

Supporting Information

Successful multiparameter optimization in CNS drug discovery: Design of pyrimido[4,5-d]azepines as potent 5-hydroxytryptamine 2C (5-HT_{2C}) receptor agonists with exquisite functional selectivity over 5-HT_{2A} and 5-HT_{2B} receptors

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1. Abbreviations - The following abbreviations and definitions have been used:

3D	Three-dimensional
7-TMD	Seven-transmembrane domain
Arbocel [®]	Filtration agent
APCI	Atmospheric Pressure Chemical Ionisation
β2AR	ADRB2_HUMAN, β2-adrenergic receptor
Boc	<i>Tert</i> -butyloxycarbonyl
br	broad
CBz	Benzyloxycarbonyl
CDCl ₃	Chloroform-d1
CD ₃ OD	Methanol-d4
Celite [®]	Filtration agent
cHex	Cyclohexane
δ	Chemical shift
d	Doublet
DMA	N,N-Dimethylacetamide
DMSO	Dimethylsulfoxide
ECL	Extracellular loop
ee	Enantiomeric excess
EI	Electron ionisation
ELSD	Evaporative Light Scattering Detector
eq	Equivalent
ent	Enantiomer
ESI	Electrospray ionisation (positive scan)
EtOAc	Ethyl acetate
Et ₂ O	Diethylether
EtOH	Ethanol
GPCR	G-protein coupled receptor

h	Hour(s)
¹ H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
HPLC	High Performance Liquid chromatography
HRMS	High resolution mass spectrum
IPA	Isopropyl alcohol
LRMS	Low resolution mass spectrum
M	Molarity
m	Multiplet
Me	Methyl
MeOH	Methanol
mg	Milligram
min(s)	Minute(s)
MHz	Megahertz
mL	Millilitre
mmol	Millimole
<i>m/z</i>	Mass spectrum peak
N	Normal concentration
NaOMe	Sodium methoxide
nm	Nanometer
NMR	Nuclear Magnetic Resonance
rac	Racemic
R _t	Retention time
s	Singlet
SDM	Site-directed mutagenesis
t	Triplet
Tf	(Trifluoromethyl)sulfonyl
TM	trans-membrane helix
THF	Tetrahydrofuran
UV-TIC	Ultraviolet-total ion count
VCD	Vibrational circular dichroism

2. General Chemistry Experimental Prodecures¹

¹H Nuclear magnetic resonance (NMR) spectra were in all cases consistent with the proposed structures. Characteristic chemical shifts (δ) are given in parts-per-million downfield from tetramethylsilane using conventional abbreviations for designation of major peaks: *e.g.* s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. The mass spectra (*m/z*) were recorded using either electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI). The following abbreviations have been used for common solvents: CDCl₃, deuteriochloroform; D₆-DMSO, deuterodimethylsulphoxide; CD₃OD, deuteromethanol; THF, tetrahydrofuran. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration

of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous magnesium sulfate or anhydrous sodium sulfate.

HPLC, unless indicated otherwise, was performed by one of the following methods:

A: Column: Sunfire C18 4.6 x 50mm; Mobile Phase A: 0.05% formic acid in water; Mobile Phase B: 0.05% formic acid in acetonitrile.

B: Column: Xterra 4.6 x 50mm; Mobile Phase A: 0.05% ammonia in water; Mobile Phase B: 0.05% ammonia in acetonitrile

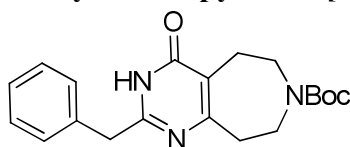
C: Column: Luna C8 4.6 x 50mm; Mobile Phase A: 10mM ammonium acetate in water; Mobile Phase B: 10mM ammonium acetate in acetonitrile

D: Column: C18 4.6 x 50mm; Mobile Phase A: 0.1% formic acid in water; Mobile Phase B: 0.1% formic acid in acetonitrile.

E: Column: XBridge C18 4.6 x 150 mm; Mobile Phase A: 0.1% TFA in water; Mobile Phase B: 0.1% TFA in acetonitrile.

3. Chemistry experimental procedures and analytical data for additional intermediates and test compounds

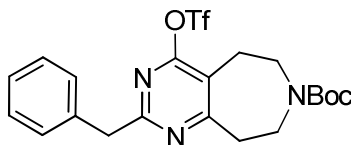
***tert*-Butyl 2-benzyl-4-oxo-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



Commercial 2-phenyl-acetamidine (750 mg, 4.4 mmol) and 1-*tert*-butyl 4-ethyl 5-oxoazepine-1,4-dicarboxylate (See WO2006029154, example 1a, p 34) (1.32 g, 4.6 mmol) were added to a solution of NaOMe (3.5 mL, 3.8M, 13 mmol) in MeOH (100 mL) at room temperature then stirred overnight. The reaction mixture was quenched by addition of water (10 mL). The residue was partitioned between water and EtOAc and the aqueous phase was extracted with EtOAc (3 x 100 mL). The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide the crude product as yellow solid. The residue was purified by flash column chromatography eluting with CH₂Cl₂:EtOAc 90:10 to 75:25 to afford the title compound as a white solid (1.3 g, 83% yield).

¹H NMR (400 MHz, CDCl₃) δ: 1.48 (s, 9H), 2.83-2.92 (m, 2H), 2.92-3.01 (m, 2H), 3.48-3.65 (m, 4H), 3.95 (s, 2H), 7.25-7.39 (m, 5H); HPLC (6 min), R_t = 2.8 min, ELSD >95% purity; LRMS (ESI, APCI) *m/z* 354 [M-H]⁻.

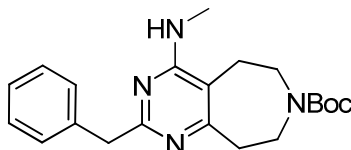
***tert*-Butyl-2-benzyl-4-[[[(trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



Triflic anhydride (4.1 g, 2.4 mL, 11.3 mmol) was added dropwise to a solution of the product of *tert*-butyl 2-benzyl-4-[[[(trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (4.0 g, 11.3 mmol) and pyridine (1.4 g, 1.4 mL, 17.0 mmol) in CH₂Cl₂ (40 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was quenched by addition of water then partitioned between 5% aqueous citric acid and EtOAc. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a yellow gum (5.5 g, 100% yield). This material was used in the subsequent reaction without further purification.

¹H NMR (400 MHz, CDCl₃) δ: 1.45 (s, 9H), 2.85-2.95 (m, 2H), 3.12-3.24 (m, 2H), 3.51-3.72 (m, 4H), 4.19 (m, 2H), 7.13-7.39 (m, 5H); HPLC (6 min), R_t = 3.8 min, ELSD >95% purity; LRMS (ESI, APCI) *m/z* 488 [M+H]⁺.

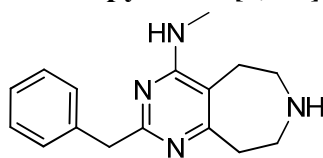
***tert*-Butyl-4-(methylamino)-2-benzyl-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



To a solution of *tert*-butyl 2-benzyl-4-[[[(trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (480 mg, 1.0 mmol) in DMA (15 mL) was added methylamine (2M, 3.0 mL, 5.9 mmol). The reaction mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL), washed with 5% aqueous sodium carbonate (2 x 20 mL) then saturated aqueous ammonium chloride (20 mL). The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield the crude product. The crude material was purified by flash column chromatography eluting with EtOAc:cHex 50:50 to 100:0 to provide the desired product as a white solid (360 mg, 98% yield).

¹H NMR (400 MHz, CD₃OD) δ: 1.39 (s, 9H), 2.62-2.69 (m, 2H), 2.91 (s, 3H), 2.92-2.99 (m, 2H), 3.58-3.66 (m, 4H), 3.95 (s, 2H), 7.11-7.18 (m, 1H), 7.19-7.27 (m, 2H), 7.30-7.35 (m, 2H); HPLC (6 min), R_t = 2.3 min, ELSD >95% purity; LRMS (ESI, APCI) *m/z* 369 [M+H]⁺.

2-Benzyl-N-methyl-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepin-4-amine hydrochloride (8)

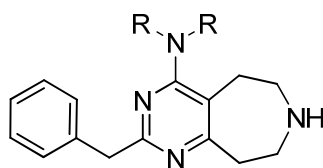


A solution of HCl in dioxane (4M, 4 mL) was added to a stirred solution of *tert*-butyl-4-(methylamino)-2-benzyl-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (724 mg, 1.9 mmol) in dioxane (5 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue dissolved in MeOH (10 mL) and aqueous sodium hydroxide (2N, 10 mL) added. The aqueous was extracted with CH₂Cl₂ (3 x 20 mL), the combined organics were dried over magnesium sulfate, filtered and concentrated. The residue was then purified by flash column chromatography eluting with CH₂Cl₂:MeOH:NH₃ 100:0:0 to 80:17:3 to yield the title product as a pale brown solid. This material was then converted to the HCl salt via addition of MeOH (10 mL) then HCl (2M in Et₂O, 5 mL). The resulting mixture was stirred for 5 mins, then concentrated under reduced pressure to yield the HCl salt of the title compound as a white solid (567 mg, 95% yield).

¹H NMR (400 MHz, CD₃OD) δ : 3.02-3.11 (m, 2H), 3.12 (s, 3H), 3.29-3.38 (m, 2H), 3.41-3.48 (m, 2H), 3.49-3.57 (m, 2H), 4.18 (s, 2H), 7.23-7.31 (m, 1H), 7.31-7.38 (m, 2H), 7.39-7.42 (m, 2H); HPLC (6 min), R_t = 0.73 min, ELSD >95% purity; LRMS (ESI, APCI) *m/z* 269 [M+H]⁺; HRMS (EI) *m/z*: Calcd for C₁₆H₂₁N₄ [M+H]⁺ 269.1761, found 269.1757.

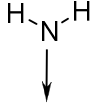
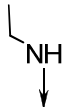

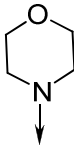
Tabulated 4-amino Examples

Compounds of general formula shown below were prepared using the following method as a parallel synthesis array:

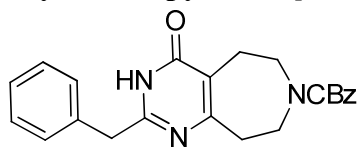


The requisite amine (6 mmol) was added to a solution of *tert*-butyl 2-benzyl-4-[[[(trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (1 mmol) in DMA (15 mL). The reaction mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure and the crude material taken on for deprotection without further purification. This material was taken up in dioxane (2 mL) and HCl in dioxane (4N, 2 eq) added. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure. The resulting solid was triturated with Et₂O to yield the product as an HCl salt.

Compound	NRR	Data	Name
6		¹ H NMR (400 MHz,	2-benzyl-6,7,8,9-tetrahydro-

		DMSO) δ : 2.82-2.95 (m, 2H), 2.99-3.08 (m, 2H), 3.10-3.21 (m, 4H), 3.81 (s, 2H), 4.0 (br s, 2H), 7.06 (t, 1H), 7.18-7.21 (m, 2H), 7.22-7.38 (m, 2H); HPLC (6 min), R_t = 1.12 min, QC >95% purity; LRMS (ESI) m/z 256 $[M+H]^+$. HRMS (EI) m/z : Calcd for $C_{15}H_{19}N_4$ $[M+H]^+$ 255.1604, found 255.1603.	5H-pyrimido[4,5- <i>d</i>]azepin-4-amine hydrochloride
9		HPLC (6 min), R_t = 3.30 min, QC >95% purity; LRMS (ESI) m/z 283 $[M+H]^+$; HRMS (EI) m/z : Calcd for $C_{17}H_{23}N_4$ $[M+H]^+$ 283.1917, found 283.1915.	2-benzyl- <i>N</i> -ethyl-6,7,8,9-tetrahydro-5H-pyrimido[4,5- <i>d</i>]azepin-4-amine hydrochloride
10		HPLC (6 min), R_t = 3.29 min, QC >95% purity; LRMS (ESI) m/z 283 $[M+H]^+$; HRMS (EI) m/z : Calcd for $C_{17}H_{23}N_4$ $[M+H]^+$ 283.1917, found 293.1917.	2-benzyl- <i>N,N</i> -dimethyl-6,7,8,9-tetrahydro-5H-pyrimido[4,5- <i>d</i>]azepin-4-amine hydrochloride
11		HPLC (6 min), R_t = 3.05 min, QC >95% purity; LRMS (ESI) m/z 325 $[M+H]^+$; HRMS (EI) m/z : Calcd for $C_{19}H_{25}N_4O$ $[M+H]^+$ 325.2023, found 325.2026.	2-benzyl-4-morpholin-4-yl-6,7,8,9-tetrahydro-5H-pyrimido[4,5- <i>d</i>]azepine hydrochloride

Benzyl 2-benzyl-4-oxo-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate

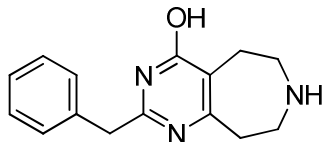


Borontrifluoride diethyletherate (67.0 g, 58.3 mL, 47 mmol) was added dropwise over 10 mins to a stirred slurry of *N*-(benzyloxycarbonyl)-4-piperidone (100 g, 43 mmol) in *tert*-butylmethyl ether (700 mL) at -25 °C, maintaining the internal temperature of the reaction below -20 °C. On

complete addition, the mixture was stirred at -20 °C for 15 mins. A solution of ethyl diazoacetate (63.6 g, 56 mmol) in *tert*-butylmethyl ether (70 mL) was added dropwise over 60 min maintaining the temperature of the reaction below -20 °C. On complete addition the mixture was stirred at -20 °C for 60 min. The reaction mixture was allowed to warm to 0 °C over 45 mins, then an aqueous solution of potassium carbonate (1M, 1070 mL) was added over 30 mins, maintaining the temperature of the reaction between 0 °C and 10 °C. The organic layer was separated and the solvent was removed by distillation under atmospheric pressure. Methanol (400 mL) was added and again the solvent (400 mL) was removed by distillation at atmospheric pressure. This step was repeated to leave a methanolic solution which was then added dropwise to a solution of sodium methoxide (69.5 g, 1.29 mmol) in methanol (550 mL) at 25 °C. The mixture was stirred at room temperature for 15 min then phenylacetamide hydrochloride (80.5 g, 47 mmol) was added. The reaction mixture was stirred for 60 mins. The solution was heated to reflux and 400 mL of solvent was distilled under atmospheric pressure. EtOAc (400 mL) was added to the solution at reflux. The solution was heated back to reflux and 400 mL of solvent was distilled under atmospheric pressure. This was repeated. The reaction mixture was cooled to 60 °C and water (685 mL) was added, and the reaction mixture was stirred for 18 h. The resulting slurry was filtered, and the cake washed with cold EtOAc (3 x 100 mL). The solid was dried at 50 °C overnight to yield the desired product (137 g, 82% yield).

¹H NMR (400 MHz, CDCl₃) δ: 2.81-3.10 (m, 4H), 3.58-3.78 (m, 4H), 3.91 (s, 2H), 5.19 (s, 2H), 7.25-7.41 (m, 10H); HPLC (6 min), R_t = 2.8min, ELSD >95% purity; LRMS (APCI) *m/z* 390 [M+H]⁺.

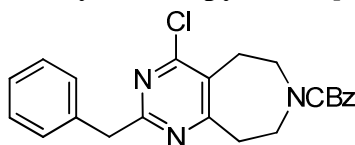
2-Benzyl-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepin-4-ol (5)



A solution of HBr (47%, 0.1 mL) as added to a solution of benzyl 2-benzyl-4-oxo-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (50 mg, 0.13 mmol) in acetic acid (5 mL). The mixture was stirred at room temperature for 15 h. The mixture was concentrated under reduced pressure and the residue triturated with Et₂O. The residue was dried to provide the desired product as a pale brown crystalline solid hydrobromide salt (40 mg, 93% yield).

¹H NMR (400MHz, CD₃OD) δ: 3.02-3.11 (m, 2H), 3.19-3.25 (m, 2H), 3.51-3.58 (m, 2H), 3.52-3.60 (m, 2H), 4.09 (s, 2H), 7.23-7.40 (m, 5H); LRMS (ESI, APCI) *m/z* 256 [M+H]⁺; HRMS (EI) *m/z*: Calcd for C₁₅H₁₈N₃O [M+H]⁺ 256.1444, found 256.1448.

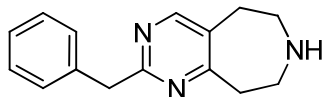
Benzyl 2-benzyl-4-chloro-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate



To a suspension of benzyl 2-benzyl-4-oxo-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (25.0 g, 64 mmol) in acetonitrile (500 ml) was added N,N-diisopropylethylamine (12.3 mL, 70 mmol), followed by careful addition of POCl₃ (47.0 ml, 524 mmol) over 30 mins. The resulting brown solution was heated at 70 °C for 1 h. The reaction mixture was cooled to room temperature, concentrated under reduced pressure to 250 mL. Acetonitrile was added and the mixture concentrated again to ~250mL (repeated twice). The resulting mixture was added dropwise to a stirred solution of HCl (1M, 375 mL) and acetonitrile (125 mL) maintaining the temperature below 5 °C. The resultant slurry was stirred at room temperature for 18 h, then at 5 °C before filtration. The residue was washed with a cold solution of water/acetonitrile (2 x 100 mL) then dried at 50 °C for 12 h to yield the desired product as a white solid (21.2 g, 81% yield).

