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Discovery of novel α_1-adrenoceptor ligands based on the antipsychotic sertindole suitable for labeling as PET ligands

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ABSTRACT

The synthesis and in vitro preclinical profile of a series of 5-heteroaryl substituted analogs of the antipsychotic drug sertindole are presented. Compounds 1-(4-fluorophenyl)-3-(1-methylpiperidin-4-yl)-5-(pyrimidin-5-yl)-1*H*-indole (Lu AA27122, **3i**) and 1-(4-fluorophenyl)-5-(1-methyl-1*H*-1,2,4-triazol-3-yl)-3-(1-methylpiperidin-4-yl)-1*H*-indole (**3l**) were identified as high affinity α_{1A}-adrenoceptor ligands with K_i values of 0.52 and 0.16 nM, respectively, and with a >100-fold selectivity versus dopamine D_2 receptors. Compound **3i** showed almost equal affinity for α_{1B}- (K_i = 1.9 nM) and α_{1D}-adrenoceptors (K_i = 2.5 nM) as for α_{1A}, as well as moderate affinity for 5-HT_{1B} (K_i = 13 nM) and 5-HT_6 (K_i = 16 nM) receptors, whereas **3l** showed >40-fold selectivity toward all other targets tested. Based on in vitro assays for assessment of permeability rates and extent, it is predicted that both compounds enter the brain of rats, non-human primates, as well as humans, and as such are good candidates to be carried forward for further evaluation as positron emission tomography (PET) ligands.

1. Introduction

The α_1-adrenoceptors are G protein-coupled receptors, existing as three subtypes (α_{1A}, α_{1B}, and α_{1D}) with widespread distribution in both the peripheral and the central nervous system (CNS).[1,2] The role of α_1-adrenoceptors in the CNS is complex and not well understood,[3] but a common feature of most antipsychotic drugs is their high in vitro affinity for these receptors in addition to their in vitro affinities for dopamine D_2 and serotonin 5-HT_{2A} receptors.[4] Studies with imaging ligands, for example, using positron emission tomography (PET), have shown that antipsychotic drugs also bind to D_2 and 5-HT_{2A} receptors in vivo.[5–7] However, no useful PET ligand or imaging methodology is currently available for imaging central α_1-adrenoceptors in vivo in rats or humans. As part of our continuing interest in antipsychotic drugs, we have been seeking a PET ligand, which would be selective for α_1-adrenoceptors and therefore suitable for studying the occupancy of putative drugs targeting this receptor. We previously reported our work on the discovery of selective α_1-adrenoceptor ligands based on sertindole (**1**).[8] Two of these sertindole derived analogs from the 1st generation (**2a** and **2b**, Fig. 1) were evaluated as PET ligands in cynomolgus monkeys, but were not progressed due to low brain uptake.[9] Herein, we report the identification of a series of novel α_1-adrenoceptor ligands (**3a**–**3m**, Table 1) that are predicted to enter the brain. Moreover, the compounds are all *N*-methyl piperidines and therefore suitable for ^{11}C labeling as PET ligands.

2. Chemistry

The 1st generation α_1-adrenoceptor selective sertindole analogs were prepared via in situ conversion of the 5-bromoindole precursors to the corresponding zinc species, after initial bromine-lithium exchange, followed by a Negishi coupling.[8] This synthetic strategy could be hampered by the facile lithium migration to the 2-position of the indole. The work reported herein was based on the 'inverted' set of coupling partners for the construction of the key bond connecting the indole nucleus at the 5-position to the heteroaromatic substituent. The bromine in compounds **5a** and **5b** would serve as a means for the Suzuki–Miyaura coupling[10] to install the heteroaromatic substituent at the 5-position of the indole, giving compounds **3a**–**3j** (Scheme 1). Such a coupling reaction is known to proceed most efficiently if the indole contains a bromine atom rather than a boronic acid at the 5-position.[11] Furthermore, boronic acids and esters, which would afford suitable heteroaryl substituents, were readily available from commercial sources.

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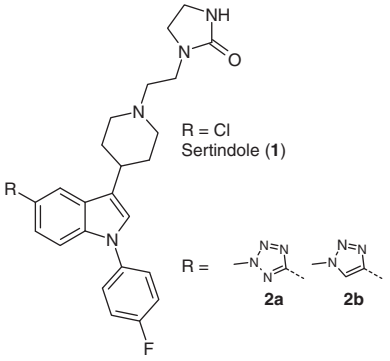
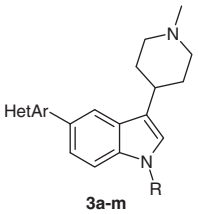


Figure 1. Sertindole and its 1st generation α_1-adrenoceptor selective analogs.

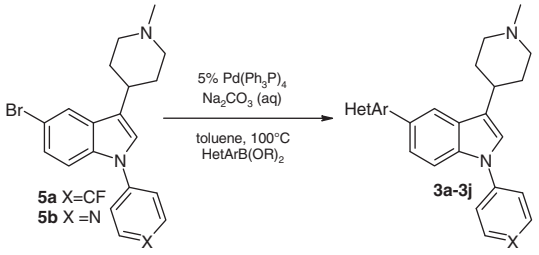
Table 1
In vitro data for compounds **3a**–**3m** and reference compounds

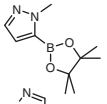
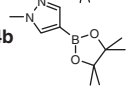
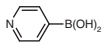
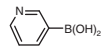
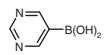


Compd	R	HetAr	K_i, nM[a]		D_2/α_{1A}
			α_{1A}	D_2	
Sertindole (**1**)			0.37	0.45	1.2
2a			0.23	140	609
2b			3.0	310	103
3a			0.32	15	47
3b			19	880	46
3c			2.7	150	56
3d			38	6400	168
3e			10	27	3
3f			24	260	11
3g			2.3	76	33
3h			22	1100	50
3i			0.52	120	230
3j			19	830	44
3k			0.28	69	246
3l			0.16	220	1375
3m			0.54	160	296

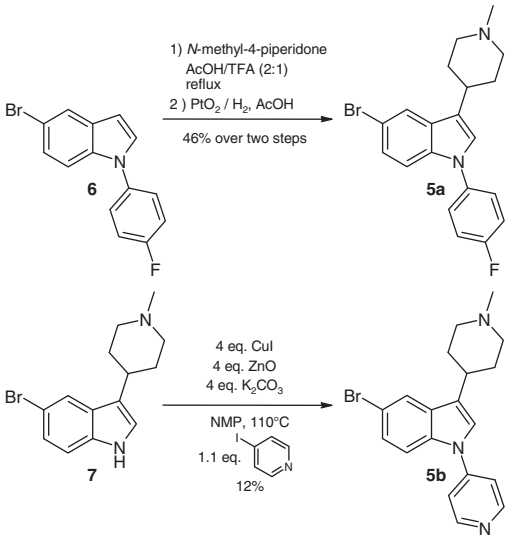
[a] K_i values are reported as the logarithmic average of at least two independent determinations. In the experiments where N >2 the SD of the pK_i were below 0.3.

The synthesis of the key building blocks **5a** and **5b** started from known compounds **6**[12] and **7**[13] as shown in Scheme 2. Indole **6** was condensed with *N*-methyl-4-piperidone, and the resulting tetrahydropyridine was hydrogenated to the desired building block **5a**. Indole **7** was *N*-arylated under classical conditions using a mixture of CuI, ZnO, and 4-iodopyridine to give **5b** in a relatively low yield.

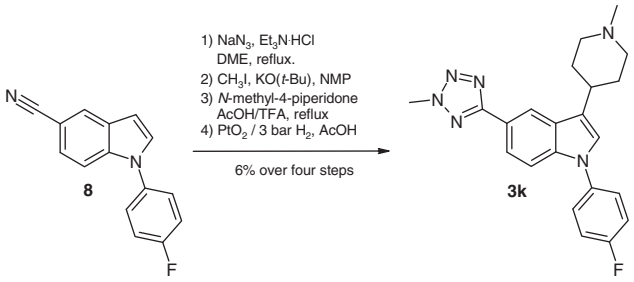


Substrate	$HetArB(OR)_2$	Product	Yield
5a	 **4a**	**3a**	>95%
5b		**3b**	80%
5a	 **4b**	**3c**	75%
5b		**3d**	63%
5a	 **4c**	**3e**	16%
5b		**3f**	82%
5a	 **4d**	**3g**	58%
5b		**3h**	47%
5a	 **4e**	**3i**	29%
5b		**3j**	17%

Scheme 1. Synthesis of **3a**–**3j** using Suzuki–Miyaura coupling reactions.



