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Short and Efficient Syntheses of Analogues of WAY-100635: New and Potent 5-HT_{1A} Receptor Antagonists

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Abstract—Simple syntheses of four new and potent analogues of the 5-HT_{1A} receptor ligand, WAY-100635 are described, namely the 6-(pyridinyl)-bromo-, the 6-(pyridinyl)-fluoro-, the pyrimidine- and the 5-(pyridinyl)-bromo-analogues. The first three analogues were obtained by aromatic nucleophilic substitution of the 2,6-dihalogenopyridine (activated or not as an *N*-oxide) or of the 2-chloropyrimidine with the corresponding amine nucleophile as a key step. The fourth analogue, the 5-(pyridinyl)-bromo-analogue, was synthesized from the 2-amino-5-bromopyridine via a progressive elongation of the skeleton. The four compounds described are all full antagonists and show good in vitro binding affinities (K_i).

Introduction

WAY-100635 **1** [*N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-pyridinyl)cyclohexanecarboxamide] is a potent and silent antagonist of the 5-HT_{1A} receptor,[1,2] a receptor which is strongly implicated in neuropsychiatric diseases (e.g. anxiety, depression and schizophrenia).[3] The labelling of WAY-100635 with ^{11}C ($t_{1/2} = 20.3$ min)[4] provided the first radioligand for imaging 5-HT_{1A} receptors in the living human brain with positron emission tomography (PET).[5,6] Carbonyl-^{11}C-labelled WAY-100635 is a very effective radioligand[6] but it is rapidly metabolised,[7] such that its input function into brain is difficult to determine accurately in the late stage of a PET experiment. A similarly effective radioligand that could be labelled easily with a longer-lived radionuclide such as ^{18}F ($t_{1/2} = 109.7$ min) or ^{76}Br ($t_{1/2} = 16.1$ h) might have considerable advantages for PET. Also labelling with γ-emitting ^{123}I ($t_{1/2} = 13.2$ h) might deliver a much-needed radioligand for studies with the more widely used imaging technique, single photon emission tomography (SPET). Therefore, we sought to develop new analogues of WAY-100635, halogenated and non-halogenated. The halogenated analogues of WAY-100635 could give an easy access to the labelled ^{18}F, ^{76}Br or ^{123}I analogues while the pyrimidine analogue labelled with ^{11}C, with its extra nitrogen, could lead to radioligands with an enhanced affinity for the receptor. We have synthesized three new halogenated WAY-analogues, namely: *N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-(6-bromo)pyridinyl)cyclohexanecarboxamide **2** (6BPWAY), *N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-(6-fluoro)-pyridinyl) cyclohexanecarboxamide **3** (6FPWAY), *N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-(5-bromo)pyridinyl)cyclohexanecarboxamide **4** (5BPWAY) and a non-halogenated analogue: *N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-pyrimidinyl)cyclohexane carboxamide **5** (PmWAY) (Fig. 1).

Chemistry

The retrosynthetic analysis of 6BPWAY **2** and 6FPWAY **3** showed that 2-amino-6-halopyridines **8** would be suitable precursors of the intermediates **6** and **7**, which could be key compounds of the synthesis (Scheme 1).

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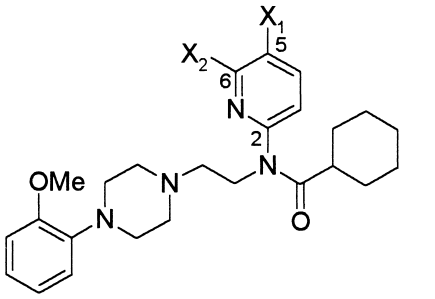
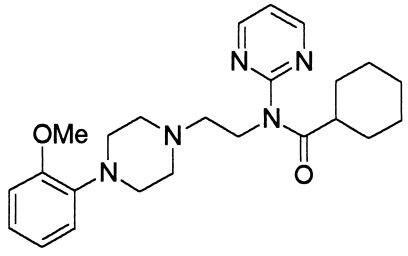


X_1 = H, X_2 = H : WAY-100635 **1**
X_1 = H, X_2 = Br : 6BPWAY **2**
X_1 = H, X_2 = F : 6FPWAY **3**
X_1 = Br, X_2 = H : 5BPWAY **4**



PmWAY **5**

Figure 1.

