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Novel 4-(Piperidin-4-yl)-1-hydroxypyrazoles as γ-Aminobutyric Acid$_A$ Receptor Ligands: Synthesis, Pharmacology, and Structure−Activity Relationships

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A series of substituted 1-hydroxypyrazole analogues of the $GABA_A$ receptor partial agonist 5-(4-piperidyl)-3-isoxazolol (4-PIOL) have been synthesized and pharmacologically characterized. Several of the analogues displayed K_i in the low nanomolar range at the native $GABA_A$ receptors and potent antagonism of the $\alpha_1\beta_2\gamma_2$ receptor. It appears that several regions situated in proximity to the core of the orthosteric binding site of the $GABA_A$ receptor are able to accommodate large hydrophobic substituents.

Introduction

γ-Aminobutyric acid (GABA,[a] Figure 1) is the major inhibitory neurotransmitter in the central nervous system, exerting its physiological effects through the ionotropic $GABA_A$ and the metabotropic $GABA_B$ receptors. The plethora of $GABA_A$ receptor subtypes is assembled from 19 different subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, θ, and ρ_{1-3}) to form pentameric channels with a central chloride-selective pore.[1,2] So far, the existence of at least 26 native $GABA_A$ receptor subtypes has been proposed,[3] of which the three most dominant synaptic subtypes are believed to be the $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_2\beta_3\gamma_2$ combinations,[4] where GABA binds at the interface of the α and the β subunits in the receptor complex.[5−7] The $GABA_A$ receptors are widely distributed in the central nervous system where they play essential roles in numerous physiological processes. Therapeutic intervention in $GABA_A$ receptor signaling has proven beneficial in several disorders such as anxiety, epilepsy, schizophrenia, and depression.[8]

The $GABA_A$ receptors belong to the Cys-loop receptor superfamily of ligand-gated ion channels, which also comprises the nicotinic acetylcholine receptors, the serotonin 5-HT_3 receptors, and the glycine receptors.[3,9] For nicotinic ligands, the elucidation of binding modes has been aided significantly by structural insight from the homologous acetylcholine binding protein, resulting in reliable templates for construction of homology models. For the other Cys-loop receptors, including $GABA_A$ receptors, homology models of the extracellular domains are still based on templates characterized by low sequence identities.[10] Consequently, structure−activity relationship (SAR) studies and pharmacophore models of orthosteric $GABA_A$ ligands are still essential to probe the structural basis for receptor−ligand interactions and the molecular determinants of ligand affinity, potency, efficacy, and subtype selectivity.

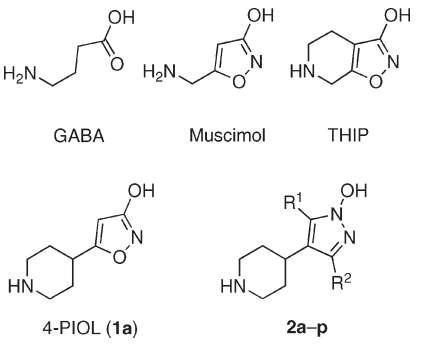

Figure 1. Structures of GABA, muscimol, THIP, 4-PIOL (**1a**) and a general structure of the new 1-hydroxypyrazole compounds **2a**−**p**.

In previous studies, we constructed a pharmacophore model of orthosteric ligand binding to the $GABA_A$ receptor based on SAR studies of analogues of the standard $GABA_A$ ligands muscimol, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-3-ol (THIP), and 5-(4-piperidyl)-3-isoxazolol (4-PIOL) (Figure 1).[13,14] By use of this model, a series of potent $GABA_A$ antagonists were developed by introducing bulky substituents in the 4-position of the 3-isoxazolol ring of 4-PIOL.[11,12,15] This suggested that there is a spacious cavity situated in the vicinity of the core of the orthosteric site of the $GABA_A$ receptor, which is able to accommodate the binding of large substituents (Figure 2).