Scheme 2. Synthesis of building blocks **5a** and **5b**.



Scheme 3. Synthesis of compound **3k**.

The final step in the synthesis was the Suzuki–Miyaura coupling of the indoles with the heteroaryl boronic acids or the corresponding esters. A side-by-side comparison identified the 'classical' $Pd(Ph_3P)_4$ catalyst as superior to its 'modern' counterparts like

the combination of $Pd(OAc)_2$ or $Pd_2(dba)_3$ with either S-Phos or P(*tert*-Bu)$_3$-HBF_4 under the conditions developed by Buchwald and Fu, respectively.[10,14–16] Intermediates **5a** and **5b** were each coupled to five heteroaromatic boronic acids/esters (cf. Scheme 1).

In addition to compounds **3a**–**3j**, we also prepared a number of analogs from more advanced intermediates. More specifically, nitrile **8**[12] was treated with sodium azide to yield the corresponding tetrazole, which was subsequently methylated with methyl iodide and condensed with *N*-methyl-4-piperidone, and finally reduced to afford piperidine **3k** as shown in Scheme 3.

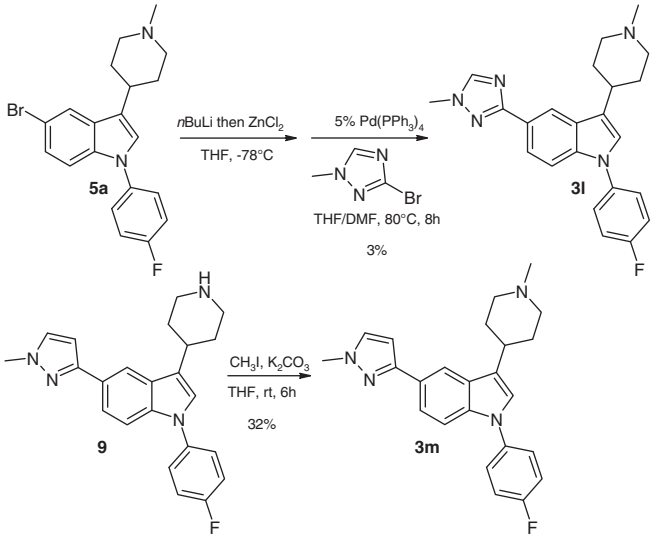
Prior to developing the Suzuki–Miyaura protocol, two other analogs were prepared as shown in Scheme 4. Compound **3l** was prepared according to the original Negishi-protocol[8] from **5a** and 3-bromo-1-methyl-1*H*-1,2,4-triazole[17] in low yield, and previously reported pyrazole **9**[8] was methylated to give **3m**.

3. Structure–activity relationships

Compounds **3a**–**3m** were evaluated in vitro for their affinity for α_{1A}-adrenoceptors versus D_2 receptors, as results from the evaluation of the 1st generation analogs **2a** and **2b** indicated that the affinity for α_{1A}-adrenoceptors essentially were parallel to those of α_{1B}- and α_{1D}-adrenoceptors.[8] The compounds were characterized in receptor-binding assays at cloned bovine α_{1A}-adrenoceptors by displacement of [^{3}H]prazosine and at cloned D_2 receptors using [^{3}H]spiperone as radioligands. The results are shown in Table 1 together with the corresponding data for reference compounds sertindole (**1**) and its heteroaryl derivatives **2a** and **2b**, which previously were investigated as potential α_1-adrenoceptor PET ligands.[9]

The pyridyl derivatives **3b**, **3d**, **3f**, **3h**, and **3j**, originally designed to lower lipophilicity compared to the 4-fluorophenyl derivatives, show generally one or two orders of magnitude lower affinity for α_{1A}-adrenoceptors when compared to the analogous 4-fluorophenyl counterparts as evident by comparing for example compounds **3a** and **3b** (Table 1). Furthermore, the structure–activity relationships of the 4-fluorophenyl-substituted derivatives are parallel to the results described for the 1st generation sertindole analogs **2a** and **2b**.[8] All 4-fluorophenyl-substituted derivatives, except the pyridin-4-yl derivative **3e** (Table 1), display single-digit nano- or sub-nanomolar affinity for α_{1A}-adrenoceptors. The slightly lower affinity of compound **3e** agrees well with previous observations, indicating the need for a hydrogen bond acceptor in the *ortho* or *meta* position, relative to the point of attachment of the heteroaryl substituent to the indole nucleus, to obtain high affinity for α_1-adrenoceptors.[18] Moreover, we have previously suggested that bulk in the plane of the indole ring would reduce affinity for D_2 receptors when compared to sertindole,[19] and this is indeed further supported by the low D_2 receptor affinity of these compounds. The 4-fluorophenyl derivatives have 60- to 500-fold reduced affinities for D_2 receptors compared to sertindole, except for the 1-methylpyrazol-5-yl derivative **3a**, for which the decrease in D_2 receptor affinity is only 30-fold and still rather high (K_i = 15 nM). Compounds **3a** and **3b** are unique in the sense that the 1-methyl group is expected to force the heteroaryl substituent out of the plane of the indole, inducing bulk above and below the plane instead of in the plane.[8] Compounds **3i** and **3l** were selected for further preclinical testing in order to evaluate their potential as PET ligands. The compounds were selected because both compounds showed high affinity for α_{1A}-adrenoceptors and good selectivity toward D_2 receptors, as well as representing compounds having either a 5- or a 6-membered ring at the 5-position of the indole moiety. Compound **3i** (Lu AA27122) was tested in a broad panel of in vitro assays at MDS pharmaservices, as well as in-house at H. Lundbeck A/S, against a total of 71 receptors, ion channels, transporters, and enzymes. Compound **3i** showed relative high affinity (>50% inhibition at 1000 nM) for D_2, 5-HT_{1B}, 5-HT_{2A}, and 5-HT_6 receptors as well as for the α_1-adrenoceptor subtypes. The 1,2,4-triazol-3-yl derivative **3l** was tested in a more limited number of assays, although in the assays relevant for direct comparison with **3i**. K_i values for **3i** and **3l** are reported herein on key targets if they were below 100 nM in the broad screen. In short, the 1,2,4-triazol-3-yl derivative **3l** showed affinity below 100 nM for 5-HT_{2A} receptors (K_i = 89 nM), whereas the pyrimidin-5-yl derivative **3i** displayed affinities below 100 nM for 5-HT_{1B} (K_i = 13 nM) and 5-HT_6 (K_i = 16 nM) receptors. Furthermore, compound **3i** showed almost equal affinity for all α_1-adrenoceptor subtypes, whereas **3l** showed selectivity for α_{1A}-adrenoceptors as high as 40- and 90-fold, respectively, compared to α_{1B} and α_{1D}, suggesting that **3l** may be termed an α_{1A}-adrenoceptor selective ligand (Table 2).

Compounds **3i** and **3l** were advanced for permeability and CNS distribution assessment, since the brain exposure of a compound is a function of both rate and extent of drug uptake into the CNS. The rate of efflux due to transporters was examined by use of Caco-2 permeability studies, whereas the extend was examined by use of equilibrium dialysis of rat brain homogenate and plasma in order to estimate the steady state in vivo brain to plasma (B/P) ratio, assuming passive distribution of compound. While the unbound fractions in brain tissue are probably comparable across species,[20] the unbound fractions in plasma can differ significantly between species. This may potentially affect the distribution of drug to the brain, that is higher protein binding of drug in plasma of a certain species will result in reduced CNS exposure when compared to another species where the degree of protein binding is lower (all other things being equal). Thus, the unbound fractions of



Scheme 4. Synthesis of compounds **3l** and **3m**.

