.1, 133.0, 131.8, 128.3, 127.9, 127.85, 127.6, 127.4, 126.7, 126.4, 126.2, 125.8, 118.3, 71.3, 60.8, 55.0, 42.3, 31.5, 26.7, 14.5. HRMS (ESI-TOF): m/z calculated for C$_{28}$H$_{31}$N$_4$O$_3$ [M+H]$^+$, 471.2391. Found, 471.2387 (∆M=0.8 ppm).

### 4.1.20 Ethyl 4-(4-(benzyloxy)-5-(napthalen-2-ylmethyl)-2H-1,2,3-triazol-2-yl)piperidine-1-carboxylate (25b). A solution of PrMgCl in THF (1.7M, 1.4 mL, 2.4 mmol) was added dropwise to a cooled solution (~10 °C) of 21 (1.0 g, 2.2 mmol) in anhydrous THF (15 mL). The mixture was stirred 1 h before a solution of 2-naphthaldehyde (0.38 g, 2.4 mmol) in anhydrous THF (5 mL) was added. The resulting mixture was allowed to reach rt. After 48 h, saturated aqueous NH$_4$Cl (10 mL) was added and the mixture stirred for 30 min before it was
evaporated in vacuo. The residue was taken up in water (50 mL) and extracted with Et₂O (3 × 50 mL). The combined organic phase was washed with brine (50 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 0%–35% EtOAc) afforded the alcohol intermediate 23b (0.64 g, 60%) as colourless oil. HRMS (ESI-TOF): m/z calculated for C₂₈H₃₀N₄O₄Na [M+Na]⁺, 509.2159. Found, 509.2167 (ΔM=1.5 ppm).

23b (0.62 g, 1.3 mmol) was dissolved in CH₂Cl₂ (45 mL) and Et₃SiH (0.33 mL, 2.0 mmol) was added. The solution was cooled at 0 °C, TFA (2.7 mL, 36 mmol) was added and the resulting mixture was allowed to reach rt and stirred for 20 h. CH₂Cl₂ was added up to 50 mL and the resulting mixture was washed with 2M NaOH (50 mL). The aqueous phase was extracted with CH₂Cl₂ (2 × 50 mL) and the combined organic phase was washed with brine (50 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 0%–20% EtOAc) afforded 25b (0.30 g, 50%) as colourless oil. ¹H NMR (600 MHz, DMSO-d₆): δ 7.85 (d, J = 7.7 Hz, 1H), 7.83–7.78 (m, 2H), 7.69 (s, 1H), 7.51–7.43 (m, 2H), 7.37–7.26 (m, 6H), 5.19 (s, 2H), 4.49 (tt, J = 10.9, 4.1 Hz, 1H), 4.07–4.00 (m, 4H), 4.00–3.92 (m, 2H), 3.09–2.93 (m, 2H), 2.07–2.00 (m, 2H), 1.79 (qd, J = 11.6, 4.4 Hz, 2H), 1.18 (t, J = 7.1 Hz, 3H). ¹³C NMR (150 MHz, DMSO-d₆) δ 157.0, 154.6, 136.5, 136.3, 133.0, 131.7, 130.5, 128.3, 128.0, 127.9, 127.8, 127.5, 127.4, 127.2, 126.4, 126.1, 125.5, 71.4, 60.8, 60.2, 41.9, 31.0, 29.5, 14.6. HRMS (ESI-TOF): m/z calculated for C₂₈H₃₁N₄O₃ [M+H]⁺, 471.2391. Found, 471.2384 (ΔM=1.3 ppm).

4.1.21 Ethyl 4-(4-(benzyloxy)-5-(3,3-diphenylpropyl)-2H-1,2,3-triazol-2-yl)piperidine-1-carboxylate (25c). A solution of PrMgCl in THF (1.7M, 1.4 mL, 2.5 mmol) was added dropwise to a cooled solution (~10 °C) of 21 (1.0 g, 2.2 mmol) in anhydrous THF (15 mL). The mixture was stirred 1 h before a solution of 3,3-diphenylpropanal (27, 0.52 g, 2.5 mmol) in anhydrous THF (5 mL) was added. The resulting mixture was allowed to reach rt. After 48
h, saturated aqueous NH₄Cl (5 mL) was added and the mixture stirred for 30 min before it was evaporated in vacuo. The residue was taken up in water (50 mL) and extracted with Et₂O (3 × 50 mL). The combined organic phase was washed with brine (50 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, gradient 0%–35% EtOAc) afforded the alcohol intermediate 23c (0.36 g, 30%) as colourless oil. 