¹H NMR (400 MHz, CDCl₃) δ: 3.02-3.18 (m, 4H), 3.61-3.72 (m, 4H), 4.13 (s, 2H), 5.15 (s, 2H), 7.18-7.37 (m, 10H); HPLC (6 min), R_t = 3.6 min, ELSD >95% purity; LRMS (ESI, APCI) *m/z* 408 [M+H]⁺.

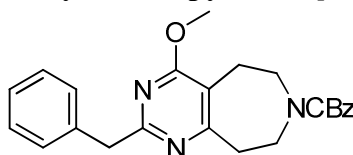
2-Benzyl-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepine and 2-benzyl-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepine, hydrochloride salt and fumarate salt (4)



Triethylamine (13.5 mL, 97.1 mmol) and Pd/C (10% w/w, 2.0 g) were added to a solution of benzyl 2-benzyl-4-chloro-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (19.8 g, 48.5 mmol) in ethanol (200 mL). The reaction mixture was hydrogenated at 50 psi / 50 °C for 15 h. The mixture was filtered through Arbocel and the cake washed with ethanol (2 x 50 mL). The solid was dried at 50 °C for 12 h to afford the title compound in quantitative yield (11.6 g, 100% yield).

¹H NMR (600MHz, CDCl₃) δ: 2.80-2.86 (m, 2H), 2.90-3.05 (m, 4H), 3.08-3.14 (m, 2H), 4.24 (s, 2H), 7.20-7.24 (m, 1H), 7.25-7.35 (m, 2H), 7.35-7.40 (m, 2H), 8.34 (s, 1H); ¹³C NMR (150MHz, CDCl₃) δ: ; HPLC (2 min), R_t = 0.45 min, ELSD 94% purity; HPLC (12 min), R_t = 5.35 min, UV 215nm >95% purity; LRMS (ESI) *m/z* 240 [M+H]⁺; HRMS (EI) *m/z*: Calcd for C₁₅H₁₈N₃ 240.1495 [M+H]⁺, found 240.1495.

2,7-Bibenzyl-4-methoxy-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepine

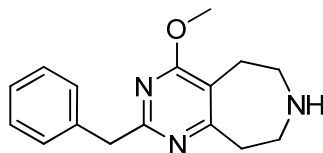


Benzyl 2-benzyl-4-chloro-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (300 mg, 0.74 mmol) was dissolved in methanol (10 mL) and potassium carbonate (112 mg, 0.81 mmol) was added. The reaction was stirred at room temperature under nitrogen for 72 h. The reaction mixture was diluted with EtOAc, filtered through Celite, then concentrated under

reduced pressure to yield the crude product as a solid. The product was purified by flash column chromatography eluting with EtOAc:cyclohexane 80:20 to yield the desired product as a white solid (226 mg, 76% yield).

^1H NMR (400MHz, DMSO d_6) δ : 2.78-2.83 (m, 2H), 2.95-3.02 (m, 2H), 3.49-3.68 (m, 4H), 3.85 (s, 3H), 3.99 (s, 2H), 5.09 (s, 2H), 7.17-7.38 (m, 10H); HPLC (6 min), R_t = 3.4 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 404 $[\text{M}+\text{H}]^+$.

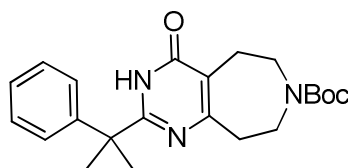
2-Benzyl-4-methoxy-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepine (7)



A solution of 2,7-dibenzyl-4-methoxy-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepine (250 mg, 0.62 mmol) in 5 mL of EtOAc then 1-methyl-1,4-cyclohexadiene (840 mg, 1.0 mL, 8.9 mmol) were added to a suspension of 10% palladium on carbon (66 mg, 0.06 mmol) in 5 mL of EtOAc. The reaction mixture was refluxed under nitrogen for 2 h. The mixture was cooled then filtered through celite and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with CH_2Cl_2 :MeOH: NH_3 (95:5:0.5 to 90:10:1), to afford the title compound as a colourless oil (167 mg, 92% yield).

^1H NMR (400MHz, DMSO- d_6) δ : 2.63-2.79 (m, 6H), 2.88-2.97 (m, 2H), 3.86 (s, 3H), 4.05 (s, 2H), 7.17-7.38 (m, 5H); HPLC (6 min), R_t = 1.82 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 270 $[\text{M}+\text{H}]^+$; HRMS (EI) m/z : Calcd for $\text{C}_{16}\text{H}_{20}\text{N}_3\text{O}$ $[\text{M}+\text{H}]^+$ 270.1601, found 270.1598.

tert-Butyl 2-(1-methyl-1-phenylethyl)-4-oxo-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate



Step 1, amidine formation:

Ammonium chloride (267 mg, 5.0 mmol) was suspended in toluene (4 mL) and cooled to 0 °C with stirring. Trimethylaluminium (2M, 2.5 mL, 5.0 mmol) was added dropwise and the mixture stirred for a further 15 mins at 0 °C before being allowed to warm to room temperature and stirred for a further 3 h. A solution of 1-phenyl-1,1-dimethyl carbonitrile (726 mg, 5.0 mmol) in toluene (2 mL) was added and the reaction mixture heated to 80 °C for 120 h. The reaction mixture was poured onto a slurry of silica (20 g) in CH_2Cl_2 (50 mL) and stirred for 5 mins then filtered washing the silica with methanol (4 x 50 mL). The filtrate was concentrated under reduced pressure and the resulting crude solid triturated with diethyl ether (100 mL), then dried

to provide a white solid (850 mg, 86% yield). This material was used in the subsequent reaction without further purification.

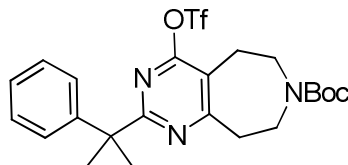
LRMS (ESI, APCI) m/z 163 $[M+H]^+$.

Step 2, pyrimidinone formation:

Ketoester, 1-*tert*-butyl 4-ethyl 5-oxoazepine-1,4-dicarboxylate (See WO2006029154, example 1a, p 34) (400 mg, 1.40 mmol) and amidine (229 mg, 1.15 mmol) were added to a solution of NaOMe (3.8M, 0.91 mL, 3.45 mmol) in MeOH (3.0 mL) at 0 °C and stirred at 0 °C to room temperature for 18 h. The reaction was quenched with water, partitioned with EtOAc and the phases separated. The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide the crude product as an orange gum. The residue was purified by flash column chromatography eluting with CH₂Cl₂:EtOAc 90:10 to 75:25 to afford the title compound as a white solid (280 mg, 64% yield).

¹H NMR (400 MHz, CDCl₃) δ : 1.48 (s, 9H), 1.67 (s, 6H), 2.80-2.86 (m, 2H), 2.98-3.07 (m, 2H), 3.53-3.60 (m, 2H), 3.61-3.66 (m, 2H); HPLC (6 min) R_t = 3.25 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 384 $[M+H]^+$.

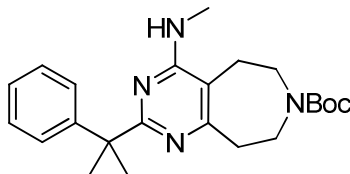
***tert*-Butyl 2-(1-methyl-1-phenylethyl)-4-[[[(trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



Triflic anhydride (191 mg, 0.11 mL, 0.68 mmol) was added dropwise to a solution of *tert*-butyl 2-(1-methyl-1-phenylethyl)-4-oxo-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (200 mg, 0.52 mmol) and pyridine (54 mg, 0.06 mL, 0.68 mmol) in CH₂Cl₂ (1.0 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was quenched by addition of water then partitioned between 5% aqueous citric acid and EtOAc. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield the crude product as a yellow gum (270 mg, 100% yield). This material was used in the subsequent reaction without further purification.

¹H NMR (400 MHz, CDCl₃) δ : 1.45 (s, 9H), 1.79 (s, 6H), 2.90-2.96 (m, 2H), 3.13-3.21 (m, 2H), 3.58-3.65 (m, 4H), 7.15-7.36 (m, 5H); HPLC (6 min), R_t = 4.18 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 516 $[M+H]^+$.

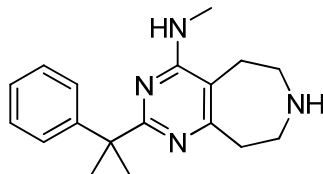
***tert*-Butyl 4-(methylamino)-2-(1-methyl-1-phenylethyl)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



A solution of methylamine (0.2M, 0.24 mL, 0.49 mmol) was added to a solution of *tert*-butyl-2-(1-methyl-1-phenylethyl)-4-[[[(trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (50 mg, 0.10 mmol) in acetonitrile (2.0 mL) at room temperature with stirring. The mixture was stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure to provide the crude compound as a brown gum (40 mg, 100 yield). The material was used in the subsequent reaction without further purification.

¹H NMR (400 MHz, CDCl₃) δ : 1.42 (s, 9H), 1.79 (s, 6H), 2.58-2.65 (m, 2H), 2.95-3.16 (m, 5H), 3.55-3.75 (m, 4H), 7.16-7.21 (m, 5H); HPLC (6 min), R_t = 2.33 min, ELSD >95% purity; LRMS (ESI APCI) m/z 397 [M+H]⁺.

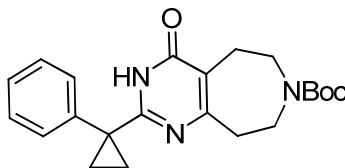
N-Methyl-2-(1-methyl-1-phenylethyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepin-4-amine (14)



A solution of HCl in dioxane (4M, 1.0 mL, 4.0 mmol) was added to a solution of *tert*-butyl 4-(methylamino)-2-(1-methyl-1-phenylethyl)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (45 mg, 0.11 mmol) in CH₂Cl₂ (2.0 mL). The mixture was stirred at room temperature for 18 h. The reaction was quenched by addition of saturated aqueous sodium bicarbonate (50 mL) and extracted into CH₂Cl₂ (3 x 50 mL). The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide the crude product as a brown gum (25 mg, 74% yield). The title compound was purified by reverse phase HPLC.

¹H NMR (400 MHz, CDCl₃) δ : 1.70 (s, 6H), 2.45-2.60 (m, 4H), 2.81-3.00 (m, 7H), 7.00-7.36 (m, 5H); HPLC (6 min), R_t = 1.41 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 295 [M-H]⁻; HRMS (EI) m/z : Calcd for C₁₈H₂₅N₄ [M+H]⁺ 297.2074, found 297.2070.

***tert*-Butyl 4-oxo-2-(1-phenylcyclopropyl)-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



Step 1, amidine formation:

Ammonium chloride (177 mg, 3.3 mmol) was suspended in toluene (3 mL) and cooled to 0 °C with stirring. Trimethylaluminium (2M, 1.7 mL, 3.3 mmol) was added dropwise and the mixture stirred for a further 15 mins at 0 °C before being allowed to warm to room temperature and stirred for a further 3 h. A solution of 1-phenyl-1-cyclopropane carbonitrile (473 mg, 3.3 mmol) in toluene (2 mL) was added and the reaction mixture heated to 80 °C for 44 h. The reaction mixture was poured onto a slurry of silica (10 g) in CH₂Cl₂ (30 mL) and stirred for 5 mins then filtered washing the silica with methanol (3 x 50 mL). The filtrate was concentrated under reduced pressure and the resulting crude solid (459 mg) triturated with diethyl ether (50 mL), then dried to provide a white solid (390 mg, 74% yield). This material was used in the subsequent reaction without further purification.

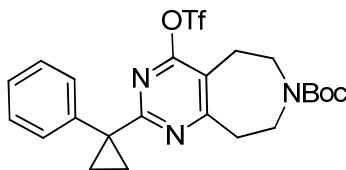
LRMS (ESI, APCI) m/z 161 [M+H]⁺.

Step 2, pyrimidinone formation:

Ketoester, 1-*tert*-butyl 4-ethyl 5-oxoazepine-1,4-dicarboxylate (see WO2006029154, example 1a, p 34) (400 mg, 1.40 mmol) and amidine from step 1 (263 mg, 1.34 mmol) were added to a solution of NaOMe (3.8M, 1.0 mL, 3.8 mmol) in MeOH (3.5 mL) at 0 °C and stirred at 0 °C to room temperature for 18 h. The reaction was quenched with water (50 mL), partitioned with EtOAc (3 x 50 mL) and the phases separated. The combined organics layer were dried over magnesium sulfate, filtered and concentrated *in vacuo* to provide the crude product as an orange gum. The residue was purified by flash column chromatography eluting with CH₂Cl₂:MeOH 98:2 to afford the title compound as a white solid (291 mg, 57% yield).

¹H NMR (400 MHz, CDCl₃) δ : 1.32-1.38 (m, 2H), 1.48 (s, 9H), 1.77-1.81 (m, 2H), 2.78-2.92 (m, 4H), 3.46-3.68 (m, 4H), 7.37-7.45 (m, 5H); HPLC (6 min) R_t = 3.16 min, ELSD >95% purity; LRMS (ESI) m/z 382 [M+H]⁺.

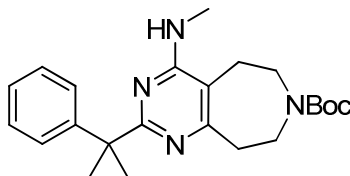
***tert*-Butyl 2-(1-phenylcyclopropyl)-4-[[[(trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



Triflic anhydride (192 mg, 0.15 mL, 0.68 mmol) was added dropwise to a solution of *tert*-butyl 4-oxo-2-(1-phenylcyclopropyl)-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (200 mg, 0.52 mmol) and pyridine (54 mg, 0.68 mmol) in CH₂Cl₂ (1.0 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was quenched by addition of water then partitioned between 5% aqueous citric acid and EtOAc. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated under vacuum to yield the crude product as a yellow gum (270 mg, 100% yield). This material was taken on to the subsequent reaction without further purification.

^1H NMR (400 MHz, CDCl_3) δ : 1.38-1.43 (m, 2H), 1.45 (s, 9H), 1.71 (m, 2H), 2.80-2.96 (m, 2H), 3.03-3.11 (m, 2H), 3.45-3.65 (m, 4H), 7.22-7.40 (m, 5H); HPLC (6 min), R_t = 2.00 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 514 $[\text{M}+\text{H}]^+$.

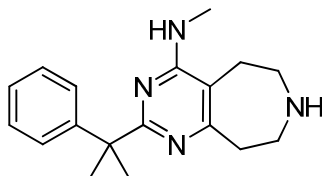
***tert*-Butyl 4-(methylamino)-2-(1-phenylcyclopropyl)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



A solution of methylamine (2.0M, 0.27 mL, 0.54 mmol) was added to a solution of *tert*-butyl 2-(1-phenylcyclopropyl)-4-[(trifluoromethyl)sulfonyl]oxy-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (55 mg, 0.11 mmol) in acetonitrile (2.0 mL) at room temperature with stirring. The mixture was stirred at room temperature for 20 h. The solvent was evaporated *in vacuo* to provide the crude title compound as a brown gum (40 mg, 98% yield). The material was used in the subsequent reaction without further purification.

^1H NMR (400 MHz, CDCl_3) δ : 1.21-1.80 (m, 4H), 1.42 (s, 9H), 2.55 (s, 2H); 2.78-2.98 (m, 5H), 3.47-3.72 (m, 4H), 7.12-7.45 (m, 5H); HPLC (6 min), R_t = 2.10 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 395 $[\text{M}+\text{H}]^+$.

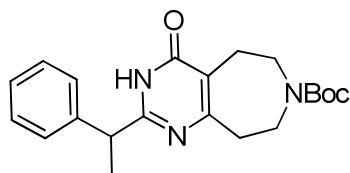
N-Methyl-2-(1-phenylcyclopropyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepin-4-amine (15)



A solution of HCl in dioxane (4M, 1.0 mL, 4.0 mmol) was added to a solution of *tert*-butyl 4-(methylamino)-2-(1-phenylcyclopropyl)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (45 mg, 0.11 mmol) in CH_2Cl_2 (2.0 mL). The mixture was stirred at room temperature for 6 h. The reaction was quenched by addition of saturated aqueous sodium bicarbonate (50 mL) and extracted into CH_2Cl_2 (3 x 50 mL). The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide the crude product as a brown gum (30 mg, 90% yield). The title compound was purified by reverse phase HPLC.