Table 2
Further in vitro characterization of compounds **3i** and **3l** and reference compound

Compd	K_i, nM[a]			
	α_{1A}	α_{1B}	α_{1D}	D_2
1	0.37[b]	0.33[b]	0.66[b]	0.45[b]
2a	0.23[b]	1.1[b]	2.0[b]	140[b]
2b	3.0[c]	6.0[c]	8.6[c]	310[b]
3i	0.52	1.9	2.5	120
3l	0.16	6.4	15	220

[a] K_i values are reported as the logarithmic average of at least two independent determinations.
[b] Balle et al.[8]
[c] Balle et al.[18]

Table 3
Summary of permeability and efflux ratios in Caco-2 cells, unbound drug fractions in plasma and brain homogenate, estimated brain/plasma (B/P) ratios at steady state and measured rat B/P ratio at 1 h for compounds **3i** and **3l** and reference compounds

	P_{app}[a] (A–B), 10^{-6} cm s^{-1}	P_{app} (B–A)/P_{app} (A–B) ratio	f_u[b] plasma (%)	f_u brain (%)	Estimated B/P ratio at steady state[c]	Measured rat B/P ratio at 1 h[d]
			Rat	*Rat*	*Rat*	*Rat*
Raclopride	66	0.5	27 ± 0.4	8.0 ± 0.4	3.4	6.4
WAY-100,635	38	0.9	10 ± 0.8	8.4 ± 0.4	1.2	1.7
2a	12	2.8	2.2 ± 0.2	0.35 ± 0.02	6.3	0.26
2b	5	19	7.2 ± 0.4	0.81 ± 0.07	8.9	>0.05
3i	13	0.7	8.8 ± 0.3	0.25 ± 0.01	35	NT
3l	18	0.7	7.4 ± 0.8	0.20 ± 0.01	37	NT
			Rhesus		*Rhesus*	*Rhesus*
3i			13 ± 0.9		51[e]	NT
3l			18 ± 1.1		87[e]	NT
			Cynomolgus		*Cynomolgus*	*Cynomolgus*
3i			10 ± 0.5		42[e]	NT
3l			16 ± 0.4		82[e]	NT
			Human		*Human*	*Human*
3i			9.6 ± 0.6		38[e]	NT
3l			17 ± 0.5		83[e]	NT

[a] P_{app}, apparent permeability ($N = 2$).
[b] f_u, Fraction unbound.
[c] Brain to plasma (B/P) ratio calculated as in vitro f_u, plasma divided by f_u, brain according to Summerfield et al.[20]
[d] Dose of 5 mg/kg, s.c.
[e] f_u Brain, rat brain homogenate used; NT, not tested.

compounds **3i** and **3l** to rhesus and cynomolgus monkeys, and human plasma as well as rat plasma and brain were determined by dialysis (Table 3). Based on these data, the estimated B/P ratio at steady state can be calculated as the fraction of unbound compound in plasma divided by the unbound fraction in rat brain.[20]

As shown in Table 3, compounds **3i** and **3l** display an apparent permeability (P_{app}) at 20 μM concentration of 13×10^{-6} and 18×10^{-6} cm s^{-1}, respectively, and a non-polarized transport as indicated by a ratio between basolateral to apical and apical to basolateral transport close to 1 for both compounds. The P_{app} value was not significantly different at 1 and 10 μM concentrations (data not shown) for any of the compounds, indicating that the transport rate was not concentration dependent. To put the apparent permeabilities into perspective, the two unsuccessful CNS PET ligands **2a** and **2b** have P_{app} values of 12×10^{-6} and 5×10^{-6} cm s^{-1}, respectively, and a highly polarized transport attributed to P-glycoprotein efflux.[9] In contrast, WAY-100,635 and raclopride, both well established CNS PET ligands, have P_{app} values of 38×10^{-6} and 66×10^{-6} cm s^{-1}, respectively, and non-polarized transport. In addition, the estimated rat B/P ratio at steady state showed a good correlation with the measured in vivo rat B/P ratio at 1 h for raclopride and WAY-100,635 (Table 3). In contrast, the estimated rat B/P ratio at steady state for compounds **2a** and **2b** is over-predicting the distribution to the brain when compared to the measured in vivo B/P ratio in rat, that is 6.3 and 8.9 versus 0.26 and >0.05, respectively. This is supporting that the active efflux is a probable course of the poor brain distribution of these two compounds. However, compounds **3i** and **3l** are expected to be superior to **2a** and **2b** based on the permeability data and apparent lack of efflux measured in Caco-2 cells. If the lack of active efflux means that estimates of the B/P ratio at steady state are predictive for the in vivo situation, both these compounds are expected to be very well distributed to rat brain. The distribution to brain of **3i** and **3l** is predicted to be even higher in rhesus and cynomolgus monkeys as well as human, as the unbound fractions in plasma of **3i** and **3l** in these species are considerably higher than in rats (Table 3).

4. Conclusion

We have prepared a series of selective α_1-adrenoceptor ligands derived from the antipsychotic drug sertindole (**1**). Compounds **3i** (Lu AA27122) and **3l** were identified as promising candidates for labeling as PET ligands based on their high affinity for α_1-adrenoceptors as well as selectivity toward other targets. Furthermore, compound **3l** displayed >40-fold selectivity for α_{1A}-adrenoceptors when compared to α_{1B} and α_{1D}, suggesting that **3l** may be termed an α_{1A}-adrenoceptor selective ligand. Both **3i** and **3l** showed good in vitro brain permeability rates and brain distribution in dialysis studies, and they are both predicted to enter the brain of non-human primates as well as humans. Moreover, compounds **3i** and **3l** are both *N*-methyl piperidines and therefore suitable for ^{11}C labeling as PET ligands.

5. Experimental

5.1. Chemistry

5.1.1. General

Reagents and solvents were obtained from commercial sources and used as received. Thin layer chromatography was carried out on Merck Silica gel 60 F254. Flash chromatography was performed using Merck Silica Gel 60 (40–63 μm). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at ambient temperature on a Bruker Avance AV-500 at 500.13 and 125.77 MHz, respectively, or a Bruker Avance AV-III-600 at 600.16 and 151.91 MHz, respectively. Chemical shifts (δ) are reported in parts per million relative to tetramethylsilane (TMS) or residual solvent, and coupling constants (J) are given in hertz. High-resolution mass spectrometry (HRMS) was recorded on a Bruker Daltonics MicrOTOF operating with external calibration with lithium formate (for ESI) and PEG200/PEG400/PEG600 (1:1:1) [for atmospheric pressure photo ionization (APPI)], using an Agilent 1100 high performance liquid chromatography (HPLC) system for inlet. LC/MS were run on a Sciex API150EX equipped with APPI-source operating in positive ion mode. The HPLC consisted of Shimadzu LC10-ADvp LC pumps, SPD-M20A PDA detector (operating at 254 nm), and SCL-10A system controller. The autosampler was a Gilson 215, the column oven was a Jones Chromatography 7990R, and the ELS detector was a Sedere Sedex 55. The mobile phase was a gradient consisting of A: water + 0.05% trifluoro acetic acid (TFA) and B: 95% acetonitrile and 5% water + 0.035% TFA. Column temperature was set to 60 °C. The methods used were one of the following: compounds **3a** and **3b**, column: Waters Symmetry C18 3.5 μ, 4.6 × 30 mm. 10% B to 100% B in 1.6 min, 100% B to 10% B in

0.05 min, 10% B for 0.05 min. Flow rate 5.2 mL/min; compounds **3c** and **3g**, column: Waters Sunfire C18 3.5 μ, 4.6 × 30 mm. 10% B to 100% B in 2.4 min, 100% B to 10% B in 0.4 min. Flow rate 3.3 mL/min; compounds **3d**–**3f**, **3h**, and **3j**, column: Waters Atlantis dC18 3 μ, 4.6 × 30 mm. 2% B to 100% B in 2.4 min, 100% B to 2% B in 0.4 min. Flow rate 3.3 mL/min; compound **3i**, column: Waters Sunfire C18 3.5 μ, 4.6 × 30 mm. 10% B to 100% B in 2.4 min, 100% B for 2 min, 100% B to 10% B in 0.4 min. Flow rate 3.3 mL/min; or compounds **3l** and **3m** column: Waters Symmetry C18 3.5 μ, 4.6 × 30 mm. 10% B to 100% B in 4 min, 100% B to 10% B in 1 min. Flow rate 2 mL/min. All pharmacologically characterized compounds were >95% pure according to ELS and >90% pure according to UV besides from **3f**, which was 89% on ELS and 78% on UV (compounds **3a**–**3j**, **3l**, and **3m**), or within 0.4% of theory using elemental analysis (compound **3k**). The compounds were also characterized by ^{1}H NMR and ^{13}C NMR, as well as HRMS. All analyses were conducted at H. Lundbeck A/S.