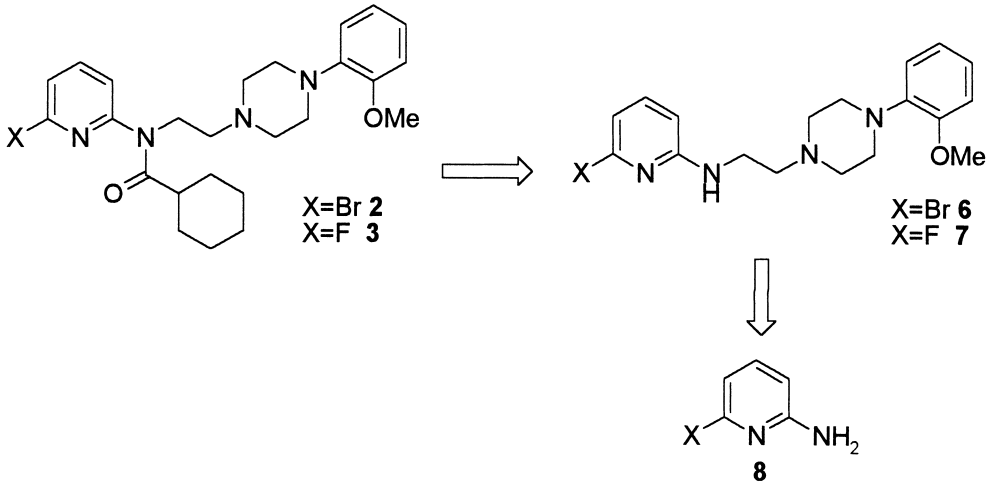
Halogenation of the pyridine ring is not equally easy to achieve at each position of the ring. The 2-aminopyridine is easily halogenated in positions *ortho* and *para* (positions 3 and 5) to the amino group due to its electronic effects. Polyhalogenated derivatives are often obtained. Selective monobromination in position 5 of 2-aminopyridine can be achieved with tetrabutylammonium tribromide[8] but no monobromination at position 6 has ever been described. In general, fluoropyridines are prepared from aminopyridines by diazotisation in hydrofluoroboric acid.[9] The yields are often low and the fluorine replaces the aminogroup. Successful substitution of chlorine by fluorine has been reported.[10] However, the reaction has to be kept for a long time at elevated temperature (200 °C) and yields are low. Hence, direct halogenation of 2-aminopyridine does not seem to be a very convenient method to use in order to obtain the 2-amino-6-bromo- and 2-amino-6-fluoropyridine.

The amination reaction of a 2-halogenopyridine[11] can be performed via the Chichibabin reaction[12] or by reaction with aqueous ammonia[13] at high temperature under pressure and for a long period. The yields are low. The Curtius rearrangement is a well-known reaction for introducing an amino group into a pyridine ring, starting from a picolinic acid.[14] However, it is sometimes difficult to have the carboxyl group and the other substituents in the desired ring positions. Hence, from the literature, it appeared difficult to prepare efficiently brominated or fluorinated compounds **8** in a reasonably high yield.

Therefore, we decided to look for a method that would be better adapted to the direct synthesis of compounds **6** and **7**. An easy method to synthesize 2-amino-6-halopyridines is aromatic nucleophilic substitution (S_NAr) of 2,6-dihalopyridines, in which one of the halogens is substituted by a suitable amine nucleophile.

The amine nucleophile, 4-(2-aminoethyl)-1-(2-methoxyphenyl)piperazine **9** was prepared by reaction of 1-(2-methoxyphenyl)piperazine and bromoacetonitrile followed by reduction with lithium aluminium hydride.[15] The amine **9** was heated with the 2,6-dibromopyridine and triethylamine.[16] No reaction occurred. However, when the nitrogen of the pyridine ring was converted into its *N*-oxide form, the electronic density in the ring was reduced and the aromatic nucleophilic substitution was facilitated.[16] This kind of reactivity has already been described by Katritkzy[17] a long time ago and has been recently reconfirmed for the 2-chloropyridine *N*-oxide by Lee et al.[18] We, therefore, decided to use 2,6-dibromopyridine *N*-oxide, as starting material for the synthesis of compounds **6** and **7** (Scheme 2).

The nitrogen of the 2,6-dibromopyridine was oxidized with hydrogen peroxide (30% in water) in trifluoro-



X=Br **2**
X=F **3**

X=Br **6**
X=F **7**

8

Scheme 1. Retrosynthesis of 6BPWAY and 6FPWAY.

acetic acid.[19] Reaction of the nucleophile **9** with the 2,6-dibromopyridine *N*-oxide **10** in *n*-butanol gave the desired compound **11** in 40% yield. No disubstitution of **10** was noticed. The *N*-oxide **11** was reduced to the pyridine **6** with iron in acetic acid at 100 °C for 2 h. Reaction of **6** with the cyclohexanecarbonyl chloride in dichloromethane then gave the desired compound **2** in 75% yield.