In the present study we have investigated the so far unexplored area of the receptor binding pocket corresponding to the area near the isoxazole oxygen in **1a** (Figure 1). For this purpose, we have applied compounds containing a new isostere to the isoxazolol moiety of **1a**, the 1-hydroxypyrazole moiety (**2a**−**p**, Figure 1), as lead structure. To verify the bioisosteric potential of the 1-hydroxypyrazole ring compared to the 3-isoxazolol ring of 4-PIOL, a series of 5-substituted 1-hydroxypyrazoles **2b**−**e**, corresponding to a selected subgroup of previously reported 4-substituted 4-PIOL analogues,[11,12]

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[a] Abbreviations: DCVC, dry column vacuum chromatography; FLIPR, fluorescent imaging plate reader; FMP, FLIPR membrane potential; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; 4-PIOL, 5-(4-piperidyl)-3-isoxazolol; SAR, structure−activity relationship; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-3-ol.

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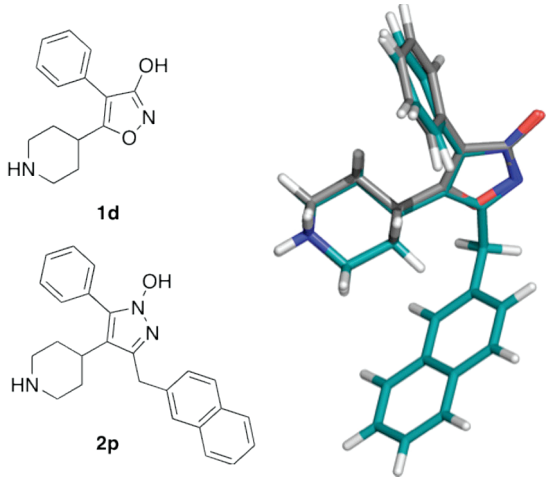
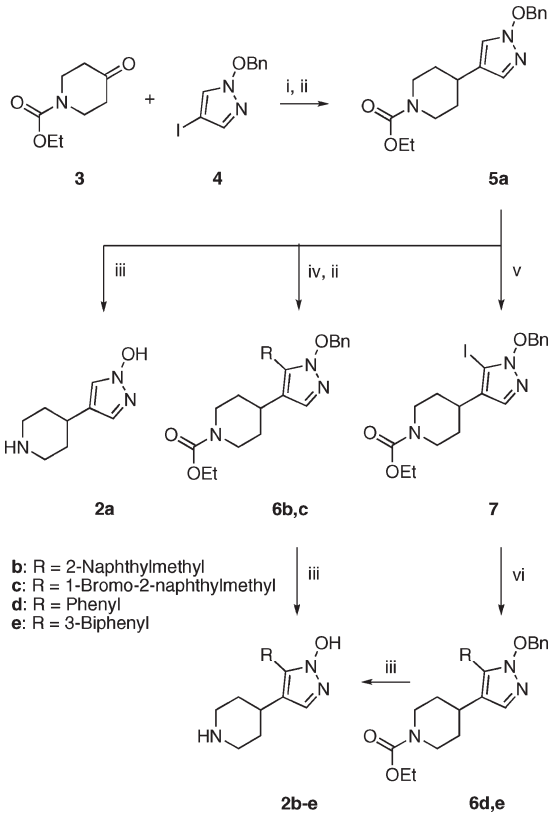


Figure 2. Structures and 3D superimposition of **1d** (gray carbons) and **2p** (cyan carbons), illustrating the new substitution position introduced by the 1-hydroxypyrazole ring compared to the isoxazole scaffold of the 4-PIOL series.