5.1.2. (1-Methyl-1*H*-pyrazol-5-yl)boronic acid pinacol ester (4a)

A solution of 1-methylpyrazole (25.0 g, 305 mmol) in tetrahydrofuran (THF) (600 mL) was cooled to 0 °C. *n*-Butyl lithium (1.6 M in hexanes, 209 mL, 335 mmol) was added drop-wise over 1 h, keeping the temperature below 7 °C. The solution was stirred at room temperature (rt) for 3 h before cooling to −70 °C. $B(OMe)_3$ (44.4 mL, 0.40 mol) was slowly added, keeping the reaction temperature below −65 °C. The resulting mixture was allowed to warm to rt, before it was quenched with 15% aqueous (aq) NH_4Cl (450 mL). The mixture was extracted with THF (3 × 500 mL), and the combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo to give a yellowish solid (15.6 g, 41%). The aq layer was concentrated in vacuo, and the resulting solid was repeatedly extracted with THF (5 × 250 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo, yielding a yellow solid (20.3 g, 52%). The combined yield of (1-methyl-1*H*-pyrazol-5-yl)boronic acid was 35.9 g (93%), and 20.3 g (161 mmol) of this material was esterified with pinacole (28.4 g, 240 mmol) in THF (200 mL). 4 Å molecular sieves (6.0 g dried in vacuo at 50 °C) were added, and the resulting mixture was stirred at rt for 2 days. The sieves were filtered off, and the filtrate was concentrated in vacuo. The resulting crude product was dissolved in heptane (500 mL) and washed with water (2 × 250 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to afford a white solid. This material was recrystallized from acetonitrile yielding white crystals (22.5 g, 67%). Mp 71.0–71.6 °C (Ivachtchenko et al.,[21] 74–76 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ, 7.46 (d, J = 1.9 Hz, pyrazole H-3), 6.62 (d, J = 1.9 Hz, pyrazole H-4), 3.98 (s, *N*-methyl), 1.30 (s, pinacol methyl groups, 12H). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 138.3, 115.9, 84.4 (2C), 41.9, 24.9 (4C). Anal. calcd for $C_{10}H_{17}BN_2O_2$: C, 57.73; H, 8.24; N, 13.46. Found: C, 57.72; H, 8.08; N, 13.40.

5.1.3. 5-Bromo-1-(4-fluorophenyl)-3-(1-methylpiperidin-4-yl)-1*H*-indole (5a)

N-Methyl-4-piperidone (460 g, 3.00 mol) was boiled under reflux in a mixture of AcOH (625 mL) and TFA (1150 mL). A solution of **6**[12] (200 g, 0.69 mol) in a mixture of AcOH (625 mL) and TFA (125 mL) was added drop-wise over 1 h. The reaction was boiled under reflux for an additional 2 h before it was cooled to rt. The crude mixture was partitioned between EtOAc and dilute aq NH_3. The aq layer was extracted with EtOAc, and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/Et_3N 96:4) to give 195 g of 5-bromo-1-(4-fluoro-phenyl)-3-(1-methyl-1,2,3,6-tetrahydro-pyridin-4-yl)-1*H*-indole sufficiently pure for the next step. This material was hydrogenated (3 bar H_2 pressure) over PtO_2 (3 g) in AcOH (1 L) for 52 h. The catalyst was filtered off, and the filtrate was hydrogenated for an additional 14 h with fresh PtO_2 (3.5 g). The catalyst was filtered off, and the solvent was removed in vacuo. The residue was purified by chromatography (EtOAc/Et_3N 96:4) to give slightly impure **5a**. The two reactions were repeated, and the combined product was recrystallized from heptane to give pure **5a** (122 g, 23% over 2 steps). ^{1}H NMR (500 MHz, DMSO-d_6) 7.86 (d, J = 1.9 Hz, indole H-4), 7.68–7.55 (m, 4-fluoro-phenyl 2 × H-2), 7.48 (s, indole H-2), 7.46–7.35 (m, 4-fluoro-phenyl 2 × H-3 and indole H-7), 7.30 (dd, J = 8.8, 1.9 Hz, indole H-6), 2.87 (d, J = 11.4 Hz, piperidine 2 × H-2a), 2.78 (ddd, J = 11.8, 8.2, 3.5 Hz, piperidine H-4), 2.22 (s, *N*-methyl), 2.10–2.00 (m, piperidine 2 × H-2b), 1.95 (d, J = 12.7 Hz, piperidine 2 × H-3a), 1.72 (qd, J = 12.7, 3.5 Hz, piperidine 2 × H-3b), ^{13}C NMR (126 MHz, $CDCl_3$): 161.3 (d, J = 246.5 Hz), 135.8, 135.7, 135.3, 126.3 (d, J = 8.6 Hz, 2C), 125.5, 125.1, 122.3, 122.2, 116.8 (d, J = 22.9 Hz, 2C), 113.2, 112.0, 56.5 (2C), 46.8, 33.2 (2C), 33.1. HRMS calcd for $C_{20}H_{21}BrFN_2$ (M+H$^+$): 387.0867. Found: 387.0870.

5.1.4. 5-Bromo-3-(1-methylpiperidin-4-yl)-1-(pyridin-4-yl)-1*H*-indole (5b)

Compound **7**[13] (15.2 g, 51.2 mmol), 4-iodopyridine (21.0 g, 102 mmol), K_2CO_3 (28.3 g, 205 mmol), CuI (39.0 g, 205 mmol), and ZnO (16.7 g, 205 mmol) were added to a 500 mL round-bottom flask, and the flask was closed, evacuated, and back-filled three times with argon. NMP (200 mL) was introduced, and the mixture was stirred at 110 °C for 24 h. The mixture was diluted with EtOAc (200 mL) and filtered through celite. Water (200 mL) and 25% aq NH_3 (50 mL) were added, and the mixture was filtered again. The organic layer was washed with saturated aq NaCl (3 × 200 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column flash chromatography (EtOAc/Et_3N 95:5, 0–10% MeOH gradient) yielding a yellowish foam (2.26 g, 12%). ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.69 (d, J = 6.1 Hz, pyridine 2 × H2), 7.90 (d, J = 1.9 Hz, indole H-4), 7.75 (d, J = 8.5 Hz, indole H-7), 7.70–7.67 (m, indole H-2 and pyridine 2 × H-3), 7.37 (dd, J = 1.9 Hz, 8.5 Hz, indole H-6), 2.88 (broad d, J = 11.8 Hz, piperidine 2 × H-2a), 2.78 (tt, J = 3.5 Hz, 11.8 Hz, piperidine H-4), 2.23 (s, *N*-methyl), 2.07 (t, J = 11.3 Hz, piperidine 2 × H-2b), 1.95 (d, J = 12.7 Hz, piperidine 2 × H-3a), 1.78–1.69 (m, piperidine 2 × H–3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 151.6 (2C), 145.7, 133.8, 131.2, 125.9, 124.9, 124.0, 122.3, 117.2 (2C), 113.7, 113.5, 56.0 (2C), 46.6, 32.5, 32.2 (2C). HRMS calcd for $C_{19}H_{21}BrN_3$ (M+H$^+$): 370.0913. Found: 370.0907.

5.1.5. General procedure for Suzuki–Miyaura couplings

The boronic acid or ester (2 equiv), aryl bromide **5a**/**5b** (1 equiv, 0.5–2.1 mmol) and $Pd(Ph_3P)_4$ (5 mol % based on **5a**/**5b**) were vigorously mixed in a 5 mL microwave vial. The vial was capped, evacuated and back-filled with argon three times. Toluene (3 mL) and 2 M aq Na_2CO_3 (2 mL) were added, and the reaction was stirred at 100 °C overnight. The reaction mixture was diluted with EtOAc (5 mL) and extracted with water (3 × 5 mL). The organic layer was dried (Na_2SO_4), evaporated in vacuo, and purified by column flash chromatography (EtOAc/Et_3N 95:5, 0–10% MeOH gradient as eluent, unless stated otherwise).

5.1.6. 1-(4-Fluorophenyl)-5-(1-methyl-1*H*-pyrazol-5-yl)-3-(1-methylpiperidin-4-yl)-1*H*-indole (3a)

Prepared according to the general procedure from (1-methyl-1*H*-pyrazol-5-yl)boronic acid pinacol ester **4a** (425 mg, 2.04 mmol) and **5a** (402 mg, 1.04 mmol). Isolated as a brownish oil (432 mg, >95%.). ^{1}H NMR (500 MHz, DMSO-d_6): δ, 7.78 (broad s, indole H-4), 7.66–7.64 (m, 4-fluoro-phenyl 2 × H-2), 7.56 (d, J = 8.5 Hz, indole H-7), 7.50 (s, indole H-2) 7.47 (broad d, pyrazole H-3), 7.43 (broad t, J = 8.5 Hz, 4-fluoro-phenyl 2 × H-3), 7.30 (broad d, J = 8.5 Hz, indole H-6), 6.37 (broad s, pyrazole H-4), 3.85 (s, pyrazole *N*-methyl), 2.92–2.87 (m, piperidine 2 × H-2a and H-4),

2.21 (s, piperidine *N*-methyl), 2.14–2.05 (m, piperidine 2 × H-2b), 2.02–1.97 (m, piperidine 2 × H-3a), 1.82–1.72 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 160.7 (d, J = 243.6 Hz), 144.1, 138.1, 135.7, 135.6, 128.3, 126.3 (d, J = 8.3 Hz, 2C), 125.6, 123.5, 122.7, 122.3, 120.1, 116.9 (d, J = 22.9 Hz, 2C), 110.9, 105.9, 56.2 (2C), 46.7, 37.6, 32.8 (2C), 32.5. HRMS calcd for $C_{24}H_{26}FN_4$ (M+H$^+$): 389.2136. Found: 389.2141.