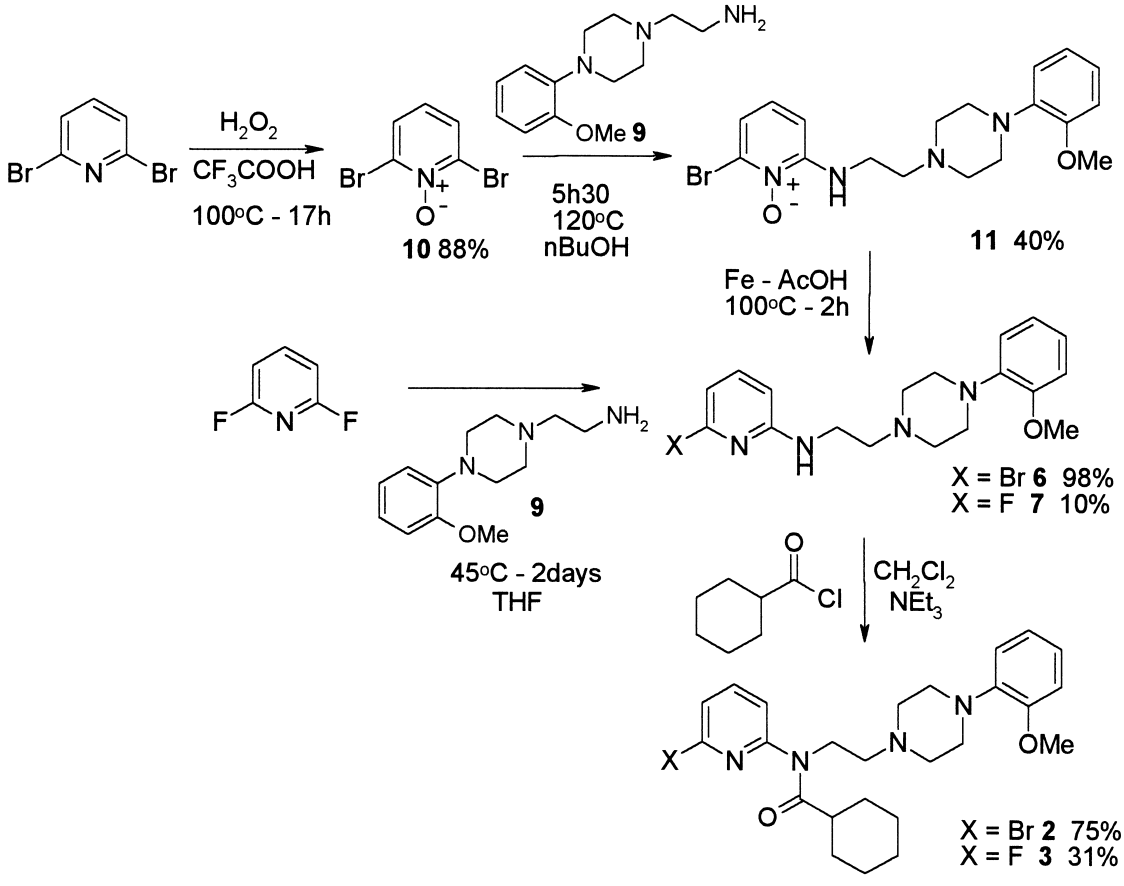
An analogous pathway was considered for the synthesis of 6FPWAY **3**, but it was impossible to isolate the 2,6-difluoropyridine-*N*-oxide. Therefore, the nucleophilic substitution was performed directly on the 2,6-difluoropyridine in THF at 45 °C for 2 days (Scheme 2). Product **7** was isolated in low yield (10%) but was successfully acylated with cyclohexanecarbonyl chloride to obtain 6FPWAY **3**. The addition of a base like triethylamine or sodium hydride did not improve the yield of compound **7**. When the reaction was performed at higher temperature, no product **7** was isolated. The 2-fluoropyridine is unstable at room temperature, it rapidly gives a pyridyl–pyridinium product.[20] Because of the electron-withdrawing effect of the two fluorine atoms, the 2,6-difluoropyridine is a quite reactive reagent. It is therefore expected to be difficult to obtain a monosubstituted product only. It has already been described that the aromatic nucleophilic subtitution of the 2,6-difluoropyridine can go even further leading to the formation of polymers.[21] Despite the low yield of the coupling reaction, a new fluorinated analogue of WAY-100635 was obtained in a straightforward two-step synthesis.

The synthesis of pyrimidine-WAY **5** is also feasible via the nucleophilic aromatic substitution of the 2-chloropyrimidine with the amine nucleophile **9** (Scheme 3). Reflux of the 2-chloropyrimidine with the amino-precursor **9** in absolute ethanol for 7 h gave the expected compound **12** with 50% yield. Reaction of **12** with the cyclohexanecarbonyl chloride in dichloromethane then gave the desired compound **5** in 91% yield in a two-step synthesis.

The synthesis of 5BPWAY **4** followed a different pathway. It was easily achieved from the commercially available 2-amino-5-bromopyridine (Scheme 4). The acylated product was treated with 1-(2-methoxyphenyl)piperazine, and the product amide **13** reduced with lithium aluminium hydride. Some expected debromination occurred during this step, limiting the yield of the desired product to 51%. This side reaction can be avoided by reduction with borane-dimethyl sulphide. Treated with cyclohexanecarbonyl chloride, the desired compound **4** was obtained with 60% yield.

Pharmacology

The new halogenated analogues of WAY-100635 **2**–**4** and the pyrimidine-WAY analogue **5** were assessed for their ability to inhibit the binding of [^{3}H]-5-CT to human cloned 5-HT$_{1A}$ receptors in vitro. The results of these binding studies are shown in Table 1.



Scheme 2. Synthesis of 6BPWAY and 6FPWAY.

The intrinsic efficacy of each compound **2**–**5** was determined in human cloned 5-HT_{1A} receptors, looking at the production of cAMP (in the presence of forskolin) with a concentration up to 10 μM of each compound.

The abilities of the new compounds to inhibit the production of cAMP (in the presence of forskolin) were also assessed in human cloned 5-HT_{1A} receptors with 1000 nM of 5-HT. The results of these efficacy assays are shown in Table 1.