^1H NMR (400 MHz, CDCl_3) δ : 1.21-1.60 (m, 2H), 1.65-2.10 (m, 2H), 2.88-3.08 (m, 2H), 3.10-3.42 (m, 4H), 3.43-3.95 (m, 5H), 7.10-7.58 (m, 5H); HPLC (6 min), R_t = 1.51 min; ELSD >95%; LRMS (ESI, APCI) m/z 295 $[\text{M}+\text{H}]^+$; HRMS (EI) m/z : Calcd for $\text{C}_{18}\text{H}_{23}\text{N}_4$ $[\text{M}+\text{H}]^+$ 295.1917, found 295.1916.

***tert*-Butyl 4-oxo-2-(1-phenylethyl)-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



Step 1, amidine formation:

Ammonium chloride (535 mg, 10.0 mmol) was suspended in toluene (8 mL) and cooled to 0 °C with stirring. Trimethylaluminium (2M, 5.0 mL, 10 mmol) was added dropwise and the mixture stirred for a further 15 mins at 0 °C before being allowed to warm to room temperature and stirred for a further 2 h. A solution of 1-phenyl-1-(*rac*)-methyl carbonitrile (1.3 g, 10.0 mmol) in toluene (5 mL) was added and the reaction mixture heated to 80 °C for 48 h. The reaction mixture was poured onto a slurry of silica (20 g) in CH₂Cl₂ (50 mL) and stirred for 5 mins then filtered washing the silica with methanol (4 x 50 mL). The filtrate was concentrated under reduced pressure and the resulting crude solid triturated with diethyl ether (100 mL), then dried to provide a pale brown solid (1.4 g, 92% yield). This material was used in the subsequent reaction without further purification.

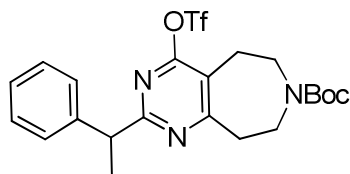
LRMS (ESI, APCI) *m/z* 149 [M+H]⁺.

Step 2, pyrimidinone formation:

Ketoester, 1-*tert*-butyl 4-ethyl 5-oxoazepine-1,4-dicarboxylate (see WO2006029154, example 1a, p 34) (695 mg, 2.44 mmol) and amidine from step 1 (428 mg, 2.32 mmol) were added to a solution of NaOMe (3.8M, 1.8 mL, 7.0 mmol) in MeOH (6.0 mL) at 0 °C and stirred at 0 °C to room temperature for 15 h. The reaction was quenched with water, partitioned with EtOAc and the phases separated. The combined organics layer were dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide the crude product as an orange gum. The residue was purified by flash column chromatography eluting with CH₂Cl₂:EtOAc 90:10 to 75:25 to afford the title compound as a white solid (780 mg, 91% yield).

¹H NMR (400 MHz, CDCl₃) δ : 1.46 (s, 9H), 1.65 (d, *J* = 7.2 Hz, 3H), 2.80-3.05 (m, 4H), 3.45-3.75 (m, 4H), 4.05 (q, 1H), 7.21-7.40 (m, 5H); HPLC (6 min), *R*_t = 3.11 min, ELSD >95% purity; LRMS (ESI) *m/z* 370 [M+H]⁺.

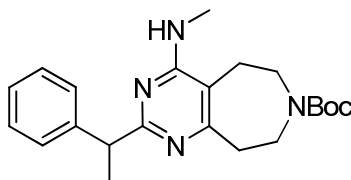
***tert*-Butyl 2-(1-phenylethyl)-4-[[[(trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



Triflic anhydride (300 mg, 0.18 mL, 1.10 mmol) was added dropwise to a solution of *tert*-butyl 4-oxo-2-(1-phenylethyl)-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (300 mg, 0.81 mmol) and pyridine (84 mg, 0.09 mL, 1.10 mmol) in CH₂Cl₂ (1.5 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was quenched by addition of water then partitioned between 5% aqueous citric acid and EtOAc. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield the crude product as a yellow gum (410 mg, 100% yield). This material was taken on to the subsequent reaction without further purification.

¹H NMR (400 MHz, CDCl₃) δ : 1.44 (s, 9H), 1.68-1.78 (m, 3H), 2.84-2.99 (m, 2H), 3.11-3.20 (m, 2H), 3.55-3.68 (m, 4H), 4.33 (q, *J* = 7.3 Hz, 1H), 7.19-7.22 (m, 1H), 7.23-7.32 (m, 2H), 7.35-7.41 (m, 2H); HPLC (6 min), *R*_t = 4.04 min, ELSD >95% purity; LRMS (ESI) *m/z* 446 [M+H-*t*Bu]⁺.

***tert*-Butyl 4-(methylamino)-2-(1-phenylethyl)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate**



A solution of methylamine (2.0M, 0.65 mL, 1.30 mmol) was added to a solution of *tert*-butyl 2-(1-phenylethyl)-4-[[[(trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (130 mg, 0.26 mmol) in acetonitrile (4.0 mL) at room temperature. The mixture was stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure to provide the crude title compound as a yellow gum. The crude material was purified by flash column chromatography eluting with CH₂Cl₂:EtOAc 100:0 to 50:50 to yield the desired product as a white crystalline solid (89 mg, 90% yield).

The racemic product was separated into individual enantiomers by preparative chiral column chromatography:

Column: Chiralcel OJ-H, *analytical* 250 x 4.6 mm id, *preparative* 250 x 21.2 mm id

Mobile phase: heptane:EtOH:diethylamine 95:5:0.1

Flow rate: *analytical* 1 mL/min, *preparative* 20 mL/min

Detection: 225 nm

Sample dissolution: *preparative* 89mg in 58 mL EtOH

Max injection volume: *preparative* 1 mL

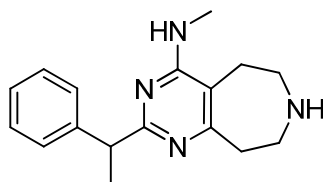
Enantiomer 1 *R*_t=13.18 min, Enantiomer 2 *R*_t=23.20 min

This method provided good separation. The first peak off the column (*R*_t=13.18 min) was concentrated to yield 30 mg, >99.5% ee of white solid (led to more potent enantiomer after

deprotection). The second peak off the column ($R_t=23.20$ min) was concentrated to yield 29 mg, >99.5% ee of white solid (led to less potent enantiomer after deprotection).

^1H NMR (400 MHz, CDCl_3) δ : 1.22 (s, 9H), 1.65 (d, $J = 7.1$ Hz, 3H), 2.52-2.54 (m, 2H), 2.95-3.05 (m, 2H), 3.03 (br s, 3H), 3.52-3.71 (m, 4H), 4.14 (q, $J = 7.1$ Hz, 1H), 4.59 (s, 1H), 7.14-7.19 (m, 1H), 7.22-7.30 (m, 2H), 7.48 (d, 2H); HPLC (6 min), $R_t = 2.29$ min, ELSD >95% purity; LRMS (ESI, APCI) m/z 383 $[\text{M}+\text{H}]^+$.

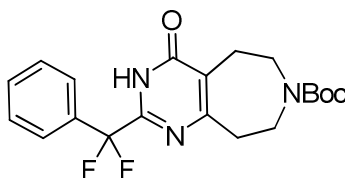
N-Methyl-2-(1-phenylethyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepin-4-amine (12/13)
Method applied to racemic and +/- enantiomers of starting material as required



A solution of HCl in dioxane (4M, 1.0 mL, 4.0 mmol) was added to a solution of *tert*-butyl 4-(methylamino)-2-(1-phenylethyl)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (50 mg, 0.13 mmol) in CH_2Cl_2 (2.0 mL). The mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of saturated aqueous sodium bicarbonate (50 mL) and extracted into CH_2Cl_2 (3 x 50 mL). The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide the crude product as a brown gum (30 mg, 99% yield). The title compound was purified by reverse phase HPLC.

^1H NMR (400 MHz, CDCl_3) δ : 1.65 (d, $J = 7.1$ Hz, 3H), 2.51-2.79 (m, 4H), 2.86-3.06 (m, 7H), 4.09-4.19 (m, 1H), 4.68 (br s, 1H), 4.79 (br s, 1H), 7.11-7.19 (m, 1H), 7.21-7.33 (m, 2H), 7.38-7.41 (m, 1H), 7.45-7.52 (m, 1H); HPLC (6 min), $R_t = 0.57$ min, ELSD >95% purity; LRMS (ESI, APCI) m/z 283 $[\text{M}+\text{H}]^+$.

***tert*-Butyl 2-[difluoro(phenyl)methyl]-4-oxo-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (22b)**



Step 1, amidine formation:

Ammonium chloride (1.6 g, 29 mmol) was added to a solution of 2,2-difluorophenylacetonitrile (4.4 g, 29 mmol) in toluene (25 mL). The resulting suspension was cooled to 0 °C under nitrogen. Trimethylaluminium (2M in toluene, 14.5 mL, 29 mmol) was added dropwise with stirring at 0 °C over 10 mins. The reaction mixture was warmed to room temperature over 15 mins, then heated to 85 °C with stirring for 17 h. The mixture was then cooled to room temperature then poured into a slurry of silica gel (140 g) in CH_2Cl_2 (150 mL).

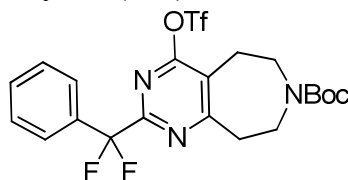
The slurry was stirred for 5 mins then filtered washing thoroughly with methanol. The filtrate was concentrated under reduced pressure to provide 2,2-difluorophenylamidine (**20b**) as a dark brown residue (4.2 g) which was used in the subsequent reaction without further purification.

Step 2, pyrimidinone formation:

A freshly prepared solution of NaOMe (sodium 2.2g, 96 mmol in methanol 25 mL) was added to a solution of 1-*tert*-butyl 4-ethyl 5-oxoazepine-1,4-dicarboxylate (**21b**) (see WO2006029154, example 1a, p34) (8.7 g, 30.7 mmol) and 2,2-difluorophenylamidine (**20b**, 4.2 g, 29 mmol) in MeOH (135 mL) at 0 °C. The mixture was allowed to warm to room temperature with stirring for 39 h. The reaction was quenched with water (10 mL), acidified with HCl (2N) and extracted with EtOAc (3 x 50 mL). The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide the crude product as an orange gum (11 g). The residue was purified by flash column chromatography eluting with CH₂Cl₂:EtOAc 100:0 to 70:30 to afford the title compound (**22b**) as a pale yellow solid (2.2 g, 20% yield over 2 steps).

¹H NMR (400 MHz, CDCl₃) δ : 1.42 (s, 9H), 2.83-2.92 (m, 2H), 2.92-2.99 (m, 2H), 3.48-3.61 (m, 4H), 7.22-7.51 (m, 3H), 7.59-7.63 (m, 2H); HPLC (6 min), R_t = 3.06 min, ELSD >95% purity; LRMS (ESI) m/z 390 [M-H]⁻, m/z 335 [M+H-*t*Bu]⁺.

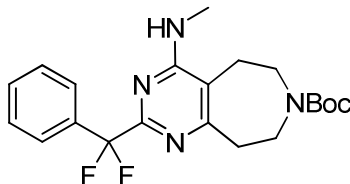
***tert*-Butyl 2-[difluoro(phenyl)methyl]-4-[(trifluoromethyl)sulfonyl]oxy}-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (**23b**)**



Triflic anhydride (1.3 mL, 7.5 mmol) was added dropwise to a solution of the *tert*-butyl 2-[difluoro(phenyl)methyl]-4-oxo-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (**22b**, 2.2 g, 5.6 mmol) and pyridine (0.8 mL, 9.9 mmol) in CH₂Cl₂ (25 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred for 2 h. The reaction mixture was quenched by addition of water then partitioned between 5% aqueous citric acid and EtOAc. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield the crude product (**23b**) as a yellow gum. This material was used in the subsequent reaction without further purification.

HPLC (6 min) R_t = 3.86 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 468 [M+H-*t*Bu]⁺.

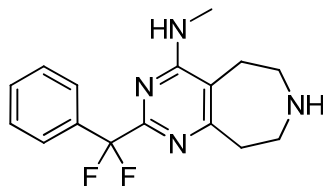
***tert*-Butyl 2-[difluoro(phenyl)methyl]-4-(methylamino)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (**24b**)**



A solution of methylamine (2.0M, 22 mL, 44 mmol) was added to a solution of *tert*-butyl 2-[difluoro(phenyl)methyl]-4-[[trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (**23b**, 3.1g, 5.6 mmol) in acetonitrile (120 mL) at room temperature with stirring. The mixture was stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure to provide the crude title compound as an orange gum. Purification of the crude material by flash column chromatography eluting with pentane:EtOAc 4:1 to 2:1 afforded the product as a white solid (**24b**, 1.4 g, 60% yield).

¹H NMR (300 MHz, CDCl₃): 1.43 (9H, br s), 2.56-2.65 (m, 2H), 3.00 (d, *J* = 5.9 Hz, 3H) 3.06-3.10 (m, 2H), 3.50-3.80 (m, 4H), 4.78 (br s, 1H), 7.35-7.45 (m, 3H), 7.70-7.80 (m, 2H); HPLC (6 min) *R*_t = 3.19 min, ELSD >95%; LRMS (ESI, APCI) *m/z* 405 [M+H]⁺.

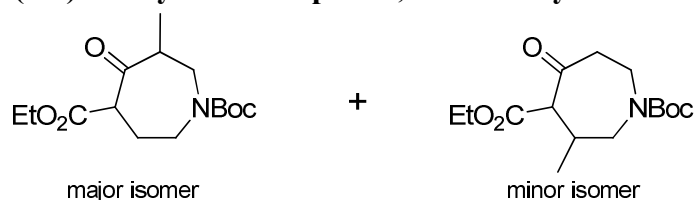
2-[Difluoro(phenyl)methyl]-N-methyl-6,7,8,9-tetrahydro-5H-pyrimido[4,5-*d*]azepin-4-amine (18**)**



A solution of HCl in EtOAc (2.0N, 25 mL, 50 mmol) was added to a solution of *tert*-butyl 2-[difluoro(phenyl)methyl]-4-(methylamino)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (**24b**, 1.4 g, 3.4 mmol) in CH₂Cl₂ (100 mL). The mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of saturated aqueous sodium bicarbonate and extracted into CH₂Cl₂. The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide the crude product as a brown gum. Purification of the crude material by flash column chromatography eluting with CH₂Cl₂:MeOH:NH₃ 30:1:0.2 to 10:1:0.2 afforded the product as a pale yellow solid (**18**, 750 mg, 2.5 mmol, 75% yield).

¹H NMR (300 MHz, CDCl₃) δ : 2.23 (br s, 1H), 2.50-2.68 (m, 2H), 2.88-3.12 (m, 9H), 4.78 (br s, 1H), 7.32-7.47 (m, 3H), 7.68-7.79 (m, 2H); HPLC (6 min) *R*_t = 1.96 min, ELSD 95% purity; HPLC (25 min) *R*_t = 9.47 min, UV >95% purity; LRMS (ESI, APCI) *m/z* 305 [M+H]⁺; HRMS (EI) *m/z*: [M+H]⁺ Calcd for C₁₆H₁₉F₂N₄ 305.1572, found 305.1568.

1-*tert*-Butyl-4-ethyl-6-(*rac*)-methyl-5-oxoazepine-1,4-dicarboxylate 21a



Solutions of borontrifluoride diethyletherate (3.9 mL, 31 mmol) in 5 mL of Et₂O and ethyl diazoacetate (4.17 g, 37) in 5 mL of Et₂O were added drop-wise to a solution of the 4-piperidinone (6.0 g, 30 mmol) in diethylether (20 mL) at -50 °C, maintaining an internal temp below -30 °C. The reaction mixture was stirred at -50 °C for 1 h, then warmed to -20 °C for 1 h. Acetone (10 mL) was added to quench any remaining ethyl diazoacetate and the resulting mixture was stirred for 10 min. Water and solid potassium carbonate were added to bring pH to 8.5 then the solution was diluted with EtOAc (300 mL). The aqueous layer was removed and the organics washed with saturated aqueous sodium bicarbonate (2 x 100 mL), brine (100 mL), then dried over MgSO₄, filtered and concentrated under reduced pressure to provide the crude product as a solid (8.0 g, 100% yield). Analysis (NMR) showed this to be a mixture (~10:1) of regioisomers in favour of the desired product. This material was taken on for pyrimidone formation without further purification.