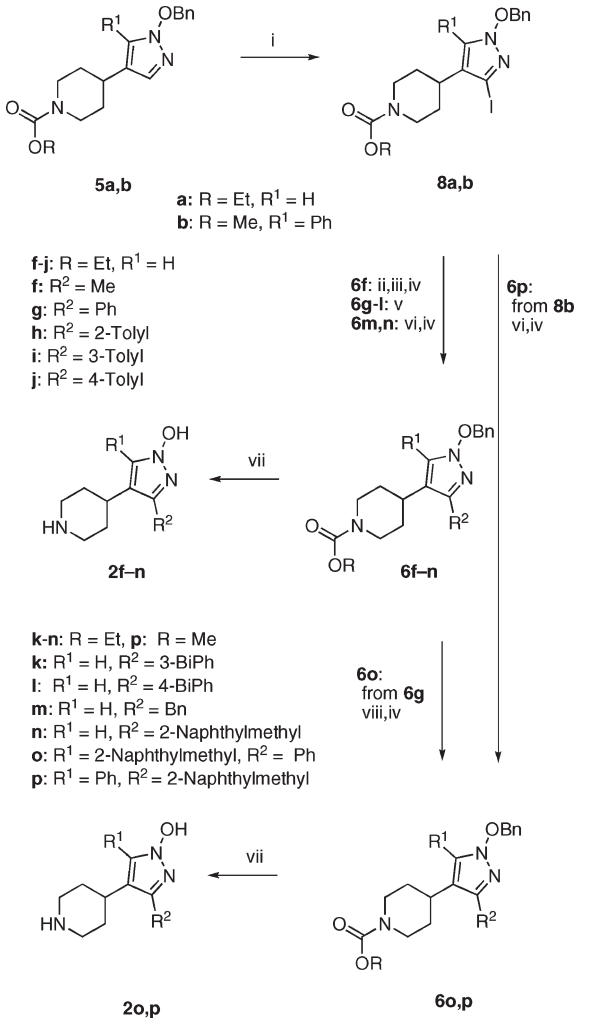
Scheme 1[a]



[a] Reagents: (i) ^{i}PrMgCl, THF, 0 °C; (ii) Et_3SiH, TFA, CH_2Cl_2, 50 °C; (iii) 35% aq HCl, 130 °C; (iv) LDA, R-CHO, THF, −78 °C; (v) LDA, I_2, THF, −78 °C; (vi) R-B(OH)$_2$, $PdCl_2(PPh)_2$, DMF, K_2CO_3 (3 M).

was first synthesized. Then, to investigate unexplored areas of the $GABA_A$ receptor ligand binding site, a series of 3-substituted and 3,5-disubstituted 1-hydroxypyrazole analogues (**2f**−**p**) was prepared. The pharmacological properties of the synthesized compounds have been characterized at native and recombinant $GABA_A$ receptors, and the findings are discussed in terms of our previously reported pharmacophore model.

Scheme 2[a]



[a] Reagents: (i) ICl, AcOH/H_2O; (ii) ^{i}PrMgCl, DMF, THF, 0 °C; (iii) $NaBH_4$, MeOH; (iv) Et_3SiH, TFA, CH_2Cl_2, 50 °C; (v) R-B(OH)$_2$, $PdCl_2(PPh)_2$, toluene/EtOH (10:1), K_2CO_3 (3 M); (vi) ^{i}PrMgCl, R-CHO, THF, 0 °C; (vii) 35% aq HCl, 130 °C or 47% aq HBr, 110 °C; (viii) LDA, 2-naphthaldehyde, THF, −78 °C.

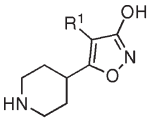
Results and Discussion

Chemistry. Compounds **2a**−**p** were all synthesized from the same lead molecules **5a**,**b** (Schemes 1 and 2). Preparation of **5a** took place through a Grignard reaction using 1-(benzyloxy)-4-iodopyrazole[16] and commercially available 1-ethoxycarbonyl-4-piperidone **3** (Scheme 1). The resulting hydroxyl group was removed using trifluoroacetic acid and triethylsilane in dichloromethane to give **5a**.[17]

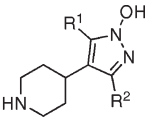
The 5-lithiated version of **5a** was prepared using lithium diisopropylamide (LDA). Addition of the corresponding aldehydes and subsequent reduction of the hydroxyl group gave rise to **6b**,**c** (Scheme 1). Compound **7** was prepared by lithiation of **5a** with LDA and subsequent addition of iodine. Cross-coupling of **7** using phenylboronic acid and 3-biphenylboronic acid resulted in **6d** and **6e**, respectively. Acidic deprotection of **6b**−**e** yielded the target compounds **2b**−**e**.