Table 1. In vitro binding affinities and intrinsic efficacies

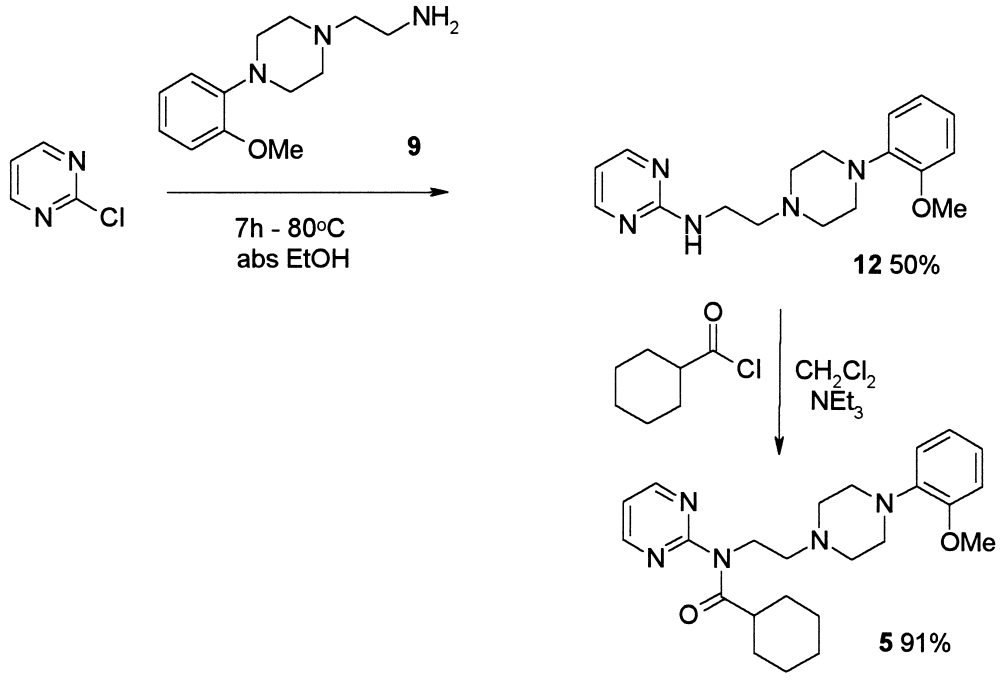
	Affinity K_i (nM)	Efficacy K_i (nM)
WAY-100635	0.17	0.029
6BPWAY **2**	0.51	4.30
6FPWAY **3**	0.33	10.0
5BPWAY **4**	6.30	2.80
PmWAY **5**	1.00	0.082

Results and Discussion

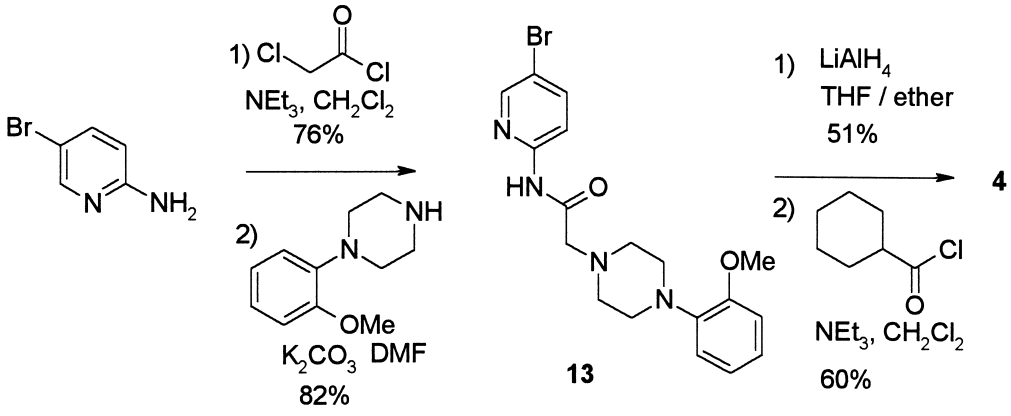
Each new analogue of WAY-100635 was found to show a good binding affinity for the 5-HT_{1A} receptor. The K_i value obtained for 5BPWAY **4** is somewhat higher than the one of 6BPWAY **2**, indicating that the position of the halogen could interfere in the binding of the compound to the receptor. The K_i value of PmWAY **5** (1.00 nM) is higher than the one of WAY-100635 (0.17 nM) or of the 6-substituted pyridine WAY (0.33 nM and 0.51 nM). The extra-nitrogen of the pyrimidine ring does not enhance the affinity of the compound for the receptor.

None of these new compounds **2**–**5** presents any intrinsic efficacy, they are all full antagonists of the 5-HT_{1A} receptor.

The lipophilicity of each compound has been calculated as a logP/logD^{22} value to get insight of their possible penetration into the brain. The values are shown in



Scheme 3. Synthesis of PmWAY.



Scheme 4. Synthesis of 5BPWAY.

Table 2. Calculated log D values at physiological pH and log P values

	Log D at pH = 7.40	Log P
WAY-100635	2.88	3.28
6BPWAY **2**	4.14	4.53
6FPWAY **3**	3.45	3.85
5BPWAY **4**	4.16	4.16
PmWAY **5**	2.30	2.70

Table 2. They are all in a good range; it is expected that they can pass the blood-brain barrier and, if labelled with a positron-emitter, can then be studied with PET.