5.1.7. 5-(1-Methyl-1*H*-pyrazol-5-yl)-3-(1-methylpiperidin-4-yl)-1-(pyridin-4-yl)-1*H*-indole (3b)

Prepared according to the general procedure from (1-methyl-1*H*-pyrazol-5-yl)boronic acid pinacol ester **4a** (208 mg, 1.00 mmol) and **5b** (192 mg, 0.519 mmol). Purified by column flash chromatography (EtOAc/Et$_3$N 95:5, 0–10% MeOH gradient) to yield an yellow oil (155 mg, 80%). ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.75–8.72 (m, pyridine 2 × H-2), 7.78 (d, J = 8.4 Hz, indole H-7), 7.72 (d, J = 1.5 Hz, indole H-4), 7.55 (d, J = 1.9 Hz, pyrazole H-3), 7.49-7.46 (m, pyridine 2 × H-3), 7.32 (dd, J = 1.5 Hz, 8.4 Hz, indole H-6), 7.24 (broad s, indole H-2), 6.34 (d, J = 1.9 Hz, pyrazole H-4), 3.89 (s, pyrazole *N*-methyl), 3.85–3.75 (m, piperidine 2 × H-2a), 3.20–3.12 (m, piperidine H-4), 3.02–2.90 (m, piperidine 2 × H-2b and *N*-methyl), 2.50–2.40 (m, piperidine 2 × H-3a), 1.82–1.72 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, CDCl$_3$): δ, 151.9 (2C), 146.9, 144.6, 138.9, 135.5, 129.8, 125.4, 124.6, 124.0, 123.6, 120.8, 117.5 (2C), 114.5, 106.5, 56.6 (2C), 47.0, 37.8, 33.5, 33.3 (2C). HRMS calcd for $C_{23}H_{26}N_5$ (M+H$^+$): 372.2183. Found: 372.2174.

5.1.8. 1-(4-Fluorophenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)-3-(1-methylpiperidin-4-yl)-1*H*-indole (3c)

Prepared according to the general procedure from (1-methyl-1*H*-pyrazol-4-yl)boronic acid pinacol ester **4b** (416 mg, 2.01 mmol) and **5a** (420 mg, 1.08 mmol). Purified by column flash chromatography (EtOAc/MeOH/Et$_3$N (90:5:5) to yield a colorless oil (317 mg, 75%). Crystallized as white crystals from acetonitrile. Mp 158.8–159.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.14 (s, pyrazole H-3), 7.87 (s, pyrazole H-5), 7.83 (broad s, indole H-4), 7.63–7.59 (m, 4-fluoro-phenyl 2 × H-2), 7.46 (d, J = 8.5 Hz, indole H-7), 7.42–7.38 (m, 4-fluoro-phenyl 2 × H-3 and indole H-2 and H-6), 3.88 (s, pyrazole *N*-methyl), 2.95–2.85 (m, piperidine 2 × H-2a), 2.81 (broad t, J = 11.5 Hz, piperidine H-4), 2.23 (s, piperidine *N*-methyl), 2.07 (broad *t*, J = 11.5 Hz, piperidine 2 × H-2b), 2.03–1.97 (m, piperidine 2 × H-3a), 1.81–1.71 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 160.4 (d, J = 243.9 Hz), 136.2, 136.0, 134.6, 128.7, 127.6, 125.8 (d, J = 8.8 Hz, 2C), 125.0, 124.8, 123.3, 122.5, 120.8, 116.7 (d, J = 22.8 Hz, 2C), 115.7, 110.9, 56.4 (2C), 46.8, 38.9, 32.8 (2C), 32.6. HRMS calcd for $C_{24}H_{26}FN_4$ (M+H$^+$): 389.2136. Found: 389.2138.

5.1.9. 5-(1-Methyl-1*H*-pyrazol-4-yl)-3-(1-methylpiperidin-4-yl)-1-(pyridin-4-yl)-1*H*-indole (3d)

Prepared according to the general procedure from (1-methyl-1*H*-pyrazol-4-yl)boronic acid pinacol ester **4b** (208 mg, 1.00 mmol) and **5b** (193 mg, 0.521 mmol). Isolated as a colorless oil (122 mg, 63%). ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.65 (d, J = 6.1 Hz, pyridine 2 × H-2), 8.14 (s, pyrazole H-5), 7.92 (s, pyrazole H-3), 7.87 (broad s, indole H-4), 7.75 (d, J = 8.5 Hz, indole H-7), 7.63 (d, J = 6.1 Hz, pyridine 2 × H-3), 7.54 (s, indole H-2), 7.45 (dd, J = 1.4 Hz, 8.5 Hz, indole H-6), 3.88 (s, pyrazole *N*-methyl), 2.86 (d, J = 11.2 Hz, piperidine 2 × H-2a), 2.79 (tt, J = 3.3 Hz, 11.8 Hz, piperidine H-4), 2.19 (s, piperidine *N*-methyl), 2.07–2.00 (m, piperidine 2 × H-2b), 2.00–1.94 (m, piperidine 2 × H-3a), 1.81–1.71 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 151.5 (2C), 146.1, 136.3, 133.7, 130.1, 127.8, 126.1, 124.8, 123.5, 123.0, 121.4, 116.6 (2C), 116.0, 111.9, 56.2 (2C), 46.7, 38.9, 32.6 (2C), 32.5. HRMS calcd for $C_{23}H_{26}N_5$ (M+H$^+$): 372.2183. Found: 372.2171.

5.1.10. 1-(4-Fluorophenyl)-3-(1-methylpiperidin-4-yl)-5-(pyridin-4-yl)-1*H*-indole (3e)

Prepared according to the general procedure from pyridin-4-ylboronic acid (246 mg, 2.00 mmol) and **5a** (396 mg, 1.02 mmol). Isolated as a colorless oil (65 mg, 16%). ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.61 (d, J = 6.1 Hz, pyridine 2 × H-2), 8.11 (s, indole H-4), 7.75 (d, J = 6.1 Hz, pyridine 2 × H-3), 7.62–7.55 (m, 4-fluoro-phenyl 2xH-2 and indole H-6 and H-7), 7.45 (s, indole H-2), 7.40 (m, 4-fluoro-phenyl 2 × H-3), 2.89–2.83 (m, piperidine 2 × H-2a and piperidine H-4), 2.22 (s, *N*-methyl), 2.10–2.02 (m, piperidine 2 × H-2b), 2.01 (broad d, J = 12.7 Hz, piperidine 2 × H-3a), 1.82–1.37 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 160.6 (d, J = 243.6 Hz), 150.5 (2C), 148.5, 136.4, 135.7, 129.2, 128.8, 126.1 (d, J = 8.2 Hz, 2C), 125.6, 123.3, 121.7, 121.6 (2C), 118.3, 166.9 (d, J = 22.5 Hz, 2C), 111.3, 56.2 (2C), 46.7, 32.9 (2C), 32.5. HRMS calcd for $C_{25}H_{25}FN_3$ (M+H$^+$): 386.2027. Found: 386.2023.

5.1.11. 3-(1-Methylpiperidin-4-yl)-1,5-di(pyridin-4-yl)-1*H*-indole (3f)

Prepared according to the general procedure from pyridin-4-ylboronic acid (123 mg, 1.00 mmol) and **5b** (179 mg, 0.483 mmol). Isolated as a colourless oil (75 mg, 42%). ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.70 (broad d, J = 4.7 Hz, *C*-bound pyridine 2 × H-2), 8.61 (broad d, J = 4.7 Hz, *N*-bound pyridine 2 × H-2), 8.12 (d, J = 1.4 Hz, indole H-4), 7.87 (d, J = 9.0 Hz, indole H-7), 7.77 (dd, J = 1.4 Hz, 4.7 Hz, *C*-bound pyridine 2 × H-3), 7.68 (dd, J = 1.4 Hz, 4.7 Hz, *N*-bound pyridine 2 × H-3), 7.66–7.64 (m, indole H-6 and H-1), 2.88–2.85 (m, piperidine 2 × H-2a and H-4), 2.20 (s, *N*-methyl), 2.09–2.02 (m, piperidine 2 × H-2b), 2.02–1.96 (m, piperidine 2 × H-3a), 1.82–1.72 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 151.6 (2C), 150.4 (2C), 148.0, 145.9, 153.5, 130.4, 130.1, 125.3, 124.4, 122.3, 121.7 (2C), 118.5, 117.0 (2C), 112.3, 56.1 (2C), 46.7, 32.6, 32.4 (2C). HRMS calcd for $C_{24}H_{25}N_4$ (M+H$^+$): 369.2074. Found: 369.2076.