For the synthesis of the 3-substituted analogues, iodine was selectively introduced by refluxing **5a** with iodine monochloride in acetic acid and water to give **8a** (Scheme 2). Compound **6f** could not be prepared by Suzuki cross-coupling and was instead synthesized through a Grignard reaction using

Table 1. Pharmacological Data for **1a**−**e** and **2a**−**p**: $GABA_A$ Receptor Binding Affinities at Rat Synaptic Membranes and Functional Characterization at the Human $\alpha_1\beta_2\gamma_2$ $GABA_A$ Receptor Transiently Expressed in tsA201Cells in the FMP Red Assay



1a–e



2a–p

compd	R_1	R_2	[^{3}H]muscimol binding K_i (μM)[a] [pK_i ± SEM]	$\alpha_1\beta_2\gamma_2$ tsA201 cell line IC_{50} (μM)[b] [pIC_{50} ± SEM]
1a	H		9.1[c]	> 500 [< 3.30][d]
1b	2-naphthylmethyl		0.049[c]	1.8 [5.74 ± 0.05]
1c	1-bromo-2-naphthylmethyl		0.010[c]	nd
1d	phenyl		0.22[c]	nd
1e	3-biphenyl		0.010[e]	0.078 [7.11 ± 0.04]
2a	H	H	10 [4.99 ± 0.02]	> 500 [< 3.30][d]
2b	2-naphthylmethyl	H	0.033 [7.48 ± 0.03]	0.79 [6.10 ± 0.05]
2c	1-bromo-2-naphthylmethyl	H	0.0095 [8.07 ± 0.09][f]	0.48 [6.32 ± 0.05]
2d	phenyl	H	0.022 [7.65 ± 0.02]	0.15 [6.81 ± 0.04]
2e	3-biphenyl	H	0.0028 [8.55 ± 0.03]	0.024 [7.62 ± 0.03]
2f	H	methyl	5.0 [5.31 ± 0.05]	66 [4.18 ± 0.04]
2g	H	phenyl	0.27 [6.57 ± 0.01]	1.9 [5.73 ± 0.06]
2h	H	*o*-tolyl	0.67 [6.17 ± 0.03]	2.6 [5.58 ± 0.02]
2i	H	*m*-tolyl	0.24 [6.62 ± 0.03]	1.9 [5.72 ± 0.06]
2j	H	*p*-tolyl	0.32 [6.50 ± 0.06]	1.4 [5.85 ± 0.03]
2k	H	3-biphenyl	0.030 [7.55 ± 0.09]	0.21 [6.67 ± 0.04]
2l	H	4-biphenyl	0.42 [6.39 ± 0.05]	1.4 [5.85 ± 0.04]
2m	H	benzyl	0.36 [6.45 ± 0.03]	8.3 [5.08 ± 0.04]
2n	H	2-naphthylmethyl	0.0030 [8.55 ± 0.10]	0.17 [6.78 ± 0.04]
2o	2-naphthylmethyl	phenyl	1.5 [5.84 ± 0.05][f]	3.2 [5.50 ± 0.06]
2p	phenyl	2-naphthylmethyl	0.022 [7.66 ± 0.02][f]	nd

[a] IC_{50} values were calculated from inhibition curves and converted to K_i values. Data are given as the mean [mean pK_i ± SEM] of three to four independent experiments. [b] For the characterization of the antagonists, assay concentrations of GABA of 10−15 μM (5- to 8-fold higher than the EC_{50} concentration) were used. nd: not determined. [c] Reference 11. [d] The compound was also tested in the agonist assay but showed no activity (>500 [<3.30]). [e] Reference 12. [f] Tested in 10% DMSO.

N,*N*-dimethylformamide followed by reduction. Suzuki cross-coupling of **8a** with the corresponding boronic acids led to **6g**−**l**. In order to introduce the alkylaryl substituents in **6m**,**n**, compound **8a** was converted into the Grignard reagent and quenched with benzaldehyde and 2-naphthaldehyde, respectively, followed by reduction. Deprotection of **6f**−**n** resulted in **2f**−**n**.

Finally, **6o** and **6p**, with substituents in the 3- and the 5-position of the 1-hydroxypyrazole ring, were prepared from **6g** and **8b**, respectively, by introduction of the 2-naphthylmethyl substituent in the 5- or the 3-position as described for **6b** and **6n**. Deprotection under acidic conditions resulted in **2o** and **2p** (Scheme 2).