Major Isomer: ¹H NMR (400 MHz, CDCl₃) δ: 1.14 (d, *J* = 7.2 Hz, 3H), 1.20-1.35 (m, 5H), 1.42 (s, 9H), 1.98-2.08 (m, 2H), 2.70-3.06 (m, 2H), 3.71-3.85 (m, 2H), 4.10-4.29 (m, 2H); HPLC (6 min) R_t = 3.03 min, ELSD >95% purity as a mixture (~10:1) of two isomers; LRMS (ESI) *m/z* 322 [M+Na]⁺, *m/z* 298 [M-H]⁻.

tert-Butyl-2-benzyl-9-(*rac*)-methyl-4-oxo-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate 22a



A solution of NaOMe (3.8M, 7.9 mL, 30 mmol) was added to a solution of keto ester, 1-*tert*-butyl-4-ethyl-6-methyl-5-oxoazepine-1,4-dicarboxylate ~10:1 of regioisomers (3.0 g, 10 mmol) in MeOH (40 mL) at room temperature followed by addition of the 2-phenyl-acetamidine (2.1 g, 12 mmol). The reaction mixture was stirred at room temperature for 16 h, then quenched by addition of water (3 mL) and evaporated to dryness. The residue was partitioned between water (20 mL) and EtOAc (3 x 20 mL) then separated. The aqueous layer was washed with EtOAc (3 x 10 mL). The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield the crude product as a solid. The material was purified by flash column chromatography eluting with CH₂Cl₂:EtOAc 100:0 to 70:30 to yield the product as an

inseparable mixture of regioisomers (~10:1) as a yellow foam (2.74 g, 74% yield). This mixture of isomers was taken on to the subsequent reaction without further separation.

Major isomer: ^1H NMR (400 MHz, CDCl_3) δ : 1.26 (d, $J = 7.3$ Hz, 3H), 1.43 (s, 9H), 2.68-2.91 (m, 1H), 3.01-3.20 (m, 2H), 3.31-3.43 (m, 1H), 3.45-3.81 (m, 3H), 3.93 (s, 2H), 7.22-7.39 (m, 5H); HPLC R_t =2.94 min, ELSD >95 purity as a mixture (~10:1) of two isomers; LRMS (APCI) m/z 370 $[\text{M}+\text{H}]^+$.

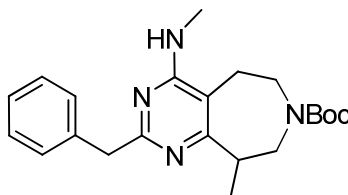
***tert*-Butyl 2-benzyl-9-(*rac*)-methyl-4-[[trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate 23a**



Triflic anhydride (1.54 g, 0.89 mL, 5.5 mmol) was added to a solution of the *tert*-butyl-2-benzyl-9-methyl-4-oxo-3,4,5,6,8,9-hexahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (1.83 g, 5.0 mmol) in CH_2Cl_2 at 0 °C under N_2 . The resulting solution was stirred for 30 mins at 0 °C then partitioned between 5% aqueous citric acid and EtOAc. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield an orange gum (2.5 g, 100% yield). This material was taken on to the next reaction without further purification.

^1H NMR (400 MHz, CDCl_3) δ : 1.30 (d, $J = 7.3$ Hz, 3H), 1.47 (s, 9H), 2.78-3.04 (m, 2H), 3.26-3.41 (m, 1H), 3.43-3.76 (m, 4H), 4.19 (s, 2H), 7.12-7.47 (m, 5H); HPLC (6 min) R_t 4.09 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 446 $[\text{M}+\text{H}-t\text{Bu}]^+$.

***tert*-Butyl-2-benzyl-9-(*rac*)-methyl-4-(methylamino)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate 24a**



A solution of methylamine (2M, 12.4 mL, 25 mmol) in THF was added dropwise to a stirred solution of triflate *tert*-butyl 2-benzyl-9-methyl-4-[[trifluoromethyl)sulfonyl]oxy]-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (2.48 g, 4.95 mmol) in acetonitrile (150 mL) at room temperature. The resulting mixture stirred at room temperature for 75 h. The mixture was concentrated under reduced pressure, then CH_2Cl_2 (50 mL) and a saturated aqueous sodium bicarbonate (50 mL) were added. The mixture was separated and the aqueous layer washed with CH_2Cl_2 (3 x 50 mL). The combined organics were dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield the crude product as an orange solid.

Purification by flash column chromatography eluting with pentane:EtOAc 100:0 to 70:30 the desired product as a yellow gum that crystallised on standing (1.64 g, 87% yield). *NB The minor methyl regioisomer which had been carried through previous steps was separated from desired product via this column chromatography.*

The desired racemic product was separated into individual enantiomers by preparative chiral column chromatography:

Column: Chiralpak AD-H, analytical 250x4.6mm id, preparative 250x21.2 mm id

Mobile phase: heptane:IPA 90:10

Flow rate: analytical 1 mL/min, preparative 15 mL/min

Detection: 225 nm and 254 nm

Sample dissolution: preparative 5 mL EtOH and 6 mL heptane

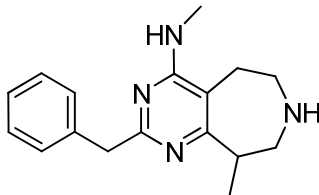
Max injection volume: preparative 1 mL

Enantiomer 1 R_t =6.10 min, Enantiomer 2 R_t =7.88 min

This method provided good separation, loaded as 150 mg batches. The combined first peak off the column (R_t =6.10 min) was concentrated to yield 480 mg, 99.7% ee of colourless oil (led to less potent enantiomer after deprotection). The second peak off the column (R_t =7.88 min) was concentrated to yield 495 mg, 99.7% ee of colourless oil (led to more potent enantiomer after deprotection).

^1H NMR (400 MHz, CDCl_3) δ : 1.23 (d, J = 7.2 Hz, 3H), 1.42 (s, 9H), 2.40-2.51 (m, 1H), 2.97 (d, J = 6.0 Hz, 3H), 3.12-3.21 (m, 1H), 3.21-3.39 (m, 1H), 3.40-3.51 (m, 1H), 3.58-3.90 (m, 2H), 4.01 (s, 2H), 4.50-4.67 (m, 1H), 7.13-7.20 (m, 1H), 7.21-7.30 (m, 2H), 7.38-7.44 (m, 2H); HPLC (6 min), R_t =1.96 min, ELSD >95% purity; LRMS (ESI, APCI) m/z 383 $[\text{M}+\text{H}]^+$.

2-Benzyl-9-methyl-4-(methyamino)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine (16 (*R*)/17 (*S*)) - Method applied to racemic and +/- enantiomers of starting material as required



A solution of HCl in dioxane (4M, 4 mL) was added to a stirred solution of *tert*-butyl-2-benzyl-9-methyl-4-(methyamino)-5,6,8,9-tetrahydro-7H-pyrimido[4,5-*d*]azepine-7-carboxylate (495 mg, 1.3 mmol) in CH_2Cl_2 (10 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure to yield the crude product as a solid HCl salt (410 mg, 95% yield).

A small amount of impurity (~5%) was evident by NMR so the material was purified by preparative HPLC chromatography:

Column: Chiralcel OD-H, *analytical* 250x4.6 mm id, *preparative* 250x21.2 mm id

Mobile phase: heptane:IPA:diethylamine 85:15:0.1

Flow rate: *analytical* 1 mL/min, *preparative* 15 mL/min

Detection: 225 nm

Sample dissolution: *preparative* 3 mL IPA and 7 mL heptane

Max injection volume: *preparative* 0.25 mL

More potent enantiomer $R_t=9.94$ min, Less potent enantiomer $R_t=11.81$ min, Impurity $R_t=8.24$ min

This method provided good separation to yield the desired product 177 mg, 99.2% ee of colourless oil free base. Enantiomeric purity check run on Chiralcel OD-H column eluting with heptane:IPA:diethylamine 85:15:0.1 afforded two enantiomers (Peak 1 more potent enantiomer, $R_t=9.94$ min) and (Peak 2 less potent enantiomer, $R_t=11.81$ min) – method developed using racemic material.

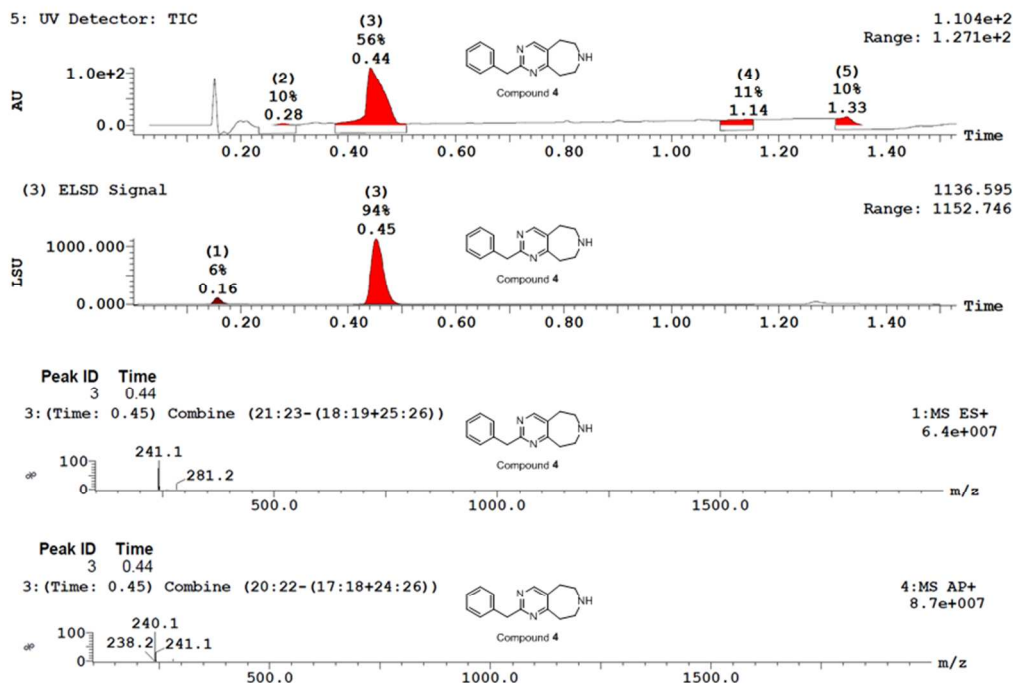
The tartrate salt of the more potent enantiomer was formed by dissolving free base (177 mg, 0.63 mmol) in methanol (5 mL) followed by addition of tartaric acid (94 mg, 0.63 mmol). The mixture was stirred until all material dissolved. The solvent was removed under reduced pressure to yield the desired tartrate salt as a yellow solid (280 mg, 100% yield).

Free base 16 (R): ^1H NMR (600 MHz, CDCl_3) δ : 1.31 (d, $J = 7.2$ Hz, 3H), 2.47-2.57 (m, 1H), 2.55-2.63 (m, 1H), 2.30-2.40 (m, 2H), 2.95 (s, 3H), 2.99-3.06 (m, 2H), 3.07-3.18 (m, 1H), 4.05 (s, 2H), 4.07 (br s, 1H), 7.16-7.21 (m, 1H), 7.26-7.36 (m, 2H), 7.45-7.51 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ : 16.2, 27.3, 28.6, 44.7, 45.9, 47.6, 52.3, 111.9, 126.1, 128.1, 129.5, 139.5, 161.0, 165.6, 169.1; HPLC (2 min), $R_t=0.42$ min ELSD >95% purity; HPLC (12 min), $R_t=5.38$ min >95% purity; LRMS (ESI, APCI) m/z 283 $[\text{M}+\text{H}]^+$; HRMS (EI) m/z : Calcd for $\text{C}_{17}\text{H}_{23}\text{N}_4$ $[\text{M}+\text{H}]^+$ 283.1917, found 283.1916; $[\alpha]_{\text{D}}^{20} = +5.14^\circ$.

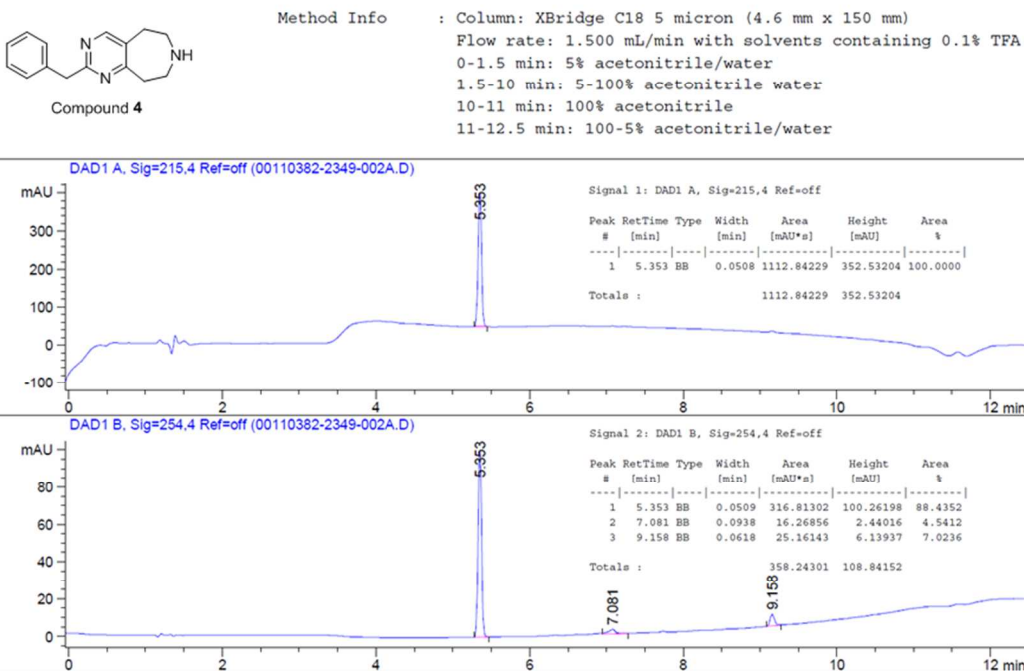
Free base 17 (S): ^1H NMR (600 MHz, CDCl_3) δ : 1.32 (d, $J = 7.1$ Hz, 3H), 2.48-2.55 (m, 1H), 2.56-2.65 (m, 1H), 2.30-2.39 (m, 2H), 2.97 (s, 3H), 2.98-3.06 (m, 2H), 3.07-3.15 (m, 1H), 4.05 (s, 2H), 4.06 (br s, 1H), 7.16-7.22 (m, 1H), 7.27-7.34 (m, 2H), 7.45-7.49 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ : 16.2, 27.3, 28.6, 44.7, 45.9, 47.6, 52.4, 111.9, 126.0, 128.1, 129.4, 139.5, 161.0, 165.6, 169.1; HPLC (2 min), $R_t=0.42$ min ELSD >95% purity; HPLC (12 min), $R_t=5.40$ min >95% purity; LRMS (ESI, APCI) m/z 283 $[\text{M}+\text{H}]^+$; HRMS (EI) m/z : Calcd for $\text{C}_{17}\text{H}_{23}\text{N}_4$ $[\text{M}+\text{H}]^+$ 283.1917, found 283.1918; $[\alpha]_{\text{D}}^{20} = -6.07^\circ$.