5.1.12. 1-(4-Fluorophenyl)-3-(1-methylpiperidin-4-yl)-5-(pyridin-3-yl)-1*H*-indole (3g)

Prepared according to the general procedure using pyridin-3-ylboronic acid (246 mg, 2.00 mmol) and **5a** (399 mg, 1.03 mmol). Purified by column flash chromatography (EtOAc/MeOH/Et$_3$N 90:5:5) to give a white foam (232 mg, 58%). Crystallized as a white solid from acetonitrile. Mp 148.6–150.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.96 (d, J = 1.4 Hz, pyridine H-2), 8.54 (dd, J = 1.4 Hz, 4.7 Hz, pyridine H-4), 8.12 (d, J = 8.0 Hz, pyridine H-6), 8.02 (d, J = 1.4 Hz, indole H-4), 7.67–7.63 (m, 4-fluoro-phenyl 2 × H-2), 7.59 (d, J = 8.5 Hz, indole H-7), 7.54 (dd, J = 1.4 Hz, 8.5 Hz, indole H-6), 7.50–7.46 (m, indole H-2 and pyridine H-5), 7.45–7.40 (m, 4-fluoro-phenyl 2 × H-3), 2.93–2.86 (m, piperidine 2 × H-2a and H-4), 2.22 (s, *N*-methyl), 2.10–2.01 (m, piperidine 2 × H-2b and 2 × H-3a), 1.82–1.72 (m, piperdine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): 160.5 (d, J = 243.2 Hz), 148.2, 147.9, 137.0, 135.8, 135.7, 134.5, 129.3, 128.9, 126.1 (d, J = 8.6 Hz, 2C), 125.4, 124.2, 123.1, 121.9, 118.2, 116.9 (d, J = 22.8 Hz, 2C), 111.3, 56.3 (2C), 46.8, 32.9 (2C), 32.5. HRMS calcd for $C_{25}H_{25}FN_3$ (M+H$^+$): 386.2027. Found: 386.2030.

5.1.13. 3-(1-Methylpiperidin-4-yl)-5-(pyridin-3-yl)-1-(pyridin-4-yl)-1*H*-indole (3h)

Prepared according to the general procedure from pyridin-3-ylboronic acid (123 mg, 1.00 mmol) and **5b** (179 mg, 0.483 mmol). Isolated as a colorless oil (87 mg, 47%). ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.98 (d, J = 1.9 Hz, *C*-bound pyridine H-2), 8.70 (d, J = 6.1 Hz, *N*-bound pyridine 2 × H-2), 8.56 (dd, J = 1.4 Hz, 4.7 Hz, *C*-bound pyridine H-6), 8.14 (dt, J = 1.9 Hz, 8.0 Hz, *C*-bound pyridine H-4), 8.04 (d, J = 1.4 Hz, indole H-4), 7.90 (d, J = 8.5 Hz, indole H-7),

7.71 (d, J = 6.1 Hz, *N*-bound pyridine 2 × H-3), 7.66 (s, indole H-2), 7.61 (dd, J = 1.4 Hz, 8.5 Hz, indole H-6), 7.48 (dd, J = 4.7 Hz, 8.0 Hz, *C*-bound pyridine H-5), 2.90–2.86 (m, piperidine 2 × H-2a and H-4), 2.22 (s, *N*-methyl), 2.08 (broad t, J = 11.5 Hz, piperidine 2 × H-2b), 2.01 (broad d, J = 12.3 Hz, piperidine 2 × H-3a), 1.82–1.73 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 151.6 (2C), 148.2, 148.1, 146.0, 136.7, 134.9, 134.6, 130.4, 130.2, 125.2, 124.2, 124.2, 122.6, 118.4, 116.9 (2C), 112.3, 56.2 (2C), 46.7, 32.6 (2C), 32.4. HRMS calcd for $C_{24}H_{25}N_4$ ($M+H^+$): 369.2074 Found: 369.2065.

5.1.14. 1-(4-Fluorophenyl)-3-(1-methylpiperidin-4-yl)-5-(pyrimidin-5-yl)-1*H*-indole (3i)

Prepared according to the general procedure from pyrimidin-5-ylboronic acid (248 mg, 2.00 mmol) and **5a** (398 mg, 1.03 mmol). Purified by column flash chromatography (EtOAc/MeOH/Et_3N 90:5:5) to give a white solid (117 mg, 29%). Re-crystallized from acetonitrile. Mp 219.8–220.5 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ, 9.21 (s, pyrimidine 2 × H-3), 9.15 (s, pyrimidine H-1), 8.14 (broad s, indole H-4), 7.66–7.60 (m, 4-fluoro-phenyl 2 × H-2 and indole H-6 and H-7), 7.49 (s, indole H-2), 7.45–7.42 (m, 4-fluoro-phenyl 2 × H-3), 2.92–2.87 (m, piperidine 2 × H-2a and H-4), 2.22 (s, *N*-methyl), 2.10–2.02 (m, piperidine 2 × H-2b and 2 × H-3a), 1.81–1.72 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 160.6 (d, J = 243.9 Hz), 150.4, 148.5 (2C), 136.4, 135.7 (d, J = 1.8 Hz), 129.3, 128.8, 126.1 (d, J = 8.4 Hz, 2C), 125.6, 123.3, 121.6, 121.6, 118.3, 116.8 (d, J = 23.2 Hz, 2C), 111.4, 56.2 (2C), 46.7, 32.8 (2C), 32.5. HRMS calcd for $C_{24}H_{24}FN_4$ ($M+H^+$): 387.1980. Found: 387.1979.

5.1.15. 3-(1-Methylpiperidin-4-yl)-1-(pyridin-4-yl)-5-(pyrimidin-5-yl)-1*H*-indole (3j)

Prepared according to the general procedure from pyrimidin-5-ylboronic acid (159 mg, 1.28 mmol) and **5b** (183 mg, 0.494 mmol). Isolated as a white solid (32 mg, 17%). ^{1}H NMR (500 MHz, DMSO-d_6): δ, 9.23 (s, pyrimidine 2 × H3), 9.17 (s, pyrimidine H-1), 8.70 (broad d, J = 4.7 Hz, pyridine 2 × H-2), 8.18 (d, J = 1.9 Hz, indole H-4), 7.94 (d, J = 9.0 Hz, indole H-7), 7.73 (broad d, J = 4.7 Hz, pyridine 2 × H-3), 7.70–7.68 (m, indole H-2 and H-6), 2.91–2.89 (m, piperidine 2 × H-2a+H-4), 2.24 (s, *N*-methyl), 2.15–2.06 (m, piperidine 2 × H-2b), 2.06–2.02 (m, piperidine 2×H-3a), 1.82–1.72 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 155.1 (2C), 151.6 (2C), 145.9, 135.2, 134.3, 130.3, 126.9, 125.3, 124.5, 122.4, 118.8, 117.1, 117.1 (2C), 112.5, 56.2 (2C), 46.7, 32.6 (2C), 32.3. HRMS calcd for $C_{23}H_{24}N_5$ ($M+H^+$): 370.2026 Found: 370.2023.