Pharmacology. The synthesized compounds were pharmacologically characterized in binding studies using rat brain membrane preparations, where the binding affinities of the compounds at native $GABA_A$ receptors were measured by displacement of [^{3}H]muscimol. The functional characterization of the compounds at the human $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors transiently expressed in tsA201 cells was performed using the FLIPR membrane potential (FMP) red assay as described in Supporting Information. Data for **2a**−**p** are listed in Table 1.

The effect of the compounds on the uptake of GABA through the GABA transporters was also examined. None of the compounds displayed inhibitory activity at the four human GABA transporters (hGAT-1, hBGT-1, hGAT-2, and hGAT-3) transiently expressed in tsA201 cells (data not shown).

Receptor Binding and Structure−**Affinity Relationships.** The 1-hydroxypyrazole analogue of 4-PIOL (**2a**) displayed $GABA_A$ receptor affinity in the low micromolar range comparable to that of 4-PIOL (**1a**). The 5-substituted 1-hydroxypyrazole analogues (**2b**−**e**) all showed high affinity for the $GABA_A$ receptor sites and exhibited similar SAR as previously described for the corresponding 3-isoxazolol analogues **1b**−**e**.[11,12]

These results support the presence of the previously suggested cavity in the orthosteric site and, considering the structural similarity of the compounds, imply a shared binding mode for the 1-hydroxypyrazole series of compounds. According to this hypothesis, the binding of the 3-substituted 1-hydroxypyrazoles, **2f**−**n**, would include an area in the binding pocket in the vicinity of the respective ring position, which has not previously been explored. Introduction of a methyl group (**2f**) in this position increased the affinity slightly compared to that of **2a**. This effect was more pronounced when introducing aromatic groups as in **2g**, where a phenyl group led to approximately 40-fold increased affinity compared to **2a**. Similar effects were observed for the tolyl analogues (**2h**−**j**) and the benzyl analogue (**2m**), all showing affinities in the same range as **2g**. This was also the case for **2l**, which is generated by enlargement of the aromatic system by a phenyl group in the 4-position of the phenyl substituent in **2g**. Interestingly, introduction of a phenyl group in the 3-position of the phenyl ring of **2g**, compound **2k**, resulted in a 10-fold increase in affinity compared to **2g**. Introduction of a naphthylmethyl group

(**2n**) led to the highest affinity of all the 3-substituted 1-hydroxypyrazole analogues. Thus, there appears to be ample space in this particular area of the orthosteric site. In fact, the area surrounding the relevant positions is clearly large enough to encompass a benzyl group and larger aromatic substituents favoring hydrophobic interactions. This is particularly evident for **2n** and **2k**, containing a 2-naphthylmethyl and 3-biphenyl substituent, respectively, exhibiting binding affinities in the low nanomolar range (K_i of 3.0 and 30 nM).

Finally, large aromatic substituents in the 3- and 5-position of the 1-hydroxypyrazole ring led, for **2o**, to significant reduction in receptor affinity compared to the monosubstituted 1-hydroxypyrazole analogues **2b**,**g**. The disubstituted analogue **2p** showed markedly higher affinity than **2o**, similar or 10-fold lower compared to the affinity shown for the corresponding monosubstituted analogues **2d** and **2n**, respectively (Table 1).

Since a certain resemblance of the overall SAR pattern for 5- and 3-substituted 1-hydroxypyrazoles exists, one could speculate that the 3-substituted 1-hydroxypyrazoles would rotate 180° around the piperidine−pyrazole bond to place the large substituents in the cavity already described in the pharmacophore model. However, several observations seem to be in conflict with this hypothesis: First, the relative binding affinities and antagonistic activities of the 2-naphthylmethyl and 3-biphenyl 1-hydroxypyrazole analogues **2b** and **2e** are similar to those of the 3-isoxazolol counterparts **1b** and **1e**. This is in contrast to the pattern observed for the 3-substituted 1-hydroxypyrazoles **2n** and **2k** with regard to binding affinity and antagonistic activity. Second, the fact that the 3,5-disubstituted analogues **2o** and **2p** bind to the receptor unequivocally suggests the presence of a relatively large pocket on both sides of the heterocyclic core. The affinity of **2o** ($K_i = 1.5\,\mu$M) is not as high as for the analogous compounds **2g** and **2b**, but since this compound has two groups fitting into different cavities, it becomes more restrained and less flexible, so the decrease is not surprising. However, the high affinity displayed by **2p** (K_i = 22 nM), which correlates well with the corresponding monosubstituted analogues **2d** and **2n**, indicates that the position of these substituents is more favorable compared to that of **2o**.