4. Selected HPLC, MS and NMR spectra

Compound 4 LCMS (2 min run):

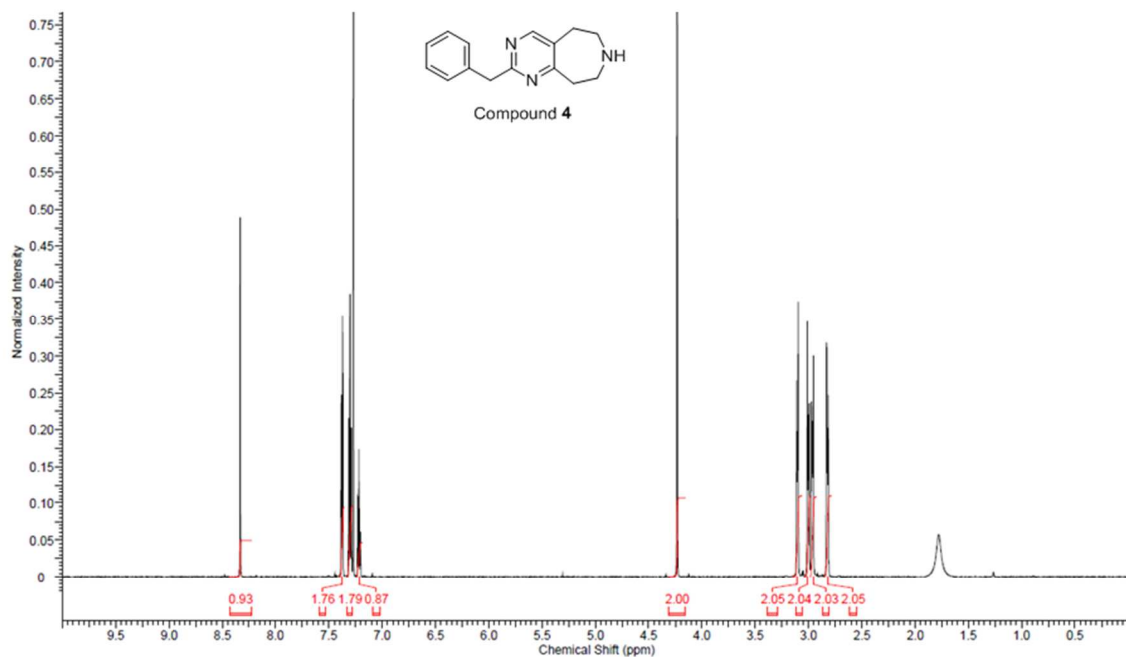


Compound 4 HPLC (12 min run):



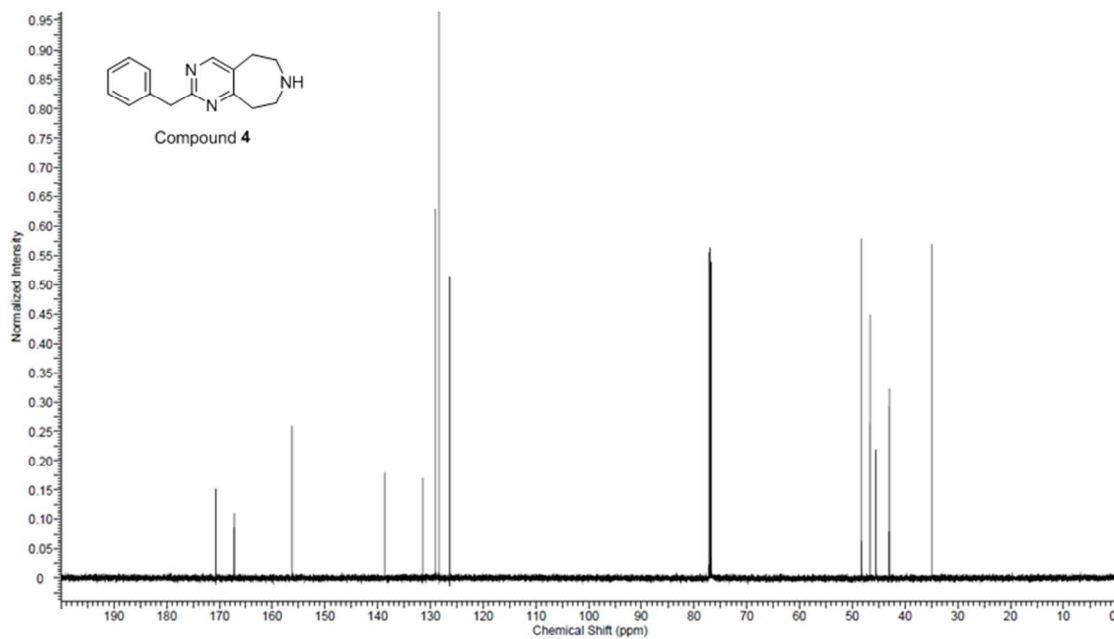
Compound 4 NMR ^1H (CDCl_3):

Frequency (MHz)	599.82	Nucleus	^1H	Number of Transients	64
Original Points Count	16384	Points Count	16384	Pulse Sequence	s2pul
Receiver Gain	30.00	Solvent	CHLOROFORM-d	Spectrum Offset (Hz)	3605.5984
Spectrum Type	STANDARD	Sweep Width (Hz)	9615.38	Temperature (degree C)	25.000

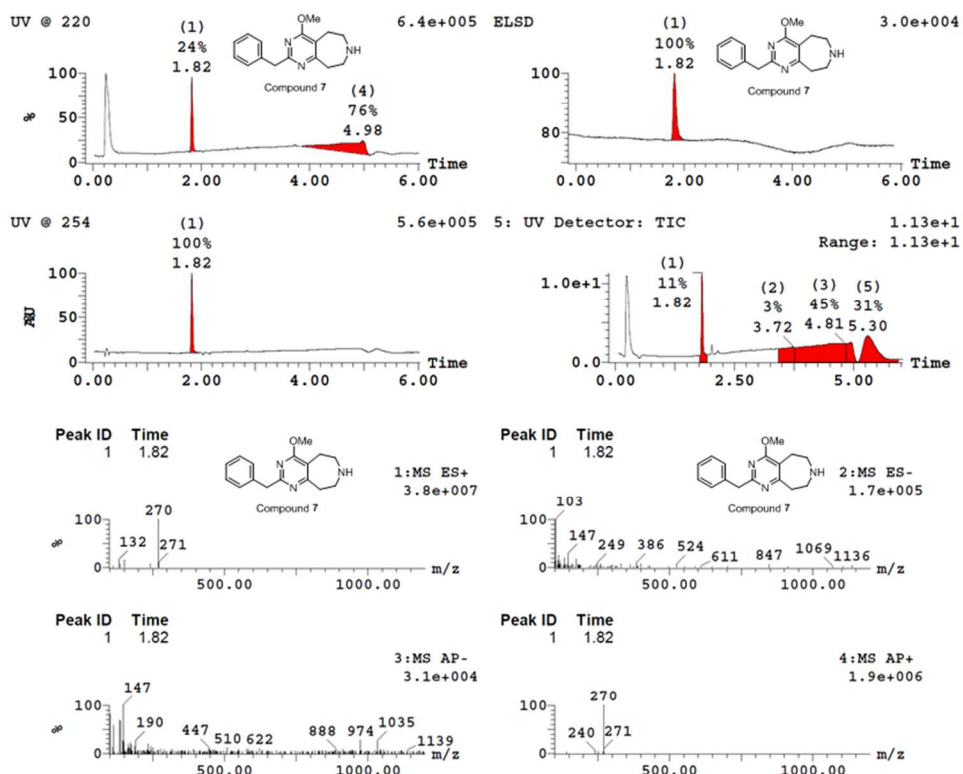


Compound 4 NMR ^{13}C (CDCl_3):

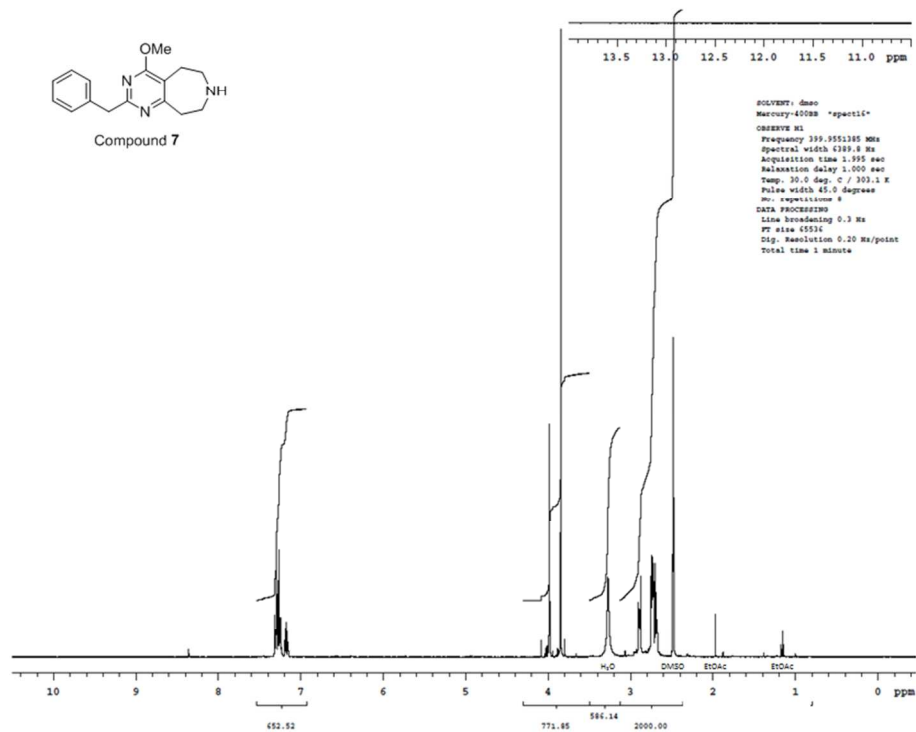
Frequency (MHz)	150.84	Nucleus	^{13}C	Number of Transients	2048
Original Points Count	32768	Points Count	32768	Pulse Sequence	s2pul
Receiver Gain	40.00	Solvent	CHLOROFORM-d	Spectrum Offset (Hz)	15080.4844
Spectrum Type	STANDARD	Sweep Width (Hz)	32894.74	Temperature (degree C)	25.000



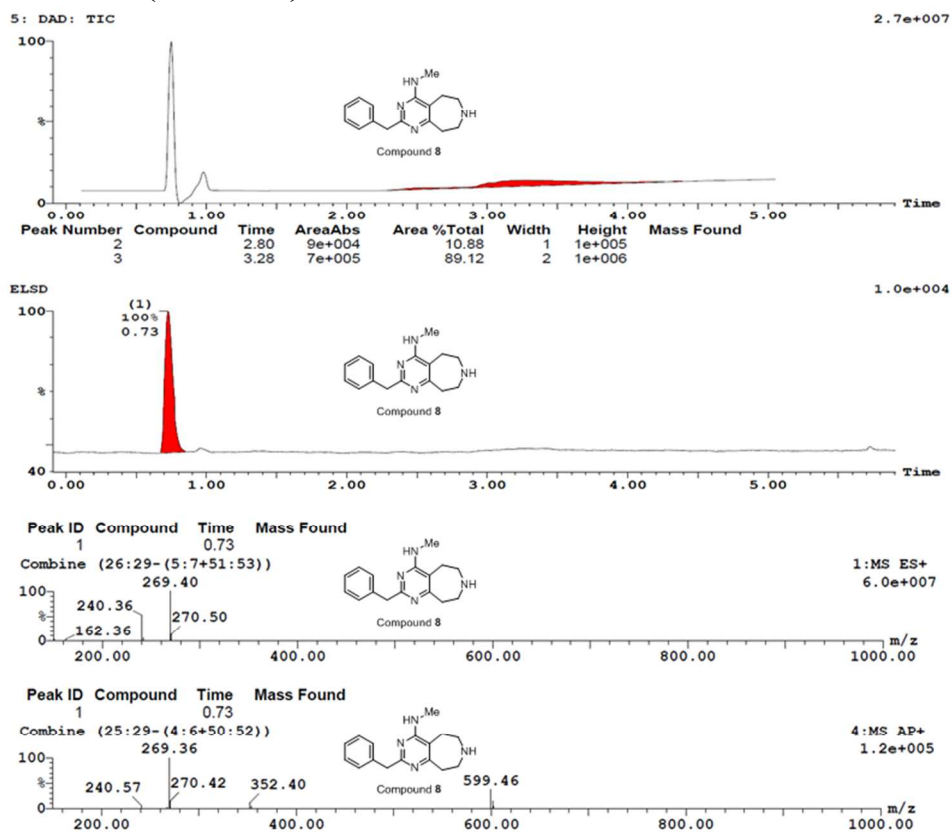
Compound 7 LCMS (6 min run):



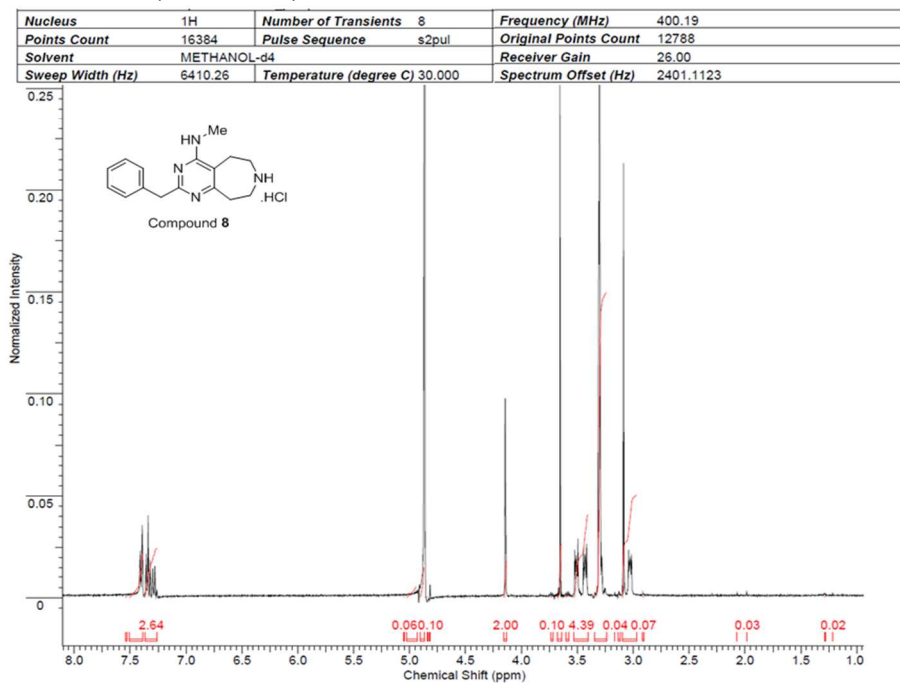
Compound 7 NMR ^1H (DMSO- d_6):



Compound 8 LCMS (6 min run):

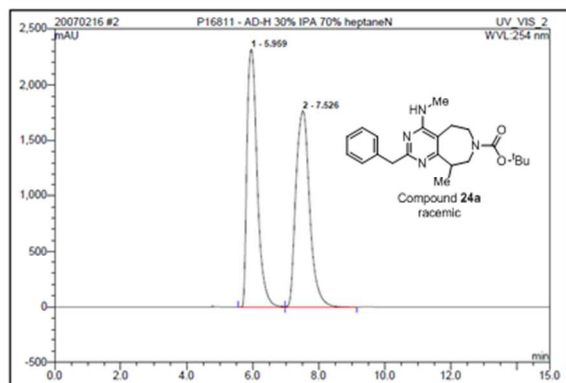


Compound 8 NMR ¹H (MeOD-d₄):



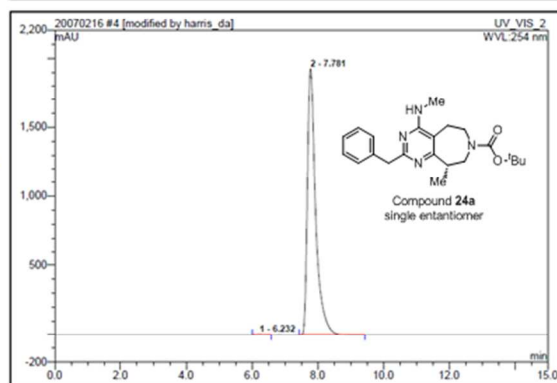
Compound 24a chiral HPLC:

Sample Name:	P16811	Injection Volume:	20.0
Conditions:	10B	Channel:	UV_VIS_2
Column:	AD-H	Wavelength:	254
Control Program:	10B	Vial Number:	GB5
Quantif. Method:	method	Sample Type:	unknown
Recording Time:	16/2/2007 10:54	System:	System_1
Run Time (min):	15.00		



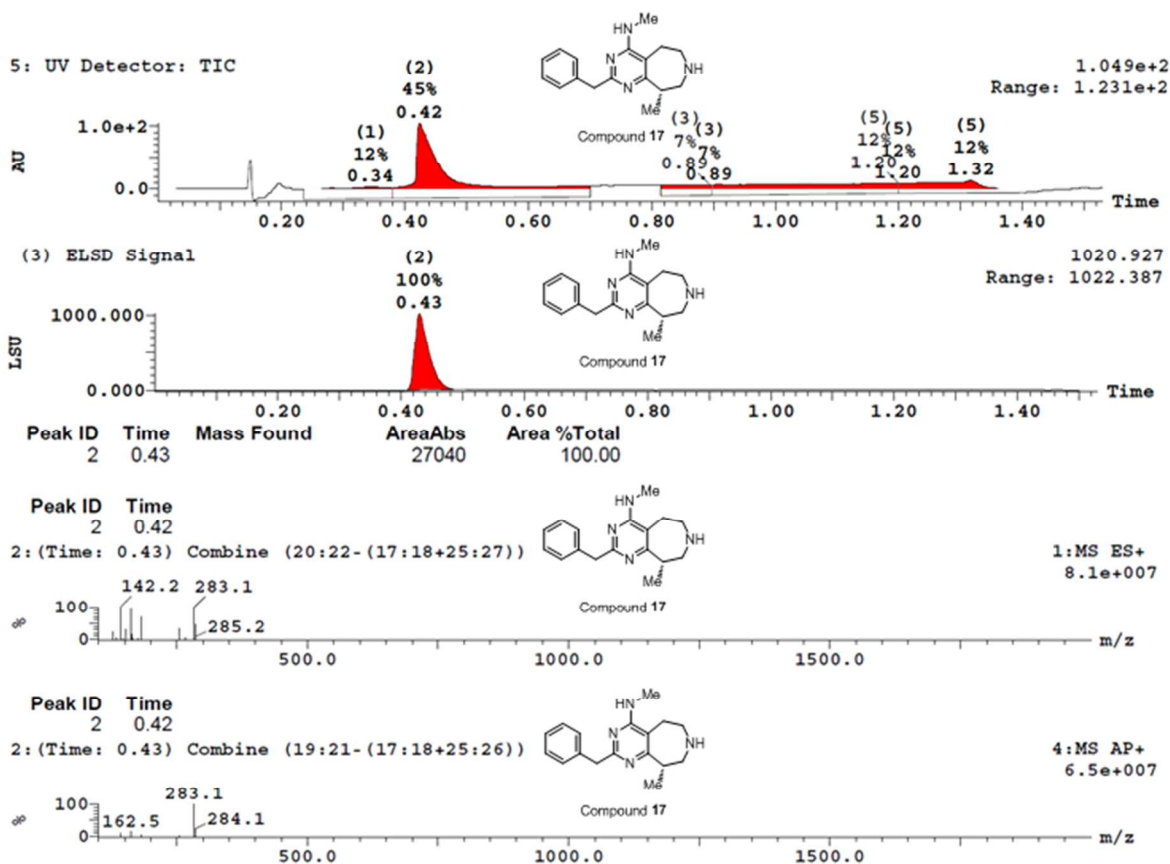
No.	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %	Amount	Type	ee (%)
1	5.96	2317.484	829.006	49.34	n.a	BM	1.3
2	7.53	1760.457	851.257	50.66	n.a	MB	
Total:		4077.941	1680.263	100.00	0.000		