1H NMR (600 MHz, DMSO-d₆): δ 7.41–7.30 (m, 5H), 7.30–7.20 (m, 8H), 7.18–7.11 (m, 2H), 5.24 (d, J = 5.2 Hz, 1H), 5.17 (s, 2H), 4.48 (tt, J = 10.7, 4.0 Hz, 1H), 4.32 (dt, J = 8.2, 5.6 Hz, 1H), 4.05 (q, J = 7.1 Hz, 2H), 4.03–4.00 (m, 1H), 3.99–3.92 (m, 2H), 3.11–2.95 (m, 2H), 2.56–2.43 (m, 2H), 2.06–2.00 (m, 2H), 1.83–1.74 (m, 2H), 1.19 (t, J = 7.1 Hz, 3H).

13C NMR (150 MHz, DMSO-d₆) δ 156.6, 154.6, 145.1, 144.3, 136.6, 134.2, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 126.1, 126.0, 71.3, 61.7, 60.8, 60.2, 46.9, 41.9, 40.9, 30.9, 14.6. HRMS (ESI-TOF): m/z calculated for C₃₂H₃₆N₄O₄Na [M+Na]+, 563.2629. Found, 563.2618 (∆M=1.8 ppm).

23c (0.34 g, 0.62 mmol) was dissolved in CH₂Cl₂ (30 mL) and Et₃SiH (0.60 mL, 3.7 mmol) was added. The solution was cooled at 0 °C and TFA (1.3 mL, 17 mmol) was added and the resulting mixture was allowed to reach rt and stirred for 72 h. CH₂Cl₂ was added up to 50 mL and the resulting mixture was washed with 2M NaOH (50 mL). The aqueous phase was extracted with CH₂Cl₂ (2 × 50 mL) and the combined organic phase was washed with brine (50 mL), dried over anhydrous Na₂SO₄, and evaporated in vacuo. Purification by flash chromatography (petroleum ether 40–60 °C/EtOAc, 85:15 v/v) afforded 25c (0.25 g, 78%) as colourless oil.

1H NMR (600 MHz, DMSO-d₆): δ 7.41–7.31 (m, 5H), 7.28–7.22 (m, 8H), 7.17–7.12 (m, 2H), 5.16 (s, 2H), 4.46 (tt, J = 10.8, 4.1 Hz, 1H), 4.05 (q, J = 7.1 Hz, 2H), 4.00–3.92 (m, 2H), 3.92 (t, J = 7.7 Hz, 1H), 3.10–2.94 (m, 2H), 2.41–2.37 (m, 2H), 2.32–2.27 (m, 2H), 2.04–1.99 (m, 2H), 1.77 (qd, J = 12.3, 4.3 Hz, 2H), 1.19 (t, J = 7.1 Hz, 3H). 13C NMR (150 MHz, DMSO-d₆) δ 156.9, 154.6, 145.4, 144.7, 136.5, 131.1, 128.4, 128.3, 128.1, 128.0, 127.6, 126.1, 71.4, 60.8, 60.0, 49.9, 41.9, 33.4, 30.9, 21.7, 14.6. HRMS (ESI-TOF): m/z calculated for C₃₂H₃₇N₄O₃ [M+H]+, 525.2860. Found, 525.2856 (∆M=0.7 ppm).
4.1.22 5-(Naphthalen-2-ylmethyl)-2-(piperidin-4-yl)-2H-1,2,3-triazol-4-ol hydrochloride (3b). A solution of 25b (0.23 g, 0.49 mmol) in EtOH/35% HCl (1:2 v/v, 15 mL) was heated at reflux for 24 h. Upon cooling to rt, the solvents were evaporated in vacuo. Purification by preparative HPLC (gradient 20%–50% solvent B over 15 min) followed by conversion of the obtained product into the hydrochloric salt using 2M HCl afforded 3b (32 mg, 20%) as pale yellow solid: mp 200–203 °C. \(^1\)H NMR (600 MHz, DMSO-\(\text{d}_6\)): \(\delta\) 10.50 (s, 1H), 9.03 (br s, 2H), 7.87–7.79 (m, 3H), 7.68 (s, 1H), 7.50–7.42 (m, 2H), 7.39 (d, \(J = 8.4\) Hz, 1H), 4.52 (tt, \(J = 9.8, 4.3\) Hz, 1H), 4.01 (s, 2H), 3.33–3.24 (m, 2H), 3.09–2.99 (m, 2H), 2.21–2.06 (m, 4H). \(^13\)C NMR (150 MHz, DMSO-\(\text{d}_6\)) \(\delta\) 156.1, 136.9, 133.0, 131.6, 130.5, 127.9, 127.5, 127.2, 126.3, 126.1, 125.5, 57.3, 41.7, 29.3, 27.9. HRMS (ESI-TOF): \(m/z\) calculated for C\(_{18}\)H\(_{21}\)N\(_4\)O [M+H]\(^+\), 309.1710. Found, 309.1711 (\(\Delta M=0.3\) ppm).