6BPWAY **2**, 6FPWAY **3** and PmWAY **5**, from their respective binding profiles, look interesting for PET studies. They can be labelled with ^{11}C and, in the case of **3**, with ^{18}F. 6BPWAY **2** has also been transformed to the 6-(pyridinyl)-stannate analogue in order to prepare ^{123}IPWAY, a new radioligand which could be studied with SPET.

5BPWAY **4** can be labelled with ^{11}C but the labelling with ^{18}F will be difficult. The electron distribution of the pyridine ring is not favorable for the displacement of the bromine atom for a ^{18}F at position 5 of the ring.

Conclusion

We have described short and efficient syntheses of four new analogues of WAY-100635 displaying potent, full antagonist effects on the 5-HT_{1A} receptor. The 6-halopyridine compounds **2** and **3**, and the pyrimidine-WAY analogue **5** have been synthesized via aromatic nucleophilic substitution as a key step on the 2,6-dibromopyridine *N*-oxide, the 2,6-difluoropyridine and the 2-chloropyrimidine, respectively. This reaction gives a general and easy access to new analogues of WAY-100635, halogenated or not. The 5-bromopyridine analogue **4** has been prepared via a linear and progressive elongation of the skeleton, starting from the 2-amino-5-bromopyridine. These ligands **2**, **3**, **5** can be labelled with carbon-11, with fluorine-18 or bromine-76. These new radioligands are now being evaluated in vivo in PET.

Experimental

Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. IR spectra were obtained on an ATI-Mattson spectrometer. NMR spectra were obtained on a Varian Gemini, 300 MHz or 200 MHz instrument. Chemical shifts are reported in δ value (ppm) relative to the deuterated solvent as internal standard. Mass spectra (ES) were recorded on a Sciex API 3000 mass spectrometer or on a Unicam 610/Automass 150 GC–MS system (EI). Elemental analyses were performed by the Microanalytical Laboratory, University of Groningen and are within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was performed on silica gel 60 PF 254 pre-coated aluminum sheet (Merck) with a layer thickness of 0.2 mm (analytical) and on silica gel 60 PF 254 pre-coated glass plate (Merck) with a layer thickness of 1 mm (preparative).

All chemicals used were commercially available (Aldrich, Acros or Fluka) and were used without purification.

2,6-Dibromopyridine-*N*-oxide[19] **10** and of 4-(2-aminoethyl)-1-(2-methoxyphenyl)piperazine[16] **9** were prepared according to described procedures.

1-(2-Methoxyphenyl)-4-(2-(2-(6-bromo)aminopyridinyl-*N*-oxide)ethyl)piperazine (11). To 2,6-dibromopyridine-*N*-oxide **10** (2.34 g, 9.25 mmol) in *n*-butanol (40 mL) was added 4-(2-aminoethyl)-1-(2-methoxyphenyl)piperazine **9** (2.18 g, 9.25 mmol) and triethylamine (1.29 mL, 9.25 mmol). The reaction mixture was heated at 120 °C for 20 h. The solvent was evaporated and the residue chromatographed on silica (CH_2Cl_2/MeOH 95/5) to afford 1.50 g (40% yield) of **11** as a brown oil. IR (neat): 1590, 1499, 1453, 1240 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.74 (m, 6H), 3.08 (br s, 4H), 3.36 (m, 2H), 3.80 (s, 3H), 6.52 (d, $J = 8$ Hz, 1H), 6.99 (m, 5H), 7.37 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 37.55, 48.90, 51.65, 53.85, 54.65, 102.20, 109.60, 114.35, 116.85, 119.50, 126.15, 150.70; MS (EI): *m*/*z* 390–392 (M–O), 218, 205, 190, 162.

1-(2-Methoxyphenyl)-4-(2-(2-(6-bromo)aminopyridinyl)-ethyl)piperazine (6). To 1-(2-methoxyphenyl)-4-(2-(2-(6-bromo)aminopyridinyl-*N*-oxide)ethyl)piperazine **11** (1.20 g, 2.90 mmol) in acetic acid (10 mL) was added Fe (0.34 g, 6.10 mmol). The mixture was stirred at 100 °C for 17 h. The acid was evaporated, the residue was basified with a solution of Na_2CO_3 (5%) and the product extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The product **6** was purified by crystallization as the hydrochloride salt and then basified to give 0.98 g of **6** as a brown solid (85% yield). IR (KBr): 3300, 1599, 1506, 1444 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.75 (br s, 6H), 3.12 (br s, 4H), 3.38 (br s, 2H), 3.81 (s, 3H), 6.31 (d, $J = 8$ Hz, 1H), 6.65 (d, $J = 7$ Hz, 1H), 6.80–6.96 (m, 4H), 7.18 (t, $J = 8$ Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 36.90, 49.00, 51.55, 53.85, 55.00, 103.25, 109.65, 113.95, 116.70, 119.50, 121.50, 137.90; MS (EI): *m*/*z* 309–392 (M), 218, 205, 190, 162; HRMS (EI) calcd for $C_{20}H_{25}BrN_4O$: 390.1055, observed: 390.1045. Anal. calcd for $C_{20}H_{25}BrN_4O.1/3\ H_2O$: C, 54.41, H, 6.00, N, 14.10. Found: C, 54.43; H, 5.62; N, 13.93.