Sample Name:	P16811-2	Injection Volume:	20.0
Conditions:	10B	Channel:	UV_VIS_2
Column:	AD-H	Wavelength:	254
Control Program:	10B	Vial Number:	GB7
Quantif. Method:	method	Sample Type:	unknown
Recording Time:	16/2/2007 11:26	System:	System_1
Run Time (min):	15.00		

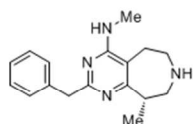


No.	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %	Amount	Type	ee (%)
1	6.23	4.050	0.976	0.17	n.a	BMB*	99.7
2	7.78	1925.591	559.986	99.83	n.a	BMB	
Total:		1929.641	560.962	100.00	0.000		

Compound 17 LCMS (2 min run):



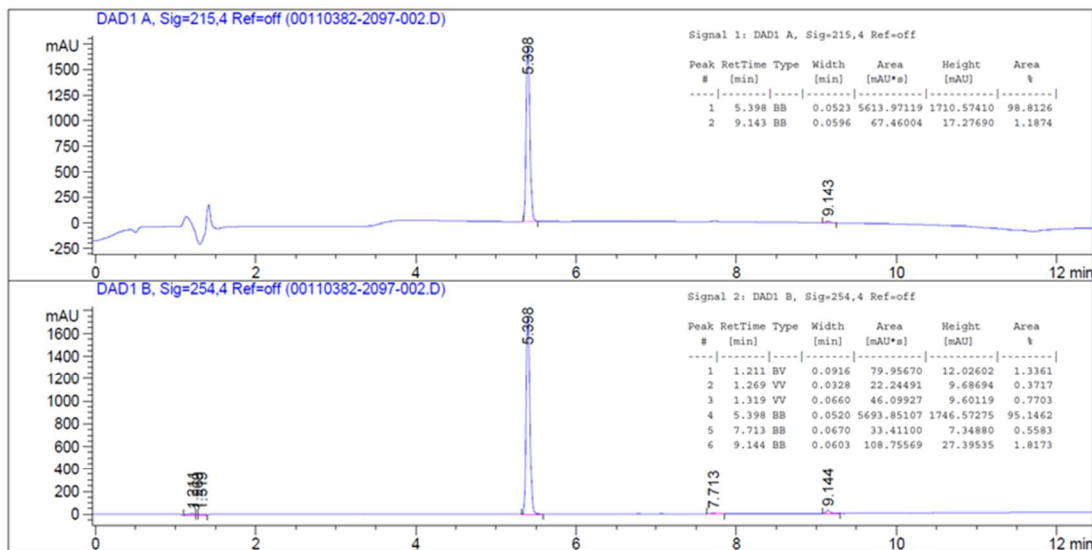
Compound 17 HPLC (12 min run):



Compound 17

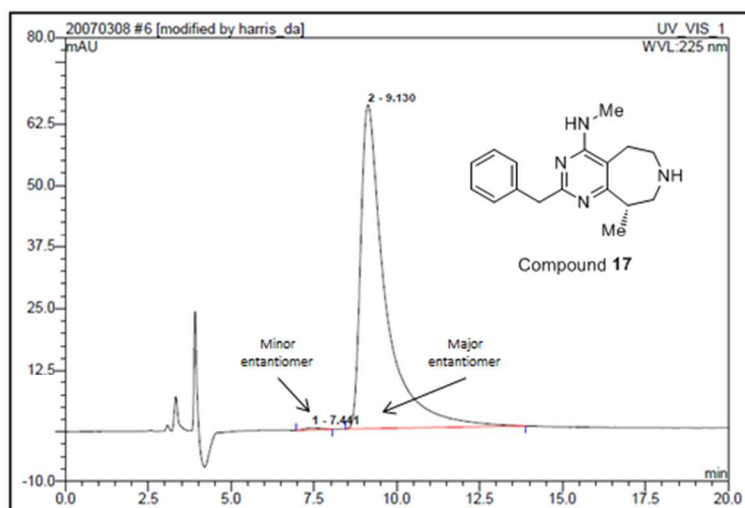
Method Info

: Column: XBridge C18 5 micron (4.6 mm x 150 mm)
Flow rate: 1.500 mL/min with solvents containing 0.1% TFA
0-1.5 min: 5% acetonitrile/water
1.5-10 min: 5-100% acetonitrile water
10-11 min: 100% acetonitrile
11-12.5 min: 100-5% acetonitrile/water



Compound 17 chiral HPLC:

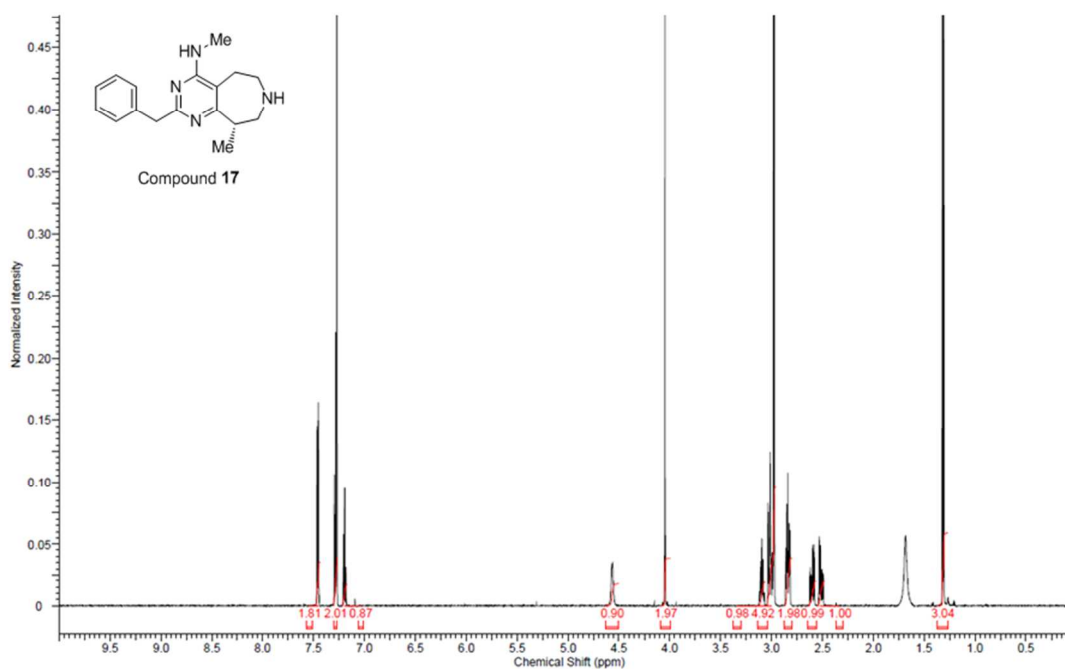
Sample Name:	P16861c	Injection Volume:	20.0
Conditions:	20B	Channel:	UV_VIS_1
Column:	OD-H	Wavelength:	225
Control Program:	20B	Vial Number:	GA2
Quantif. Method:	method	Sample Type:	unknown
Recording Time:	8/3/2007 12:51	System:	System_1
Run Time (min):	20.00		



No.	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %	Amount	Type	ee (%)
1	7.44	0.392	0.215	0.38	n.a.	BMB*	99.2
2	9.13	65.651	56.842	99.62	n.a.	BMB	
Total:		66.043	57.057	100.00	0.000		

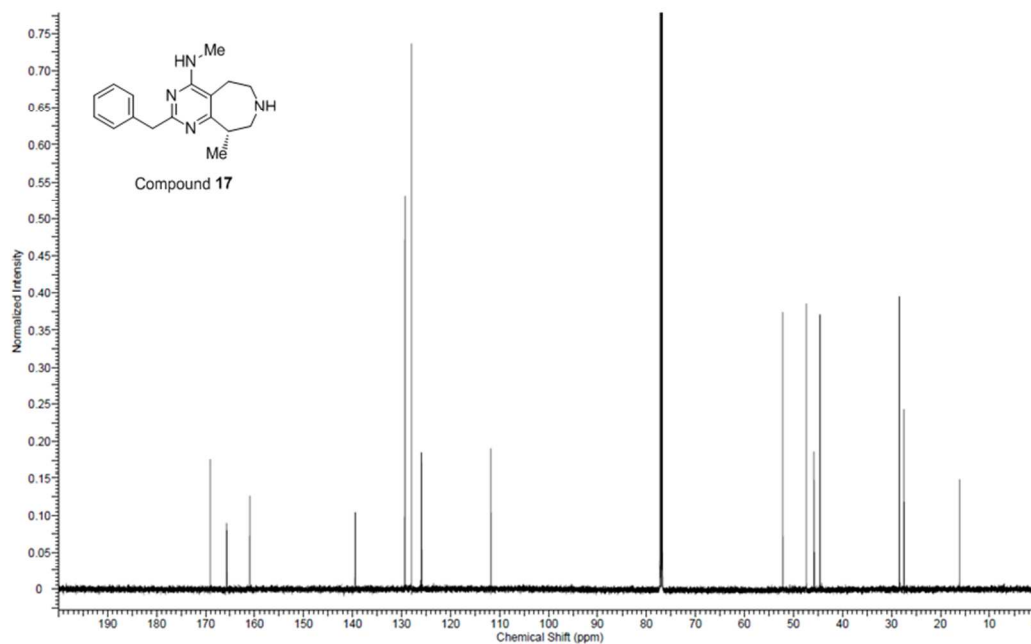
Compound 17 NMR ^1H (CDCl_3):

Frequency (MHz)	599.82	Nucleus	^1H	Number of Transients	16	Original Points Count	16384
Points Count	16384	Pulse Sequence	s2pul	Receiver Gain	30.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	3605.5984	Spectrum Type	STANDARD	Sweep Width (Hz)	9615.38	Temperature (degree C)	25.000

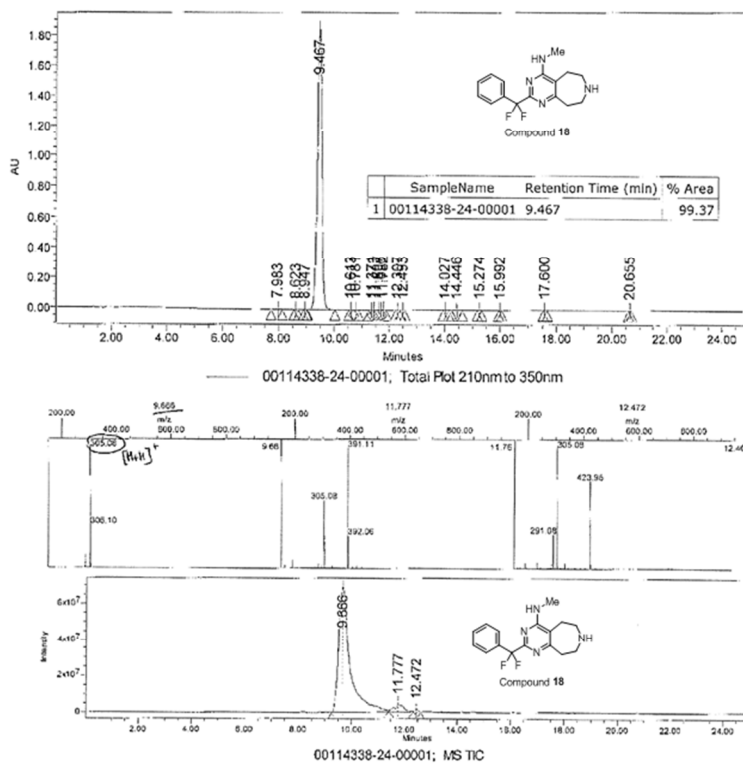


Compound 17 NMR ^{13}C (CDCl_3):

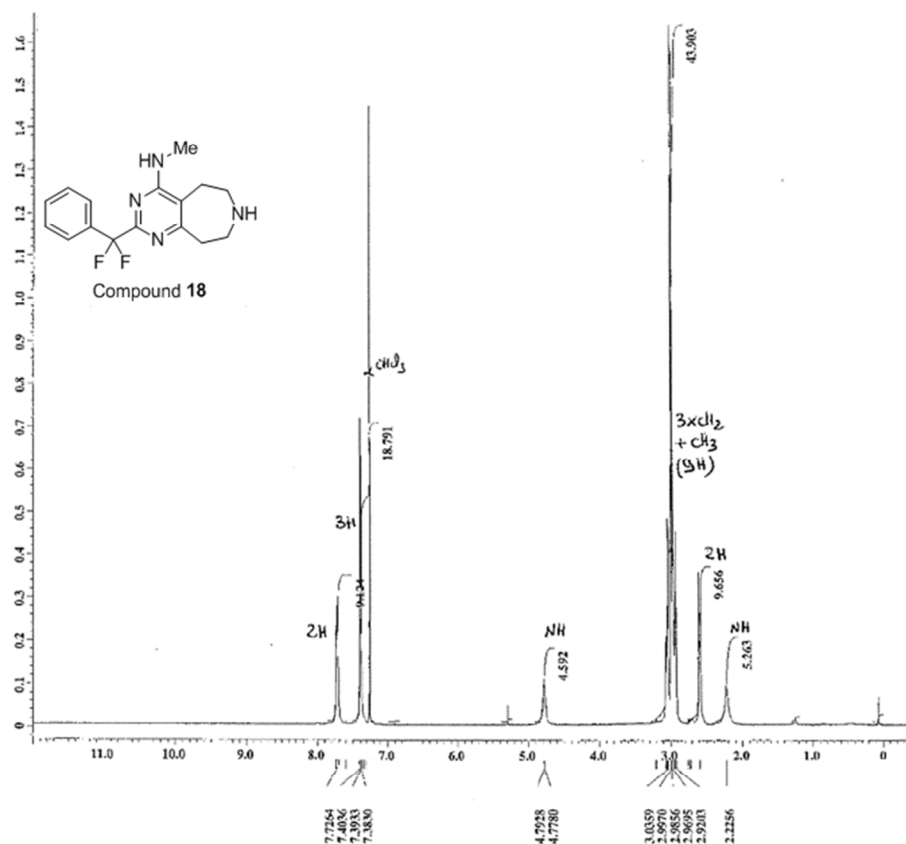
Frequency (MHz)	150.84	Nucleus	^{13}C	Number of Transients	8192	Original Points Count	32768
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	15082.4922	Spectrum Type	STANDARD	Sweep Width (Hz)	32894.74	Temperature (degree C)	25.000



Compound 17 HPLC (25 min run):



Compound 18 NMR 1H (CDCl₃):



```

Spectrometer      = DELTA2_NMR
Field_strength    = 7.0586013 [T] (300 [MHz])
X_acq_duration    = 2.90717896 [s]
X_domain          = 1H
X_freq            = 300.52965592 [MHz]
X_offset          = 5 [ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.34397631 [Hz]
X_sweep           = 5.63570784 [kHz]
Irr_domain        = 1H
Irr_freq          = 300.52965592 [MHz]
Irr_offset        = 5 [ppm]
Tri_domain        = 1H
Tri_freq          = 300.52965592 [MHz]
Tri_offset        = 5 [ppm]
Clipped           = FALSE
Mod_return        = 1
Scans              = 16
Total_scans       = 16

X_90_width        = 11 [us]
X_acq_time        = 2.90717696 [s]
X_angle           = 35 [deg]
X_atn              = 2.7 [dB]
X_pulse           = 4.27777778 [us]
Irr_mode          = Off
Tri_mode          = Off
Dante_present     = FALSE
Initial_wait      = 1 [s]
Relaxation_delay   = 1 [s]
Repetition_time    = 3.90717696 [s]
Solvent           = CHLOROFORM-D
Spin_action        = SPIN ON
Spin_state        = SPIN ON
Spin_status       = SPIN ON
Spin_get          = 14 [Hz]
Spin_set          = 15 [Hz]
Spin_gas_source    = AIR
Sample_action      = LOADED
Sample_state      = LOAD
Sample_status      = LOADED
Changer_sample     = 38
  