4.1.23 5-(3,3-Diphenylpropyl)-2-(piperidin-4-yl)-2H-1,2,3-triazol-4-ol hydrochloride (3c). A solution of 25c (0.21 g, 0.38 mmol) in EtOH/35% HCl (1:2 v/v, 15 mL) was heated at reflux for 24 h. Upon cooling to rt, the solvents were evaporated in vacuo. Recrystallization from MeOH/Et\(_2\)O afforded 3c (82 mg, 52%) as white solid: mp 246–249 °C. \(^1\)H NMR (600 MHz, DMSO-\(\text{d}_6\)): \(\delta\) 10.27 (s, 1H), 9.21 (s, 2H), 7.38–7.21 (m, 8H), 7.21–7.09 (m, 2H), 4.49 (tt, \(J = 10.0, 4.8\) Hz, 1H), 3.96 (t, \(J = 7.5\) Hz, 1H), 3.32–3.22 (m, 2H), 3.12–2.98 (m, 2H), 2.42–2.25 (m, 4H), 2.21–2.06 (m, 4H). \(^13\)C NMR (150 MHz, DMSO-\(\text{d}_6\)) \(\delta\) 155.8, 144.8, 131.1, 128.4, 127.6, 126.0, 57.0, 50.1, 41.6, 33.5, 27.8, 21.8. HRMS (ESI-TOF): \(m/z\) calculated for C\(_{22}\)H\(_{27}\)N\(_4\)O [M+H]\(^+\), 363.2179. Found, 363.2182 (\(\Delta M=0.8\) ppm).

4.1.24 5-(Naphthalen-2-ylmethyl)-1-(piperidin-4-yl)-1H-1,2,3-triazol-4-ol hydrochloride (4b). A solution of 26b (0.21 g, 0.46 mmol) in EtOH/35% HCl (1:2 v/v, 15 mL) was heated at reflux for 24 h. Upon cooling to rt, the solvents were evaporated in vacuo. Purification by
preparative HPLC (gradient 20%–40% solvent B over 10 min) followed by conversion of the obtained product into the hydrochloric salt using 2M HCl afforded 4b (73 mg, 46%) as pale yellow solid: mp 258–261 °C. 1H NMR (600 MHz, DMSO-d$_6$): δ 9.34 (br s, 1H), 8.99 (br s, 1H), 7.91–7.81 (m, 3H), 7.71 (s, 1H), 7.51–7.44 (m, 2H), 7.37 (dd, J = 8.4, 1.7 Hz, 1H), 4.67 (tt, J = 10.9, 3.9 Hz, 1H), 4.19 (s, 2H), 3.31–3.25 (m, 2H), 3.03–2.94 (m, 2H), 2.21–2.11 (m, 2H), 1.85–1.77 (m, 2H). 13C NMR (150 MHz, DMSO-d$_6$) δ 155.8, 135.7, 133.0, 131.8, 128.3, 127.6, 127.4, 126.8, 126.3, 126.0, 125.7, 116.8, 52.3, 42.0, 28.4, 26.6. HRMS (ESI-TOF): m/z calculated for C$_{18}$H$_{21}$N$_4$O [M+H]$^+$, 309.1710. Found, 309.1708 (ΔM=0.5 ppm).

4.1.25 5-(3,3-Diphenylpropyl)-1-(piperidin-4-yl)-1H-1,2,3-triazol-4-ol hydrochloride (4c). A solution of 28c (93 mg, 0.21 mmol) in EtOH/35% HCl (1:2 v/v, 15 mL) was heated at reflux for 24 h. Upon cooling to rt, the solvents were evaporated in vacuo. Recrystallization from MeOH/Et$_2$O afforded 4c (43 mg, 51%) as white solid: mp 232–234 °C. 1H NMR (600 MHz, DMSO-d$_6$): δ 9.82 (s, 1H), 9.16 (br s, 1H), 8.88 (br s, 1H), 7.37–7.24 (m, 8H), 7.23–7.13 (m, 2H), 4.28 (tt, J = 11.0, 4.0 Hz, 1H), 3.97 (t, J = 7.8 Hz, 1H), 3.40–3.37 (q, J = 7.0 Hz, 0.6H, (CH$_3$CH$_2$)O), 3.37–3.33 (m, 2H), 3.00–2.91 (m, 2H), 2.48–2.45 (m, 2H), 2.30–2.24 (m, 2H), 2.22–2.13 (m, 2H), 2.02–1.95 (m, 2H), 1.09 (t, J = 7.0 Hz, 0.9 H, (CH$_3$CH$_2$)O). 13C NMR (150 MHz, DMSO-d$_6$) δ 155.4, 144.5, 128.5, 127.6, 126.2, 117.2, 64.9, 51.9, 50.1, 42.1, 33.0, 28.4, 19.7, 15.1. HRMS (ESI-TOF): m/z calculated for C$_{22}$H$_{27}$N$_4$O [M+H]$^+$, 363.2179. Found, 363.2179 (ΔM=0.2 ppm). Anal. calcd (C$_{22}$H$_{26}$N$_4$O·1.25HCl·0.1Et$_2$O): C, 64.76; H, 6.85; N, 13.49. Found: C, 65.14; H, 6.45; N 13.18.