These data strongly imply that the binding site can accommodate large/bulky substituents in the region corresponding to the 3- and 5-position of the 1-hydroxypyrazole ring.

Functional Characterization. The functional characterization of the compounds was performed using the FMP red assay (Table 1). In previous studies we have applied the FMP blue assay in the ρ_1 $GABA_A$ receptor and other Cys-loop receptors,[18−21] and in recent studies the FMP assay has been validated for the functional characterization of the $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor.[22,23] The functional data displayed by **1b** and **1e** at the $\alpha_1\beta_2\gamma_2$ receptor transiently expressed in tsA201 cells in the FMP assay were found to be in good agreement with findings of previous electrophysiological studies.[12] The measurements are given as IC_{50} instead of K_i because of the mixed antagonist profile we observed in a previous study.[15] The GABA concentrations used for the characterization of the antagonist in the assay were 5- to 8-fold higher than the EC_{50} for the agonist. Thus, if the compounds are indeed completely competitive antagonists, as we expect them to be, the respective functional K_i for the compounds would be ~10 times lower than their IC_{50}.

The 1-hydroxypyrazole analogue **2a** and the corresponding 3-isoxazolol **1a** were essentially devoid of activity in the FMP red assay in concentrations up to 500 μM. All of the 3- and 5-substituted 1-hydroxypyrazole analogues, **2b**−**o**, and the 3-isoxazolol analogues, **1b**,**e**, were characterized as antagonists with potencies in the range of moderate to highly potent (IC_{50} = 66−0.024 μM). In general, the functional data are in good agreement with the binding affinity data.

Conclusions

We have identified the 1-hydroxypyrazole ring system as a bioisostere for the 3-hydroxyisoxazole ring in 4-PIOL within the $GABA_A$ receptor area. A series of 1-hydroxypyrazoles (**2a**−**p**) has been synthesized and characterized pharmacologically at $GABA_A$ receptors. All of the 3- and 5-substituted 1-hydroxypyrazoles (**2b**−**p**) were shown to be moderately to highly potent antagonists (K_i of 5 μM to 3 nM). On the basis of the similar SARs obtained for the 5-substituted 1-hydroxypyrazoles (**2f**−**n**) and 4-substituted 3-hydroxyisoxazoles, a hypothesis of a common binding mode in the orthosteric site of the $GABA_A$ receptor for the two series of compounds was proposed, indicating space above and below the orthosteric binding site able to accommodate large hydrophobic substituents. These results offer new knowledge to the architecture of the binding site and pharmacological tools for focused studies regarding sequence alignment and receptor flexibility. In particular, the SAR data are being employed in ongoing efforts to create a validated structural model of the orthosteric $GABA_A$ receptor binding site and to propose detailed binding modes for this series of ligands, with the ultimate goal of being able to perform structure-based ligand design and better understand the mechanisms underlying agonism and antagonism at the $GABA_A$ receptors.

Experimental Section

Chemistry. The syntheses of selected compounds are described below as representative. Procedures for the chemistry in general, detailed experimentals, and syntheses of all other compounds are in the Supporting Information. Elemental analyses were performed at Analytical Research Department, H. Lundbeck A/S Denmark or by J. Theiner, Department of Physical Chemistry, University of Vienna, Austria, and the results were within ±0.4% of the calculated values unless otherwise stated.

General Procedures for Aryl−Heteroaryl Coupling. Procedure A (6b,c,o). Diisopropylamine (1.2 equiv) was dissolved in THF and cooled to −30 °C where *n*-BuLi (1.2 equiv) was added. The mixture was cooled to −78 °C where **5a** (for **6b**,**c**) or **6g** (for **6o**) (1 equiv) in THF was added. After 10 min the appropriate aldehyde (1.2 equiv) in THF was added and the mixture was allowed to slowly reach room temperature overnight. The reaction was quenched with saturated NH_4Cl/H_2O (1:1) and extracted with 3 × Et_2O. The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo.