***N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-(6-bromo)pyridinyl) cyclohexanecarboxamide (2) 6BPWAY.** To 1-(2-methoxyphenyl)-4-(2-(2-(6-bromo)aminopyridinyl)ethyl)piperazine **6** (0.77 g, 1.96 mmol) in CH_2Cl_2 (25 mL) was added cyclohexanecarbonyl chloride (0.39 mL, 2.95 mmol, 1.5 equiv) and triethylamine (0.41 mL, 2.95 mmol, 1.5 equiv). The mixture was stirred at room temperature under N_2 for 17 h. The reaction mixture was quenched with 10% Na_2CO_3 and extracted with CH_2Cl_2. The combined organic layers were dried

over $MgSO_4$, filtered and concentrated. Column chromatography on silica (CH_2Cl_2/MeOH 98/2 and then with hexane/ethyl acetate 5/5) provided 0.74 g of **2** as a pure yellow-red oil (75% yield). IR (neat): 1664, 1499, 1433 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.03–1.19 (m, 3H), 1.50–1.81 (m, 8H), 2.35 (m, 1H), 2.56–2.63 (m, 5H), 2.94 (s, 4H), 3.81 (s, 3H), 3.99 (t, $J=4$ Hz, 2H), 6.80–6.96 (m, 4H), 7.26–7.36 (m, 2H), 7.55 (t, $J=8$ Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.05, 28.15, 41.15, 43.50, 49.05, 51.85, 53.80, 55.00, 109.65, 116.55, 118.50, 119.40, 121.35, 124.15, 138.25, 138.60, 139.70, 150.70, 154.30, 174.95; MS (ES): m/z 501–503 $(M+H)^+$, 457, 391–393; HRMS (EI) calcd for $C_{27}H_{35}BrN_4O_2$: 500.1786, observed: 500.1763.

1-(2-Methoxyphenyl)-4-(2-(2-(6-fluoro)aminopyridinyl)-ethyl)piperazine (7). To 2,6-difluoropyridine (0.32 g, 2.79 mmol) in dry THF (10 mL) was added 4-(2-aminoethyl)-1-(2-methoxyphenyl)piperazine **9** (0.66 g, 2.79 mmol) and triethylamine (1.94 mL, 13.9 mmol, 5 equiv). The reaction mixture was heated at 45 °C for 3.5 h. The solvent was evaporated, the residue dissolved in water and extracted with CH_2Cl_2. The combined organic layers were washed with 10% Na_2CO_3 and with brine, dried over $MgSO_4$, filtered and concentrated. Purification of the residue by column chromatography on silica (CH_2Cl_2/MeOH 98/2) followed by preparative TLC plate (CH_2Cl_2/MeOH 95/5) provided 92 mg (10% yield) of the pure product **7** (yellow oil). IR (neat): 1590, 1499, 1449 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.73 (m, 6H), 3.13 (br s, 4H), 3.40 (q, $J=5$ Hz, $J=11$ Hz, 2H), 3.86 (s, 3H), 5.42 (br s, 1H), 6.12 (dd, $J=2$ Hz, $J=8$ Hz, 1H), 6.24 (dd, $J=2$ Hz, $J=8$ Hz, 1H), 6.84–7.01 (m, 4H), 7.44 (dd, $J=8$ Hz, $J=16$ Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 36.65, 48.80, 51.60, 53.85, 55.05, 93.45, 94.20, 101.65, 109.65, 116.75, 119.50, 121.60, 139.95, 140.15, 151.00; ^{19}F NMR (50 MHz, $CDCl_3$): δ −70.0; MS (EI): m/z 330 (M), 277, 263, 235, 205, 190, 162; HRMS (EI) calcd for $C_{20}H_{25}FN_4O$: 330.1856, observed: 330.1861.

***N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-(6-fluoro)pyridinyl) cyclohexanecarboxamide (3) 6FPWAY.** To 1-(2-methoxyphenyl)-4-(2-(2-(6-fluoro)aminopyridinyl)ethyl)piperazine **7** (50 mg, 0.15 mmol) in CH_2Cl_2 (5 mL) was added cyclohexanecarbonyl chloride (30 μL, 0.23 mmol, 1.5 equiv) and triethylamine (32 μL, 0.23 mmol, 1.5 equiv). The mixture was stirred at room temperature under N_2 for 17 h. The reaction mixture was quenched with 10% Na_2CO_3 and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica (CH_2Cl_2/MeOH 98/2) and preparative TLC plate (CH_2Cl_2/acetone 97/3, CH_2Cl_2/isopropanol 98/2) provided 22 mg (31% yield) of the pure product **3** (colorless oil). IR (KBr): 1667, 1597, 1499, 1447 cm^{-1}: ^1H NMR (200 MHz, $CDCl_3$): 1.03–1.89 (m, 11H), 2.31–2.37 (br s, 1H), 2.61–2.68 (br s, 5H), 3.00 (s, 4H), 3.84 (s, 3H), 3.99 (t, $J=7$ Hz, 2H), 6.82–7.01 (m, 5H), 7.21 (d, $J=8$ Hz, 1H), 7.82 (dd, $J=8$ Hz, $J=16$ Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.05, 28.10, 41.00, 43.30, 48.80, 51.65, 53.80, 54.60, 109.65, 116.60, 117.00, 119.40, 121.45, 139.00, 140.95, 150.50, 163.20, 175.00; ^{19}F NMR (50 MHz, $CDCl_3$): δ −67.75; MS (EI): m/z 440 (M), 218, 205, 190, 162; HRMS (EI) calcd for $C_{27}H_{35}FN_4O_2$: 440.2587, observed: 440.2592.