5.1.16. 1-(4-Fluorophenyl)-5-(2-methyl-2*H*-tetrazol-5-yl)-3-(1-methylpiperidin-4-yl)-1*H*-indole (3k)

Nitrile **8**[12] (20 g, 84.6 mmol) was dissolved in 1,2-dimethoxyethane (250 mL), and triethyl amine hydrochloride (35.0 g, 254 mmol) and NaN_3 (20.0 g, 769 mmol) were added. The reaction was boiled under reflux overnight, cooled to rt, filtered, and concentrated in vacuo. The residue was partitioned between EtOAc (250 mL), water (250 mL), and AcOH (30 mL). The aq layer was extracted with EtOAc (400 mL), and the combined organic layers were washed quickly with water (400 mL), sat. aq NaCl (400 mL), dried (Na_2SO_4), filtered, and concentrated to afford 17.2 g of 1-(4-fluorophenyl)-5-(2*H*-tetrazol-5-yl)-1*H*-indole as a solid sufficiently pure for the next step. A portion of this material (11.7 g, 41.9 mmol) was dissolved in NMP (150 mL), and KO(*t*Bu) (5.20 g, 46.1 mmol) was added at 10-20 °C under a N_2 atmosphere. CH_3I (6.0 mL, ~96 mmol) was added, and the mixture was stirred at rt for 2 h. The crude mixture was poured into a mixture of water (200 mL) and EtOAc (200 mL). The organic layer was washed with water (200 mL) and sat. aq NaCl (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column flash chromatography (CH_2Cl_2 and then EtOAc) to give 8.7 g of 1-(4-fluorophenyl)-5-(2-methyl-2*H*-tetrazol-5-yl)-1*H*-indole as a solid material sufficiently pure for the next step. A portion of this material (4.0 g, 13.6 mmol) was added portion-wise to a boiling suspension of *N*-methyl-4-piperidone hydrochloride hydrate (12.5 g, 81.6 mmol) in a mixture of AcOH (70 mL) and TFA (70 mL) under reflux. The resulting mixture was boiled under reflux for 1.5 h. After cooling to rt, the crude mixture was concentrated in vacuo, and the residue was partitioned between water (250 mL) and EtOAc (2 × 100 mL) after basification with NaOH. The combined organic extracts were washed with sat. aq NaCl (2 × 250 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo to afford 1-(4-fluorophenyl)-3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-5-(2-methyl-2*H*-tetrazol-5-yl)-1*H*-indole 1-(4-fluorophenyl)-3-(1-methyl-1,2,3,6-tetrahydro-pyridin-4-yl)-5-(2-methyl-2*H*-tetrazol-5-yl)-1*H*-indole (3.7 g) sufficiently pure for the next step. A portion of this material (2.2 g, 5.7 mmol) and PtO_2 (150 mg) were added to AcOH (50 mL), and the resulting mixture was treated with H_2 (3 bar) for 10 h at rt. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was partitioned between water (50 mL) and EtOAc (2 × 100 mL) after basification with NaOH. The combined organic layers were washed with sat. aq NaCl (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column flash chromatography (EtOAc/EtOH/Et_3N 100:25:4) to afford the title compound as a solid material, which was re-crystallized from acetone to give pure **3k** (0.34 g, 16%). Mp 148–150 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.36 (d, J = 1.6 Hz, indole H-4), 7.89 (dd, J = 1.6 Hz, 8.7 Hz, indole H-6), 7.68–7.63 (m, 4-fluoro-phenyl 2 × H-2), 6.62 (d, J = 8.7 Hz, indole H-7), 7.51 (s, indole H-2), 7.42 (app. t, J = 8.7 Hz, 4-fluoro-phenyl 2 × H-3), 4.41 (s, tetrazole-*N*-methyl), 2.94–2.80 (m, piperidine 2 × H-2a and H-4), 2.22 (s, piperidine-*N*-methyl), 2.08 (app. dt, J = 1.7 Hz, 11.4 Hz, piperidine 2 × H-2b), 2.01–1.95 (m, piperidine 2 × H-3a), 1.81 (app. dq, J = 3.6 Hz, 12.9 Hz, piperidine 2 × H-3b), ^{13}C NMR (126 MHz, DMSO-d_6): δ, 165.8, 161.0 (d, J = 242.5 Hz), 137.3, 135.8, 128.6, 126.7 (d, J = 8,5 Hz, 2C), 126.5, 123.3, 121.4, 119.4, 118.3, 117.2 (d, J = 23.0 Hz, 2C), 111.8, 56.5 (2C), 47.0, 33.2 (2C), 33.1. HRMS calcd for $C_{22}H_{24}FN_6$ ($M+H^+$): 391.2041. Found: 391.2050.

5.1.17. 1-(4-Fluorophenyl)-5-(1-methyl-1*H*-1,2,4-triazol-3-yl)-3-(1-methylpiperidin-4-yl)-1*H*-indole (3l)

Prepared following the general procedure described in Balle et al.[8] starting from **5a** (4.2 g, 10.9 mmol) and 3-bromo-1-methyl-1*H*-1,2,4-triazole[17] (1.75 g, 10.8 mmol). Compound **3l** was obtained as an off-white solid (150 mg, 3%). Mp 286–288 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ, 8.50 (s, triazole H-5), 8.35 (broad s, indole H-4), 7.89 (dd, J = 1.4 Hz, 8.6 Hz, indole H-6), 7.68–7.63 (m, 4-fluoro-phenyl 2 × H-2), 7.57–7.52 (m, indole H-2 and H-7), 7.46–7.42 (m, 4-fluoro-phenyl 2 × H-3), 3.93 (s, triazole-*N*-methyl), 3.55 (broad s, piperidine 2 × H-2a), 3.17 (broad s, piperidine 2 × H-2b and H-4), 2.81 (s, piperidine-*N*-methyl), 2.21 (broad d, J = 13.2 Hz, piperidine 2 × H-3a), 2.10–1.98 (m, piperidine 2 × H-3b). ^{13}C NMR (151 MHz, DMSO-d_6): δ, 162.4, 160.8 (d, J = 243.9 Hz), 145.9, 136.5, 135.7, 127.9, 126.5 (d, J = 8.9 Hz, 2C), 126.0, 123.9, 121.4, 120.9, 117.2, 117.1 (d, J = 23.1 Hz, 2C), 111.1, 54.3 (2C), 43.3, 36.4, 30.8, 30.3 (2C). HRMS calcd for $C_{23}H_{25}FN_5$ ($M+H^+$): 390.2089. Found: 390.2097.

5.1.18. 1-(4-Fluorophenyl)-5-(1-methyl-1*H*-pyrazol-3-yl)-3-(1-methylpiperidin-4-yl)-1*H*-indole (3m)

Compound **9**[8] (0.94 g, 2.5 mmol) was dissolved in THF (45 mL), and K_2CO_3 (0.52 g, 3.7 mmol), Et_3N (1 mL), and CH_3I (0.19 mL, 3.0 mmol) were added. The mixture was stirred at rt for 6 h. The crude mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by column flash chromatography

(EtOAc/EtOH/Et_3N 70:25:5) to give a semisolid, which was crystallized as a white product from Et_2O/($iPr)_2O$ (0.31 g, 32%). Mp 137–139 °C. ^1H NMR (500 MHz, DMSO-d_6): δ, 8.05 (d, J = 1.4 Hz, pyrazole H-5), 7.70 (d, J = 2.2 Hz, indole H-4), 7.65–7.59 (m, 4-fluorophenyl 2 × H-2 and indole H-6), 7.46 (d, J = 8.7 Hz, indole H-7), 7.43–7.38 (m, 4-fluoro-phenyl 2 × H-3 and indole H-2), 6.69 (d, J = 1.4 Hz, pyrazole H-4), 3.90 (s, pyrazole-*N*-methyl), 2.92–2.78 (m, piperidine 2 × H-2a and H-4), 2.21 (s, piperidine-*N*-methyl), 2.12–1.92 (m, piperidine 2 × H-2b and 2 × H-3a), 1.85–1.72 (m, piperidine 2 × H-3b). ^{13}C NMR (126 MHz, DMSO-d_6): δ, 160.5 (d, J = 243.1 Hz), 151.3, 135.8, 135.8, 135.4, 132.4, 128.4, 126.1 (d, J = 8.5 Hz, 2C), 126.0, 125.0, 122.7, 120.8, 116.9 (d, J = 22.9 Hz, 2C), 110.7, 102.5, 56.2 (2C), 46.8, 38.8, 32.9 (2C), 32.8. Anal. calcd for $C_{24}H_{25}FN_4$: C, 74.20; H, 6.49; N, 14.42. Found C, 74.05; H, 6.61; N, 14.23.

5.2. ADME assays

5.2.1. Efflux in Caco-2 cells

The polarized transport of the compounds was studied in Caco-2 cells grown on permeable filters (Transwell®, Corning Costar) for 21–28 days. Culture conditions were as published elsewhere.[22] For the experiment, a 2 mM DMSO stock solution was diluted to the appropriate concentrations (1–20 μM) with Hanks-Hepes buffer, pH 7.4. The test solution was added to either the upper chamber (apical-to-basolateral transport, A–B) or the lower chamber (basolateral-to-apical transport, B–A), and from the chamber opposite to the test solution, samples were removed during 240 min and replaced with an equal amount of fresh buffer.[23] The stop plates were centrifuged for 10 min at 3300 rpm and 4 °C, before being analyzed by liquid-chromatography coupled to a tandem mass spectrometer (LC–MS/MS, Waters QuattroMicro, Manchester, UK). The apparent permeability coefficient, P_{app} was calculated as:

$$P_{app} = d_Q/d_t \times (1/(A \times C_0))$$

where d_Q/d_t is the steady state flux across the monolayer, A is the area of the monolayer (1.1 cm^2) and C_0 is the initial concentration in the test solution.[24] A P_{app} (B–A)/P_{app} (A–B) ratio of one is indicative of pure passive diffusion, whereas a ratio significantly higher than one indicates polarized transport in favour of the B–A direction, suggesting involvement of an efflux system such as P-glycoprotein.