4.2. Determination of ionization constants.

The ionization constants of compounds 2a–b, 3a–c and 4a–c were determined by potentiometric titration with the GLpK$_a$ apparatus (Sirius Analytical Instruments Ltd, Forest Row, East Sussex, UK). The pK$_a$ values were obtained as mean of four titrations: aqueous
solutions (ionic strength adjusted to 0.15M with KCl) of the compound (20 mL, about 1 mM) were initially acidified to pH 1.8 with 0.5 N HCl and then titrated with standardized 0.5N KOH to pH 12.2 at constant temperature of 25(±0.1) °C under argon atmosphere.

4.3 Molecular modelling

4.3.1 Docking of selected compounds. A model of the extracellular domain of GABA$_A$R constructed using an iterative approach with the orthosteric binding site optimized using an induced fit docking protocol,[38-41] has previously been reported,[26] and is used here with the compounds 3a and 4a. Subsequently ligands 3b–c, and 4b–c, were docked into the binding site as described previously,[26] except 200 poses per ligand were included in the post-docking minimization step. The attained docking poses were subsequently refined using the “None (refine only)” ligand sampling option in the Glide 7.7 docking program.[42-45] Finally the obtained models were minimized using the MacroModel 11.8 program.[46]

4.3.2 Calculation of solvation energies of 4-PHP and 2a. Solvation energies were calculated in Jaguar[35, 47] version 9.8 on B3LYP/6-31+G** optimized geometries using the Poisson Boltzmann Finite[48-50] element method as implemented in Jaguar. Gas phase optimized geometries (B3LYP/6-31+G**) were used as reference. Default settings were used except for the SCF convergence threshold, which was set to “ultrafine”. Calculations were performed on the anionic forms the triazole moiety of the compounds.

4.4 Pharmacology

Characterization of compounds 2a–b, 3a–c, and 4a–c in muscimol binding: the binding assay was performed using rat brain synaptic membranes of cortex and the central hemispheres from male SPRD rats with tissue preparation as described in the literature.[51] On the day of the experiment, the membrane preparation was quickly thawed, homogenized in 50 volumes of ice-cold buffer (50 mM Tris–HCl buffer, pH 7.4), and centrifuged at 48,000g for 10 min at 4
°C. This washing step was repeated four times and the final pellet was re-suspended in buffer.
The assay was carried out in 96-wells plates, by incubation of membranes (70–80 µg protein) in 200 µL buffer, 25 µL [³H]muscimol (5 nM final concentration), and 25 µL test substance in various concentrations, for 60 min at 0 °C. The reaction was terminated by rapid filtration through GF/C filters (Perkin Elmer Life Sciences), using a 96 well Packard FilterMate cellharvester, followed by washing with 3 × 250 µL of ice-cold buffer. The dried filters were added Microscint scintillation fluid (PerkinElmer Life Sciences), and the amount of filterbound radioactivity was quantified in a Packard TopCount microplate scintillator counter. The experiments were performed in triplicate at least three times for each compound. Non-specific binding was determined using 1.0 mM GABA. The binding data was analysed by a non-linear regression curve-fitting procedure using GraphPad Prism v. 6.00 (GraphPad Software, CA, USA). IC₅₀ values were calculated from inhibition curves and converted to Kᵢ values using the modified Cheng–Prusoff equation.[52]

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Notes. The authors declare no competing financial interest.

ABBREVIATIONS USED
γ-Aminobutyric acid (GABA), GABA type A receptor (GABAₐR), 5-(piperidin-4-yl)-3-isoxazolol (4-PIOL), 4-(piperidin-4-yl)-1-hydroxypyrazole (4-PHP), and 5-(piperidin-4-yl)-3-hydroxypyrrozol (aza-4-PIOL).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at: XXXXXX

References