```


5. VCD to determine stereochemistry of compounds **16** & **17**²

The measured and calculated IR and VCD spectra of molecules **16** and **17** are shown in Figures S1 and S2. VCD and IR spectra of compounds **16** and **17** were obtained using ChiralIRTM - Vibrational Circular Dichroism (VCD) Spectrometer from BioTools Inc. (www.biotoools.com). Samples were analyzed at an approximate concentration of 0.06 M in deuterated dimethyl sulfoxide using a 100- μ m pathlength BaF₂ Cell. Spectra were collected in blocks over a 24 hour period at a resolution of 4 cm⁻¹ from 800 to 2000 cm⁻¹ using a single PEM VCD spectrometer from Biotoools Inc. IR and VCD spectra calculations were carried out by using the Gaussian 09 software package.³ Conformational sampling was performed using molecular dynamics (MD) simulations of each enantiomer in solution, with atomistic representation of solvent molecules. To select the most populated (representatives) conformations in solution, we carried out a hierarchical clustering analysis based on the first five dihedral principal components, which account for more than 95% of the structural variance in our data, over the MD trajectories. Five dominant conformations were selected for VCD calculation for each enantiomer. Geometry optimizations were performed using the B3LYP functional combined with basis set 6-31G(d) basis set for other atoms. Vibrational frequencies and VCD intensities were calculated at the same level of theory. Theoretical VCD and IR spectra were simulated from the calculated rotatory strengths and wavenumbers (scaled by a factor of 0.976) with Lorentzian curves using width-controlling parameter (half width at half-height) values of 6 cm⁻¹. Theoretical VCD and IR spectra were obtained as population-weighted sums (based on the Boltzmann distribution) of the calculated spectra of individual conformers. Clustering and trajectory analysis were performed on bio3d.⁴

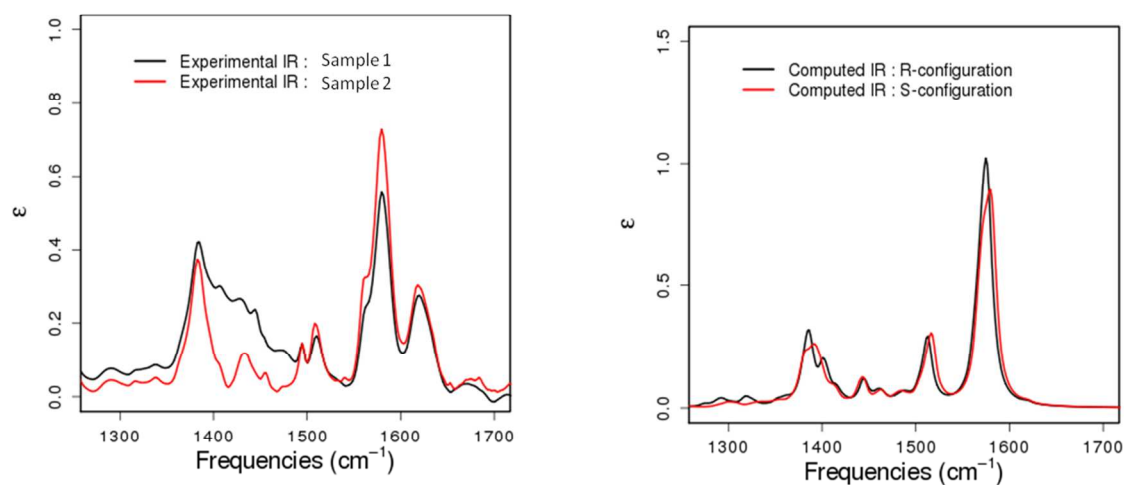


Figure S1: Comparison of experimental (left) and theoretical IR (right)

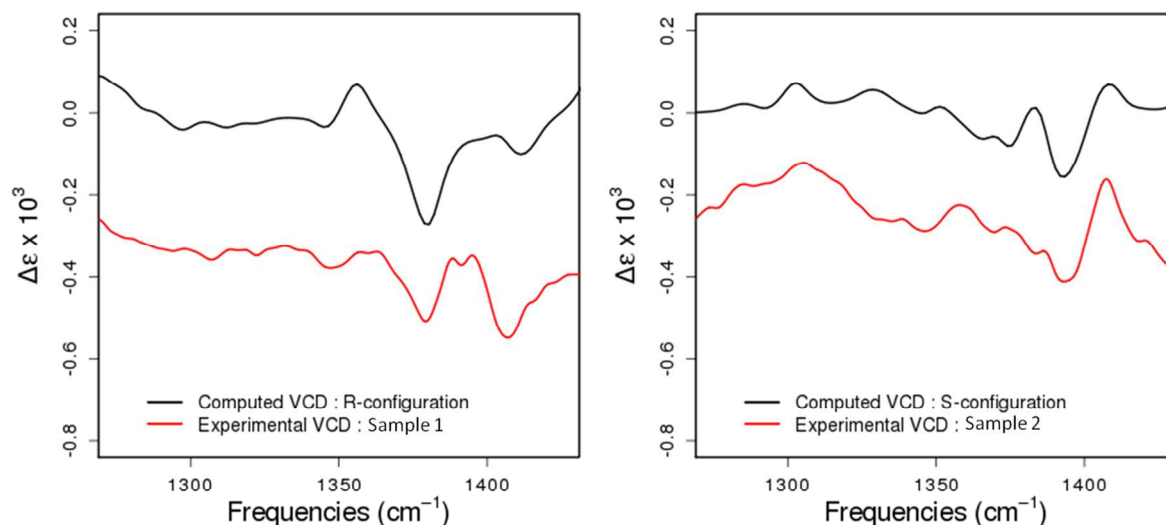


Figure S2: Comparison of experimental and theoretical VCD of compounds **16** and **17**. The experimental VCD spectrum is appropriately shift vertically for clarity.

All of the MD simulations were carried out with the Desmond module in the Schrodinger suite of programs using the OPLS all-atom force field.⁵ Conformations were collected every 10ps over MD trajectories of 100ns. The SHAKE algorithm was applied to all bonds involving hydrogen atoms, and an integration time step of 2.0fs was used in solving the equations of motion. All of the simulations were carried out at a constant pressure of 1 bar and temperature of 300K. The electrostatic interactions were treated using the Particle Mesh Ewald Method. Based on the alignment and analysis of the experimental and computed VCD spectra, compound **16** was assigned as *R* stereoisomer and compound **17** as *S* stereoisomer.

6. Computational models

cMDR model

The model was built based on 36,208 unique MDCK MDR1 efflux measurements in an analogous manner to that described by Gao, H. et al.⁶ Analysis of the features that contribute most to the cMDR efflux was performed. It was consistently observed that salvation energy, bond count, polar surface area, and hydrogen bond donor count are key contributors towards the cMDR BA/AB prediction. Evaluation of the calculated P-gp mediated efflux ratio across a diverse range of compounds from multiple projects showed that compounds with cMDR BA/AB <2.5 had a high probability of CNS penetration as measure by CSF levels or efficacy at low multiples of in vitro relevant concentrations (IC₅₀ or EC₅₀).

Homology Modeling

Residue Numbering

The position of each amino acid residue was identified both by its sequence number and by its generic number proposed by Ballesteros and Weinstein.⁷ In this nomenclature, amino acid residues in the 7TMD are given two numbers; the first is the trans-membrane (TM) helix number (1 to 7), while the second number indicates the residue position relative to a highly conserved residue in class A GPCRs in that TM, which is arbitrarily assigned to be 50. The numbering of the loops is done in a similar manner, for example extracellular loop 2 (ECL2) is labeled 45 to indicate its location between helices 4 and 5, and the conserved cysteine (thought to be part of a disulfide-bond) is given the index number 45.50. The residues within the ECL2 loop are then numbered relative to this position.

Site-directed mutagenesis (SDM) data for 5-HT_{2C}

Several mutagenesis studies⁸ have been performed on the 5-HT_{2C} and on the other members of 5-HT₂ subfamily: 5-HT_{2A} and 5-HT_{2B}. These receptors share >87% sequence similarity of their 7TM regions (see Figure S3).



Figure S3: Multiple sequence alignment between 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, the colour coding for TM 1 to 7 is dark orange, pink, red, purple, dark red, orange and light yellow respectively. The similarity or dissimilarity of each amino-acid compared to 5-HT_{2C} is shown by the bar; the strength of similarity is represented by the spectrum from blue (max. similarity) to red (min. similarity), bar length and its directionality (up – similarity, down dissimilarity).

These published SDM studies performed with several of agonists and antagonists provided insights on the key residues involved in ligand binding and functionality of 5-HT_{2C}. It was observed that conserved residue D134^{3,32} is essential for agonist 5-HT_{2C} binding.⁹ The mutation of S138^{3,36} to arginine reduces the ligand binding affinity.¹⁰ It was also reported that mutation of W337^{6,48} into alanine in 5-HT_{2B} (W324^{6,48} in 5-HT_{2C}) resulted in abolishing of serotonin binding.¹¹ Mutation studies with F341^{6,52} in 5-HT_{2B} and F340^{6,52} in 5-HT_{2A} receptor (F328^{6,52} in 5-HT_{2C}) have shown dramatic effect on ligand binding and efficacy. Medium effect on binding of serotonin was observed for mutation of Q344^{6,55} into Alanine in 5-HT_{2B} (Q331^{6,55} in 5-HT_{2C}).^{8a} These SDM results indicated that the 5-HT_{2C} agonists binding site is located between the TM3, 5 and 6.

Homology modeling procedure of active and inactive conformations of 5-HT_{2C} receptor

We modeled active and inactive conformations of 5-HT_{2C} (named 5-HT_{2C}^{active} and 5-HT_{2C}^{inactive} respectively). The model of 5-HT_{2C}^{active} was based on the crystal structure of active conformation of β 2-adrenergic receptor (β 2AR, PDB entry 3SN6)¹² and model of 5-HT_{2C}^{inactive} was based on the crystal structure of inactive conformation of β 2-adrenergic receptor (β 2AR, PDB entry 2RH1)].¹³ The β 2-adrenergic receptor was selected as template for modeling of 5-HT_{2C} receptor because it was the only receptor that had crystal structures available for both active and inactive conformations. The sequence alignment between 5-HT_{2C} receptor and β 2AR are shown in Figure S4, display 47.4% sequence similarity in the 7TMD.

(1) *Sequence Alignment* between 5-HT_{2C} and β 2-adrenergic receptor was performed using sequence alignment tool implemented in MOE version 2010.10 (Chemical Computing Group, version 2010.10). The conserved residues and conserved GPCR motifs were constrained to ensure their proper alignment. The amino acid sequence for human 5-HT_{2C} (P28335) was retrieved from the SwissProt database. The position of each amino acid was identified by its sequence number and by the generic number proposed by Ballesteros and Weinstein.^{7a}

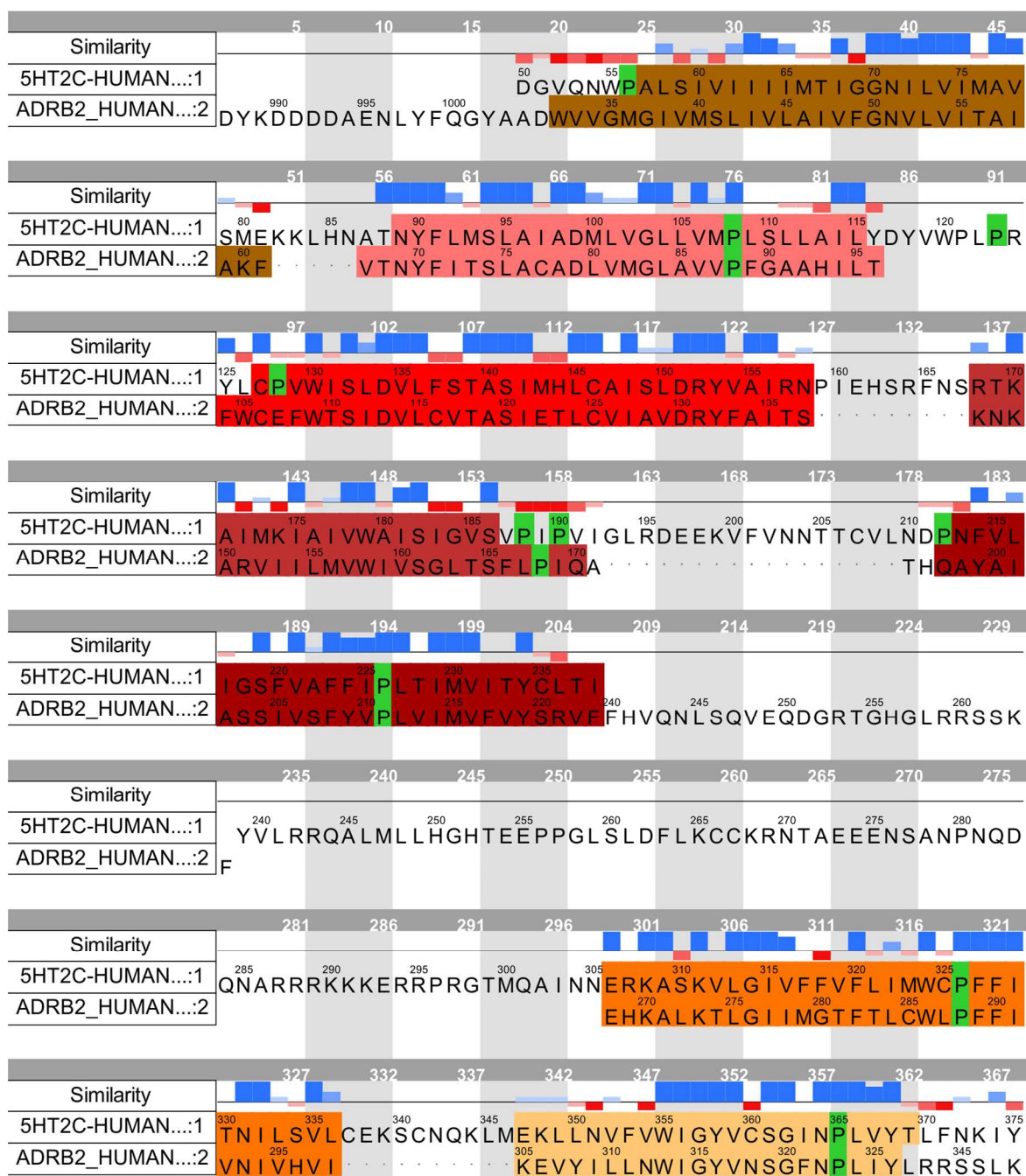


Figure S4: Multiple sequence alignment of 5-HT_{2C} receptors aligned with β₂-adrenergic receptor, the colour coding for TM 1 to 7 is dark orange, pink, red, purple, dark red, orange and light yellow respectively. The similarity or dissimilarity of each amino-acid compared to 5-HT_{2C} is shown by the bar; the strength of similarity is represented by the colour from blue (max. similarity) to red (min. similarity), bar length and directionality (up – similar, down dissimilar).

(2) Homology modeling of 5-HT_{2C}^{active} and 5-HT_{2C}^{inactive} was performed using the homology modeling tool as implemented in the MOE software package. Due to insertions of the 5-HT_{2C} sequences with respect to the template, some residues, particularly in the loop regions, did not

have assigned backbone geometries based on the template. These insertions were modeled from segments of high-resolution chains from the protein data bank (PDB) which superposed well onto anchor residues on each side of the insertion area, after the method described by Fechteler et al.¹⁴ Following the selection of appropriate loop templates, multiple model candidates for each loop were constructed and scored using an OPLS-AA energy function.¹⁵ The coordinates of the top ranked loop model were added to the global model. After all of the loops had been added, the side chains were modeled. Sidechain data is assembled from an extensive rotamer library generated by systematic clustering of high-resolution PDB data. After all of the backbone segment and sidechain conformations were chosen, the model was minimized using the OPLS-AA force-field.¹⁶

Flexible docking of key compounds into the binding site of 5-HT_{2C}^{active} and 5-HT_{2C}^{inactive}

In the final modeling step we aimed to predict the binding mode of compound **4**, with 5-HT_{2C}^{active} and 5-HT_{2C}^{inactive} and to optimize the 5-HT_{2C} binding site. We used SDM data to identify the location of 5-HT_{2C} binding site and the list of key residues responsible for ligand binding and activation. We flexibly docked compounds **4** into binding sites of 5-HT_{2C}^{active} and 5-HT_{2C}^{inactive} using GOLD docking package (Cambridge Crystallographic Data Centre, version 5.0). The default GOLD rotamer library was used to assign flexibility to key residues taken from SDM. Key residues selected to be flexible were: D134^{3,32}, S138^{3,36}, W324^{6,48}, F328^{6,52} and Q331^{6,55}.