Procedure B (6g−l). A mixture of **8a** (1 equiv), $RB(OH)_2$ (2.4 equiv), and $PdCl_2(PPh)_2$ (0.08 equiv) in toluene/EtOH (10:1) and K_2CO_3 (3 M) was stirred at 100 °C for 20 h and then cooled to room temperature. Et_2O was added, and extraction was performed using H_2O, NaOH (1 M), and then H_2O. The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by DCVC.

Procedure C (6f,m,n,p). Compound **8a** (for **6f**,**m**,**n**) or **8b** (for **6p**) (1 equiv) was dissolved in THF and cooled to 0 °C where ^{i}PrMgCl (1.5 equiv) was added. The reaction was monitored by TLC, and after 1 h the appropriate aldehyde (1.5 equiv) was added upon which the mixture was removed from the ice bath. After 2 h at room temperature, saturated NH_4Cl and water (1:1)

were added and the mixture was poured into a separating funnel and extracted with 3 × Et_2O. The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo.

General Procedures for Deprotection. Procedure E (2a−e, 2g−o). A mixture of the protected compound in concentrated aqueous HCl was stirred vigorously while heated at 130 °C for 1 h followed by evaporation and recrystallization.

Ethyl 4-(1-(Benzyloxy)-1*H*-pyrazol-4-yl)piperidine-1-carboxylate (5a). 1-(Benzyloxy)-4-iodopyrazole **4**[16] (5.35 g, 17.8 mmol) was dissolved in THF (50 mL) and cooled to 0 °C where ^{i}PrMgCl (16.5 mL, 21.4 mmol) was added. The reaction was monitored by TLC. After 1 h, 1-ethoxycarbonyl-4-piperidone **3** (4.0 mL, 26.7 mmol) was added upon which the mixture was removed from the ice bath. The water phase was further extracted with diethyl ether (3 × 50 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. The raw product was dissolved in CH_2Cl_2 (50 mL) where Et_3SiH (5.7 mL, 35.6 mmol) and trifluoroacetic acid (TFA) (41 mL, 0.53 mol) were added. The mixture was heated for 2 h at 50 °C. When the mixture was cooled, water (50 mL) was added and the mixture was extracted with Et_2O (3 × 50 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. DCVC was applied and provided **5a** as a viscous colorless oil (3.72 g, 63% over two steps).

Ethyl 4-(1-(Benzyloxy)-5-iodo-1*H*-pyrazol-4-yl)piperidine-1-carboxylate (7). Diisopropylamine (0.84 mL, 6.0 mmol) was dissolved in THF (5 mL) and cooled to −30 °C where *n*-BuLi (4.3 mL, 6.0 mmol) was added. The mixture was cooled to −78 °C where **5a** (1.66 g, 5.0 mmol) was added. After 10 min, I_2 (3.8 g, 15.0 mmol) in THF (10 mL) was added and the mixture was allowed to slowly reach room temperature overnight. The reaction was quenched with Na_2SO_3 and extracted with EtOAc (3 × 50 mL). DCVC was applied, and product was isolated as white crystals (1.25 g, 54%): mp 66−68 °C.

Ethyl 4-(1-(Benzyloxy)-3-iodo-1*H*-pyrazol-4-yl)piperidine-1-carboxylate (8a). To a solution of **5a** (0.99 g, 3.0 mmol) in AcOH (5 mL) a solution of ICl (0.59 g, 3.6 mmol) in AcOH (5 mL) was added followed by water (15 mL). The mixture was stirred for 18 h at 85 °C, and upon cooling the reaction was quenched with $Na_2S_2O_3$. Water (30 mL) was added, and the mixture was extracted with Et_2O (3 × 30 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. DCVC resulted in pure product as white crystals (0.95 g, 70%): mp 78−81 °C.

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Supporting Information Available: Synthesis details, ^{1}H NMR and ^{13}C NMR of all synthesized compounds, elemental analyses data of all new target compounds, and pharmacological methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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