1-(2-Methoxyphenyl)-4-(2-(2-aminopyrimidinyl)ethyl)piperazine (12). To 2-chloropyrimidine (0.10 g, 0.88 mmol) in absolute ethanol (10 mL) was added 4-(2-aminoethyl)-1-(2-methoxyphenyl)piperazine **9** (0.21 g, 0.88 mmol). The reaction mixture was heated at reflux for 7 h. The solvent was evaporated, the residue dissolved in water and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica (CH_2Cl_2/MeOH 95/5 and 90/10) provided 137 mg of the desired product **12** as a yellow oil (50% yield). IR (neat): 3365, 1589, 1500, 1452 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.63–2.72 (m, 6H), 3.07 (m, 4H), 3.52 (q, $J=6$ Hz, $J=11$ Hz, 2H), 3.83 (s, 3H), 5.85 (br s, 1H), 6.48 (t, $J=5$ Hz, 1H), 6.81–6.98 (m, 5H), 8.25 (d, $J=4$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 36.40, 49.05, 51.60, 53.80, 55.25, 108.85, 109.60, 109.95, 116.70, 119.45, 121.45, 139.70, 150.70, 156.50, 160.90; MS (EI): m/z 313 (M), 298, 218, 205, 162, 120; HRMS (EI) calcd for $C_{17}H_{23}N_5O$: 313.1902, observed: 313.1890.

***N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-pyrimidinyl)cyclohexane carboxamide (5) PmWAY.** To 1-(2-methoxyphenyl)-4-(2-(2-aminopyrimidinyl)ethyl)-piperazine **12** (0.10 g, 0.32 mmol) in CH_2Cl_2 (5 mL) was added cyclohexanecarbonyl chloride (64 μL, 0.48 mmol, 1.5 equiv) and triethylamine (67 μL, 0.48 mmol, 1.5 equiv). The mixture was stirred at room temperature under N_2 for 18 h. The reaction mixture was quenched with 10% Na_2CO_3 and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica (CH_2Cl_2/MeOH 99/1) provided 0.16 g of **5** as a yellow oil (91% yield). IR (neat): 1670, 1565, 1499 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ 1.02–1.84 (m, 11H), 2.82 (br s, 4H), 2.98 (br s, 5H), 3.79 (s, 3H), 4.24 (br s, 2H), 6.78–7.02 (m, 5H), 8.58 (d, $J=5$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.65, 24.05, 24.30, 26.85, 27.70, 28.40, 42.30, 44.25, 48.90, 51.70, 53.80, 55.35, 109.60, 115.25, 116.55, 119.40, 121.30, 139.75, 150.65, 156.30, 160.35, 170.50; MS (CI): m/z 424 $(M+H)^+$.

2-Chloro-*N*-(2-(5-bromo)pyridinyl)acetamide. To 2-amino-5-bromopyridine (1.00 g, 5.75 mmol) in CH_2Cl_2 (50 mL) was added triethylamine (1.20 mL, 872 mg, 8.62 mmol, 1.5 equiv) and 2-chloroacetyl chloride (0.69 mL, 975 mg, 8.62 mmol, 1.5 equiv) under nitrogen. The mixture was stirred at room temperature for 17 h and quenched with 25 mL of 10% $NaHCO_3$ solution. The reaction mixture was extracted with CH_2Cl_2, the combined organic layers dried over $MgSO_4$, filtered and concentrated. The product was purified by crystallization in the mixture CH_2Cl_2/MeOH and obtained pure as a white powder with 76% yield (1.09 g). mp: 154 °C, IR (KBr): 1671 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 4.20 (s, 2H), 7.84 (d, $J=9$ Hz, 1H), 8.15 (d, $J=9$ Hz, 1H), 8.38 (s, 1H), 8.83 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 41.20, 113.55, 121.20, 139.45, 146.50, MS (ES): m/z 249–251 $(M+H)^+$, 173–175; HRMS (EI) calcd for $C_7H_6BrClN_2O$: 247.9351, observed: 247.9339.

2-(1-(4-(2-Methoxyphenyl)piperazinyl))-*N*-(2-(5-bromo)-pyridinyl)acetamide (13). To 1-(2-methoxyphenyl)piperazine (0.80 g, 4.15 mmol) and 2-chloro-*N*-(2-(5-bromo)-pyridinyl)acetamide (1.04 g, 4.15 mmol) in DMF (20 mL) was added K_2CO_3 (1.43 g, 10.4 mmol, 2.5 equiv). The mixture was stirred at 50 °C for 60 h. The reaction mixture was quenched with water (25 mL), extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude was purified by column chromatography on silica with the mixture hexane/ethyl acetate 7/3 to provide 1.38 g of **13** as a yellow oil (82% yield). IR (neat): 1700, 1500, 1455 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.82 (br s, 4H), 3.72 (br s, 6H), 3.82 (s, 3H), 6.82–6.98 (m, 4H), 7.73 (d, $J=8$ Hz, 1H), 8.10 (br s, 1H), 8.47 (s, 1H), 9.61 (br s, 1H); MS (ES): m/z 405–407 $(M+H)^+$, 205; HRMS (EI) calcd for $C_{18}H_{21}BrN_4O_2$: 404.0847, observed: 404.0860.