5.2.2. Equilibrium dialysis measurements

The methodology employed in this study was a modification of a previously reported method.[25] Briefly, a 96-well equilibrium dialysis apparatus was used to determine the free-fraction in the plasma and brain for each drug (HT Dialysis LLC, Gales Ferry, CT, USA). Membranes (3 kDa cut-off) were conditioned in deionized water for 40 min, followed by conditioning in 80:20 deionised water:ethanol for 20 min, and then rinsed in deionised water before use. Plasma was diluted 1:1 with phosphate-buffered saline (PBS), while the brain tissue was homogenised with PBS to a final composition of 1:2 brain:PBS, by means of ultrasonication (Tomtec Autogiser, Receptor Technologies, Adderbury, Oxon, UK) in an ice bath. Plasma and brain homogenate were spiked with the test compound (1000 ng/g), and 99 aliquots (n = 6 replicate determinations) were loaded into the 96-well equilibrium dialysis plate. Dialysis versus PBS (100 μL) was carried out for 5 h in a temperature-controlled incubator at 37 °C. The samples were analyzed by liquid-chromatography coupled to a tandem mass spectrometer (LC–MS/MS, Waters QuattroMicro, Manchester, UK). The free fraction in plasma ($f_{u,plasma}$) and brain tissue ($f_{u,brain}$) was calculated according to Kalvass and Maurer[25] with their dilution factor of the brain taken into account. Brain/plasma (B/P) distribution ratios were predicted from the in vitro free fraction data under the assumption of steady-state conditions and that passive diffusion govern the brain distribution. Under these assumptions, unbound plasma concentrations, expressed as $C_{total,plasma} \times f_{u,plasma}$, equals unbound brain concentrations, expressed as $C_{total,brain} \times f_{u,brain}$. Thus, in theory, the in vivo brain/plasma ratio ($C_{total,brain}/C_{total,plasma}$) equals $f_{u,plasma}/f_{u,brain}$,[20,25] which was how the estimated B/P ratios were calculated.

5.3. In vitro receptor binding assays

5.3.1. Assays performed in-house

CHO cell lines expressing the rat α_{1D}-adrenoceptor and the human D_2 receptor, and BHK cell lines expressing the bovine α_{1A}-adrenoceptor were generated in-house at H. Lundbeck A/S using standard stable transfection techniques. Rat-1 cells expressing hamster α_{1B}-adrenoceptors were obtained from the University of Utah, Salt Lake City, UT. Hela cells expressing human 5-HT_{1B} receptors were obtained from Seattle Veterans Affairs Medical Center. Hela cells expressing human 5-HT_6 receptors were obtained from NIH. CHO cells expressing human 5-HT_{2C} receptors (VSV) were purchased from Euroscreen, Brussel, Belgium.

The cells were grown to 90% confluence, detached and homogenized in ice-cold 50 mM Tris, pH 7.4, using an Ultra-Turrax, and the homogenates were either kept on ice or stored at −80 °C until use.

In all α_1-adrenoceptor binding assays, 50 mM Tris, pH 7.7 was used as assay buffer, and [^3H]prazosine was used as radioligand at a concentration of 0.3 nM (α_{1A} and α_{1D}) or 0.5 nM (α_{1B}). Non-specific binding was defined as the binding in the presence of 1 μM 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane (WB-4101), and mixtures were incubated at 22 °C for 20 min.

In the 5-HT_6 receptor binding assay, 50 mM Tris, pH 7.7 was used as assay buffer, [^3H]LSD was used as radioligand at a concentration of 1 nM. Non-specific binding was defined as the binding in the presence of 10 μM 1-(2-{4-[5-fluoro-1-(4-fluoro-phenyl)-1*H*-indol-3-yl]-piperidin-1-yl}-ethyl)-imidazolidin-2-one, and the mixtures were incubated at 22 °C for 60 min.

In the D_2 receptor binding assay, 50 mM Tris, pH 7.4 was used as assay buffer, and [^3H]spiperone was used as radioligand at a concentration of 0.1 nM. Non-specific binding was defined as the binding in the presence of 10 μM haloperidol, and the mixtures were incubated at 37 °C for 30 min.

In the 5-HT_{2C} receptor binding assay, 50 mM Tris, pH 7.7 was used as assay buffer, and [^3H]Mesulergine was used as radioligand at a concentration of 0.5 nM. Non-specific binding was defined as the binding in the presence of 100 μM 3-(2-aminoethyl)-5-hydroxyindole creatinin sulphate, and the mixtures were incubated at 37 °C for 60 min.

In the α_1, D_2, 5-HT_6 and 5-HT_{2C} assays, bound and free radioactivity were separated by vacuum filtration on GF/B filters. The filters were dried, scintillation fluid (Optiphase Supermix, Perkin-Elmer) was added, and the samples were counted in a scintillation counter (Trilux, Wallac).

In the 5-HT_{1B} binding assay, 50 mM Tris, pH 7.7 with 0.8 mg/mL WGA SPA beads were used as assay buffer, and [^3H]CT (TRK 1038) was used as radioligand at a concentration of 1.5 nM. Non-specific binding was defined as the binding in the presence of 10 μM 3-(2-aminoethyl)-5-hydroxyindole creatinin sulphate, and the mixtures were incubated at room temperature for 60 min and subsequently counted in a scintillation counter (Trilux, Wallac).

Data shown in the tables from the above assays are means of a minimum of two full concentration-response curves using 10 concentrations of drugs (covering 4 decades). The results are given as K_i values (nM) derived from computer-fitted IC_{50} values converted

to K_i values using the Cheng–Prusoff equation (($K_i = IC_{50}/(1 + (L/K_D))$). Absolute deviation from the mean for the pK_i values were below 0.3 for all reported compounds.

5.3.2. Assays performed externally at Cerep and MDS pharmaservices

External testing in human 5-HT_{2A} receptor binding was done at Cerep (www.cerep.fr, catalog Ref. 808-2ah). Compounds were tested at two concentrations (1 μM and 100 nM), and K_i values were subsequently estimated from computer-fitted IC_{50} values converted to K_i values using the Cheng–Prusoff equation (($K_i = IC_{50}/(1 + (L/K_D))$). The compounds were only tested once, so the standard errors are not known.

Broad in vitro profiling was performed by MDS pharmaservices (Taiwan), and compounds were assayed for their ability to displace radioligand binding to the assayed targets at a concentration of 1000 nM. The following targets were tested at MDS Pharmaservices: Adenosine A_1, adenosine A_{2A}, adenosine A_3, adrenergic α_{1A}, adrenergic α_{1B}, adrenergic α_{1D}, adrenergic α_{2A}, adrenergic β_1, adrenergic β_2, transporter: norepinephrine (NET), bradykinin B_1, bradykinin B_2, calcium channel L-type: benzodiazepine, calcium channel L-type: dihydropyridine, calcium channel N-type, dopamine D_1, dopamine D_{2S}, dopamine D_3, dopamine $D_{4.2}$, transporter: dopamine (DAT), endothelin ET_A, endothelin ET_B, epidermal growth factor (EGF), estrogen ERalpha, transporter: GABA, $GABA_A$: agonist site, $GABA_A$: benzodiazepine: central, $GABA_{B1A}$, glucocorticoid, Glutamate: Kainate, Glutamate: NMDA: Agonism, Glutamate: NMDA: Glycine, glutamate: NMDA: phencyclidine, histamine H_1, histamine H_2, histamine H_3, imidazoline I_2: central, interleukin IL-1, leukotriene: cysteinyl cysLT1, melatonin MT_1, muscarinic M_1, muscarinic M_2, muscarinic M_3, tachykinin NK_1, neuropeptide Y Y_1, neuropeptide Y Y_2, nicotinic acetylcholine, nicotinic acetylcholine: bungarotoxin-sensitive: neuromuscular, opiate delta (OP_1: DOP), opiate kappa (OP_2: KOP), opiate mu (OP_3: MOP), phorbol ester, platelet activating factor (PAF), potassium channel [KATP], potassium channel HERG, prostanoid EP_4, purinergic P_{2X}, purinergic P_{2Y}, rolipram (PDE4), serotonin 5-HT_{1A}, serotonin 5-HT_3, transporter: serotonin (SERT), sigma$_1$, sigma$_2$, sodium channel: site 2, testosterone, thyroid hormone.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.10.049. These data include MOL files and InChiKeys of the most important compounds described in this article.

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