1-(2-Methoxyphenyl)-4-(2-(2-(5-bromo)aminopyridinyl)-ethyl)piperazine. To 2-(1-(4-(2-methoxyphenyl)piperazinyl))-*N*-(2-(5-bromo)pyridinyl)acetamide **13** (1.39 g, 3.42 mmol) in dry THF (40 mL) was added, 0.26 g of $LiAlH_4$ (6.84 mmol, 2 equiv). The mixture was stirred at room temperature for 17 h under nitrogen. The reaction mixture was quenched with water and NaOH 10% and extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica (CH_2Cl_2/MeOH 95/5) provided 0.68 g of the desired compound with 51% yield. IR (neat): 3365, 1592, 1500, 1455 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.85 (br s, 6H), 3.17 (br s, 4H), 3.45 (q, $J=5$ Hz, $J=11$ Hz, 2H), 3.80 (s, 3H), 5.62 (br s, 1H), 6.37 (d, $J=9$ Hz, 1H), 6.80–7.00 (m, 4H), 7.40 (dd, $J=3$ Hz, $J=9$ Hz, 1H), 8.03 (d, $J=3$ Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): 36.15, 48.00, 51.65, 53.85, 55.30, 105.60, 108.15, 109.65, 116.90, 119.55, 122.00, 138.10, 138.90, 146.85, 155.40; MS (ES): m/z 391–393 $(M+H)^+$, 199–201; HRMS (EI) calcd for $C_{18}H_{23}BrN_4O$: 390.1055, observed: 390.1049.

***N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-(5-bromo)pyridinyl) cyclohexanecarboxamide (4) 5BPWAY.** To 1-(2-methoxyphenyl)-4-(2-(2-(5-bromo)aminopyridinyl)ethyl)piperazine (0.34 g, 0.87 mmol) in CH_2Cl_2 (25 mL) was added cyclohexanecarbonyl chloride (0.17 mL, 1.30 mmol, 1.5 equiv) and triethylamine (0.18 mL, 1.30 mmol, 1.5 equiv). The mixture was stirred at room temperature under N_2 for 17 h. The reaction mixture was quenched with 10% Na_2CO_3 and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica (CH_2Cl_2/MeOH 95/5) and crystallization as an hydrochloric salt provided 0.37 g of the pure product **4** (85% yield). IR (neat): 1660 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.02–1.18 (m, 3H), 1.25–1.71 (m, 8H), 2.28 (t, $J=9$ Hz, 1H), 2.69 (br s, 5H), 3.03 (br s, 4H), 3.84 (s, 3H), 4.00 (t, $J=7$ Hz, 2H), 6.83–7.04 (m, 4H), 7.30 (s, 1H), 7.84 (dd, $J=8$ Hz, $J=2$ Hz, 1H), 8.54 (d, $J=2$ Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.05, 28.05, 40.95, 43.35, 48.80, 51.80, 53.85, 54.70, 109.65, 116.65, 119.45, 121.50, 121.80, 131.90, 139.05, 148.55, 150.70, 152.90; MS (ES): m/z 501–503 $(M+H)^+$; HRMS (EI) calcd for $C_{28}H_{35}BrN_4O_2$: 500.1786, observed: 500.1788.

In vitro receptor binding assays

Membranes from HeLa cells expressing human 5-HT_{1A} receptors are incubated in 50 mM Tris, pH 7.7 with 1.5 nM [^{3}H]5-CT at 37 °C for 15 min. The incubation is terminated by rapid filtration through unifilter GF/B filters. Filters are counted in a Packard Topcounter. Non-specific binding is determined by including 10 μM Metergoline.

Efficacy assays

HeLa cells transfected with the human 5-HT_{1A} receptor are seeded in 96-well plates. After 4–6 days media is removed and cells are washed once with assay buffer (PBS + $MgCl_2$ + $CaCl_2$ + IBMX). The cells are then incubated for 20 min with assay buffer containing forskolin and test compound. The incubation is terminated by aspiration and cAMP produced during the incubation is extracted and measured in a RIA based system. Antagonism is detected by including 1000 nM 5-HT in the incubation mixture. Compounds which are active on the 5-HT_{1A} receptor are tested in 10 concentrations to determine the IC_{50} value. This value depends on the affinity and concentration of 5-HT present in the assay. To calculate the value that corresponds to Ki or K_D obtained in binding experiments, the IC_{50} value is transformed to Ki by the Cheng-Prusoff equation: $Ki = IC_{50}/(1 + L/K_D)$), where L and K_D refer to concentration and affinity constant of 5-HT.

The intrinsic activity of each compound was measured for a concentration up to 10 μM.

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