The docking itself was performed once for each molecule, with the 10 top ranked docking poses scored by the GOLD default scoring function retained.¹⁷ We then rescored and re-ranked these 10 docked poses using AMBER interaction energy. We used the MM_PBSA/GBSA approach to calculate the AMBER interaction energy in order to take the entropy and solvation energy change upon binding into account, as well as the binding enthalpy between protein and ligand.¹⁸ As was recently published the AMBER interaction energy, while subject to the same limitations as all force field based methods, was able to accurately predict relative binding affinities between ligand and protein and was therefore selected as a reliable method to re-score and to rank docking poses.¹⁹ The top-ranked docking pose, according to the AMBER interaction energy, was taken for further optimization.

To further optimise the interaction geometry between key residues and compound **4**, and to optimise the conformation of the ECL2 loop, we used the LowModeMD protocol.²⁰ The LowModeMD protocol is a stochastic conformation generation protocol implemented in MOE. The starting point for this refinement step was the top ranked GOLD docking poses with optimised 7TMD key residues. During this process, the positions of the TM backbone atoms were restrained. The OPLS-AA force-field was used for the conformational search, with the dielectric constant set to 3. A LowModeMD constant temperature molecular dynamics simulation was performed at 300K, using the Berendsen thermostat and the velocity Verlet algorithm.²¹ The default value of the energy minimisation gradient (0.001 kcal mol⁻¹ Å⁻²) was

used. The LowModeMD and stochastic searches were terminated after 200 failed attempts to generate a new conformation, with a maximum of 10,000 iterations.

Compounds **18** was docked into in same flexible-docking procedure like prototype compound **4** but not followed by LowModeMD.

7. General biology experimental procedures

Calcium Mobilisation Assays²²

The agonist potency and efficacy of the compounds were tested by measuring their ability to induce a fluorescent based Ca^{2+} mobilization signal in a FLIPR assay using CHO K1 cells expressing recombinant human 5-HT_{2C} receptor. Both agonist affinity (EC_{50}) and efficacy (E_{max}) were determined.

Cell culture

Chinese hamster ovary cells (CHO K1) stably transfected with human 5-HT_{2c} or 5-HT_{2B} receptor were cultured under standard cell culture techniques. Specifically, cells were grown at 37 °C and 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM) culture media supplemented with 10% dialysed foetal calf serum (FCS), 1% non-essential amino acids, 1mM sodium pyruvate, 800µg/mL geneticin and 50µg/ml zeocin. Cells were harvested for passaging and storage using trypsin-EDTA, centrifugation and resuspension in culture medium. Cells were grown to 60-80% confluency, harvested and adjusted to 15-20 x 10⁶ cells/ml/vial in medium/10% DMSO and stored long-term at -80 °C.

Preparation of cell plates

Cells were seeded into black-walled clear-bottomed 384 well plates 24h before use. Frozen cells were defrosted in a 37 °C water bath and immediately transferred into 1mL/vial 37 °C culture medium, diluted to 10ml and DMSO removed by centrifugation. The cells were re-suspended in 15ml/vial of cell culture medium, counted and adjusted to give 500,000 cells/mL (10,000 cells/well). 20µL/well of cell suspension was added to the 384 well plates which were then incubated overnight at 37 °C.

Preparation of compound plates

Test compounds were prepared at 4mM in 100% dimethyl sulphoxide (DMSO) and diluted in Dulbeccos PBS (+CaCl₂, +MgCl₂) with 0.9% DMSO and 0.05% pluronic F-127 to give appropriate test concentrations. The maximum agonist response was determined with 5-HT at a final assay concentration of 10µM in the diluent above. The minimum response was determined with Dulbeccos PBS (+CaCl₂, +MgCl₂) with 0.9% DMSO and 0.05% pluronic F-127. Test compounds, maximum and minimum controls were added to a 384 well polypropylene plate.

Preparation of FLIPR dye

The FLIPR calcium assay reagent was diluted with assay buffer (Hank's Balanced Salt Solution (HBSS))/20mM HEPES and 2.5mM probenecid (diluted with 1M aqueous sodium hydroxide and DPBS (+CaCl₂, +MgCl₂)).

Running the assay using FLIPR

20μL/well FLIPR calcium assay reagent was added to the cell plates, which were incubated for 1h at 37 °C. Cell plates and compound plates were then transferred onto the FLIPR. The assay was run using the appropriate FLIPR program, which initiates the reaction by transferring 15μL compound into the corresponding well of the cell plate.

Data analysis

The statistical parameter exported from each well was the max peak height of the response. The mean minimum was subtracted from all values and then the activity was expressed as a percentage of the mean maximal response to 10μM 5-HT and dose-response curves plotted from which both agonist affinity (EC₅₀) and efficacy (E_{max}) were determined.

Radioligand Binding

The compounds were tested for biological activity by their ability to inhibit binding of the radioligand ³H-meserlgine at the human recombinant 5-HT_{2C} receptor expressed in the Swiss 3T3 cells using a scintillation proximity assay (SPA) technology.

Cell culture

Swiss 3T3 stably transfected with the 5-HT_{2C} receptor were cultured under standard cell culture techniques. Specifically, cells were grown in 50mL growth medium (Dulbecco's Modified Eagle's Medium (DMEM) culture media supplemented with 10% dialysed foetal calf serum (FCS), 2mM penicillin/streptomycin and 20μg/mL geneticin) in 225 cm² flasks at 37 °C and 5% CO₂. Cells were grown to 60-80% confluency, harvested using trypsin-EDTA and pelleted by centrifugation for long-term storage at -80 °C.

Cell membrane preparation

Cell pellets were thawed on ice and resuspended in 3 mL of membrane preparation buffer (see Media and Buffers for composition) per 1 mL of packed cell. The suspension was homogenised on ice for several 5s intervals using a hand-held homogeniser. The homogenate was then centrifuged at 1,000rpm for 5 min at 4 °C.

The supernatants were then collected and retained. Initial cell & nuclei pellets (P1) were subsequently rehomogenised and centrifuged using the conditions cited above, and the supernatants collected and pooled with those retained from the first spin.

The pooled supernatants were centrifuged at 19,500 rpm for 45 min at 4 °C, and the supernatants discarded. The pellets (P2) were then resuspended in 3 mL of membrane preparation buffer per 1 mL of the original packed cell volume. Protein concentrations were subsequently measured and the membrane suspension was finally frozen in aliquots of set volume and stored at -80 °C prior to use in assays.

Determination of optimal assay conditions for individual membranes

For each batch of membrane used, optimal concentrations of Polylysine YSi SPA beads and membrane were determined. The assay free radioligand concentration was expressed as a percentage of the total free radioligand concentration to give an estimate of the radioligand depletion. The radioligand depletion in the assay was less than 10% to ensure that there was sufficient radioligand available for binding.

The affinity of ^3H -meselurgine for the human recombinant 5-HT_{2c} receptor was determined for each membrane batch at the selected protein and bead concentrations. This was achieved by the determination of the K_D , the concentration of free radioligand at which 50 % of the receptor binding sites were occupied. The mean K_D for ^3H -meselurgine at a batch of membranes was determined from data from a minimum of three separate assays. The mean K_D was subsequently used for all assays using the membrane batch profiled to enable determination of K_i values of compounds studied using the method determined by Cheng and Prussoff (Cheng YC and Prussoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition of an enzymatic reaction. *Biochem Pharmacol* 1973; 22:2099-3108.)

Assay protocol²²

Bead/receptor membrane complex preparation

The required amount of membrane was thawed on ice and added to a pre-determined volume of bead suspension in 50mM HEPES buffer, pH7.4. The beads were then pre-coupled by incubating the predetermined protein quantity per mg of bead on a roller 4 °C for 1 h. Subsequently, the bead/membrane complex was spun down at 1000 rpm for 5 min. The resulting pellet was resuspended in assay buffer at the specific concentration required for the final assay.

Ligand preparation

An aliquot of [^3H]-meselurgine stock was diluted in binding assay buffer to give a pre-determined final assay concentration less than the equilibrium dissociation constant (K_D) value.

Compound plate preparation

All test compounds were prepared at a concentration of 4mM in 100% dimethyl sulphoxide (DMSO) from dry samples. Compounds were diluted in 2.5% DMSO in Dulbeccos PBS with 0.05% Pluronic F-127 to give appropriate test concentrations in a 384 well plate to give a final volume of 5 μL .

The same volume of assay buffer was added to specific wells of the plate to enable subsequent measurement of total radioligand binding. Furthermore, 5 μL of Mianserin at 25 μM (2.5 μM final

assay concentration was subsequently added to pre-determined wells to determine non-specific binding (NSB).

For the assay, 20 μ L of the corresponding bead/membrane complex was added to each well of the final assay plate, ensuring that the suspension was mixed well. 25 μ L of 3 H-meselurgine was added to each well of the final assay plates (containing compound solutions). Subsequently, 25 μ L of 3 H-meselurgine was added to each well of the final assay plates (containing compound solutions). The plates were then sealed and incubated, with shaking, for 2 h 30 min at room temperature. The plates were subsequently left to settle for 10 min with dark adaptation, prior to reading.

Data analysis

The assay window (specific binding) per plate was calculated by subtracting the mean NSB readings (in counts per minute, or cpm) from the mean of total binding readings. Subsequently the cpm read per well (with mean NSB subtracted) was expressed as a percentage of the plate window to determine the amount of radioligand bound to the receptor-bead complex.

These values were plotted against the concentration of the compound tested and a sigmoidal inhibitory concentration effect curve was fitted to the data using a four-parameter logistic equation and free-fitting parameters to give an IC_{50} value (the concentration of compound required to inhibit 50% of the specific binding at the 5-HT_{2C} receptor).

The inhibitory dissociation constant (K_i) value was then calculated from the IC_{50} value using the Cheng-Prusoff equation.

Following determination of individual K_i values for compounds tested, an overall geometric mean was calculated together with 95% confidence intervals and n values, where n is the total number of individual K_i values.

Buffers

Membrane Preparation Buffer

50mM HEPES, pH 7.4 at room temperature, stored at 4 °C. Prior to use, one complete protease inhibitor tablet was dissolved per 50 mL of buffer.

Binding Assay buffer

50mM HEPES pH 7.4

10mM CaCl₂

0.1mM Pargyline

0.1% Ascorbic Acid

0.05% Pluronic

In vitro dog Femoral Artery tissue preparations (functional 5-HT_{2A} assay)²³

Femoral arteries were removed from euthanased beagle dogs (male and female 17-19kg), dissected into 3mm rings, denuded of endothelium and placed under 1g of tension in an organ bath containing modified Krebs' solution (37°C, gassed with 95% O₂/ 5% CO₂) of the following

composition (mM): NaCl (120), KCl (4.7), NaHCO₃ (25), MgSO₄·7H₂O (1.2), KH₂PO₄ (1.2), Glucose (11), containing cocaine (1μM) corticosterone (100nM) and naproxen (1μM). After a period of equilibration (1h) tissues were challenged sequentially with 80mM KCl and 10μM 5-HT, a 30min wash period following each. Subsequently a cumulative concentration response curve (CCRC) was constructed for each agonist investigated (1x10⁻⁹-3x10⁻⁴M). Sigmoidal curves were fitted for each individual tissue and EC₅₀ (concentration producing 50% maximal response) and E_{max} (maximum response of agonist as a % of the tissue response to 10μM 5-HT) values derived. Only a single CCRC was carried out on each tissue. EC₅₀ values are reported as geometric mean with 95% confidence intervals and E_{max} values as arithmetic mean ± standard deviation. Tension values are reported in g as the mean ± standard deviation.

8. Preclinical *in vivo* model

In vivo Investigation of dog urethral pressure profilometry²⁴

All experiments described were conducted according to UK legislation (Animals (Scientific Procedures) Act, 1986) and are subject to local ethical review.

Adult, female, nulliparous beagle dogs (9–17 kg) were initially anesthetized with sodium pentobarbitone to induce surgical anesthesia (30 mg/kg i.v.). Animals were intubated, and respiration maintained at a constant rate of 14 breaths per minute. Tidal volume was adjusted to maintain expired air within normal physiological limits. Throughout the experiment the animal was maintained at a core body temperature of approximately 37°C by use of a thermocouple-heated blanket (Harvard Apparatus, Holliston, MA). Hartmann's solution (Animalcare Ltd., York, North Yorkshire, UK) was administered as a constant slow-drip infusion (approximately 7 ml/h) throughout the experiment, via the brachial vein cannula, to replace any fluids lost during surgery and maintain blood volume.

The right femoral vein and artery were cannulated for administration of compounds and for blood sampling, respectively. The right brachial vein was cannulated for infusion of anesthetic and Hartmann's solution, and the right brachial artery for monitoring of arterial pressure (Millar, 7F; Millar Instruments, Houston, TX). A midline incision was made in the abdomen, and underlying abdominal tissue and muscle layers were divided with blunt dissection and retracted to expose the bladder. The ureters were cannulated to drain urine from the kidneys throughout the experiment. The dome of the bladder was cannulated (Portex 7G), and the cannula fed through the bladder to the external urethra. This catheter was subsequently used to introduce the urethral pressure catheter (Millar SUPC-380C; Millar Instrument) into the urethra. The bladder was filled with saline to achieve an intravesical pressure of approximately 8 to 10 mm Hg, and bladder pressure was measured by connecting the bladder catheter to a pressure transducer (model DTX plus; BD UK Ltd, Oxford, UK). After completion of surgery, animals were transferred to α-chloralose anesthetic (70–100 mg/kg i.v. induction followed by constant infusion to deliver 10–15 mg/kg/h i.v. for the duration of the experiment). After completion of surgery,

animals were allowed to stabilize for at least 60 min before starting urethral pressure profilometry measurements.

Urethral pressure profilometry (UPP) was measured by withdrawing the Millar pressure transducer through the urethra at a constant rate. This was achieved by fixing the urethral catheter to a pump set to withdraw the catheter at a constant rate (Harvard Apparatus). A full profile measurement was obtained approximately every 6 min, and readings were taken continuously throughout the experiment. Baseline measurements were performed until four consistent measurements were obtained, after which drug administration commenced.

Compounds were administered as continuous infusions in appropriate vehicle, doses and infusion rate were calculated based on previously obtained pharmacokinetic (IV bolus) data in order to achieve plasma concentrations in the region of between 3 and 10 times the in vitro EC₅₀ against the human 5-HT_{2c} receptor from cell-based assays. Each dose was infused over a 30-60-min period and blood samples were obtained at regular intervals to measure drug plasma levels throughout.

Continuous measurement and recording of bladder pressure, urethral profilometry, and arterial blood pressure was made via Notocord-Hem software (v4.1) (Notocord Systems). Heart rate was derived from the arterial pressure signal. From the profilometry data, peak urethral pressure (PUP, mm Hg) was measured. Changes in PUP were compared with mean baseline values and expressed as the percentage change from baseline, recorded over the four consecutive profiles obtained before test drug or vehicle administration. From multiple experiments, mean \pm S.E.M. was calculated. For PUP data, biological significance was assumed when the increase in PUP was $\geq 20\%$, based on clinical translation studies. This method of analysis is appropriate because statistical analysis has shown that very small changes in peak urethral pressure will be deemed statistically significant, despite being biologically irrelevant.

At the end of experiments, animals were euthanized, without recovery, with an intravenous overdose of pentobarbitone anesthetic.

Three animals were excluded from the current study, all of which had received compound 8 during the investigation. One animal was removed due to an unusually high baseline maximal urethral pressure of 42.8 ± 1.3 mmHg (mean \pm standard deviation); under the current experimental procedures the average maximal urethral pressure measurements at baseline in adult, female, nulliparous beagle dogs (9–17 kg) has been shown to be 23.4 ± 5.6 (mean \pm standard deviation, n=34), animals showing a baseline maximal urethral pressure $>$ than 2 standard deviations from the mean are considered to be displaying biologically significant variation from the normal population and excluded. A further 2 animals were considered non-responders, i.e. changes in PUP were $\leq \pm 5\%$ from baseline. CSF samples were not available for analysis from these animals, given the known potential for reduced CNS penetration with compound 8 the data was not included in graphical representations.

Urethral anatomy and neural innervation in the female dog are known to be similar to that of human females.²⁵ In addition female dogs are known to suffer from stress urinary incontinence (SUI) in old age;²⁶ as such the dog has been extensively utilised as a model of human urethral function, in particular urethral pressure profilometry in the dog is known to reflect the integrity of urethral function and is known to increase in a similar manner to that seen in humans with exposure to specific or a class of therapeutic agent such as duloxetine, reboxetine and α -adrenoceptor agonists, which have shown promise in the treatment of SUI.^{24, 27} Furthermore, we have shown that urethral pressure profilometry in the dog is highly predictive for drugs acting within the CNS on the neural control of urethral function in order to increase urethral pressure, in particular 5-HT_{2C} agonists, as such urethral pressure profilometry in the dog is an extremely predictive model of human urethral function and of 5-HT_{2C} agonist pharmacology which allows translation of in vitro agonist activity to an in vivo endpoint and hence provides valuable information on the potential of early drug candidates.²⁷ Potential clinical utility for 5-HT_{2C} agonists includes SUI, obesity, erectile dysfunction and psychotherapeutic disorders.²⁸

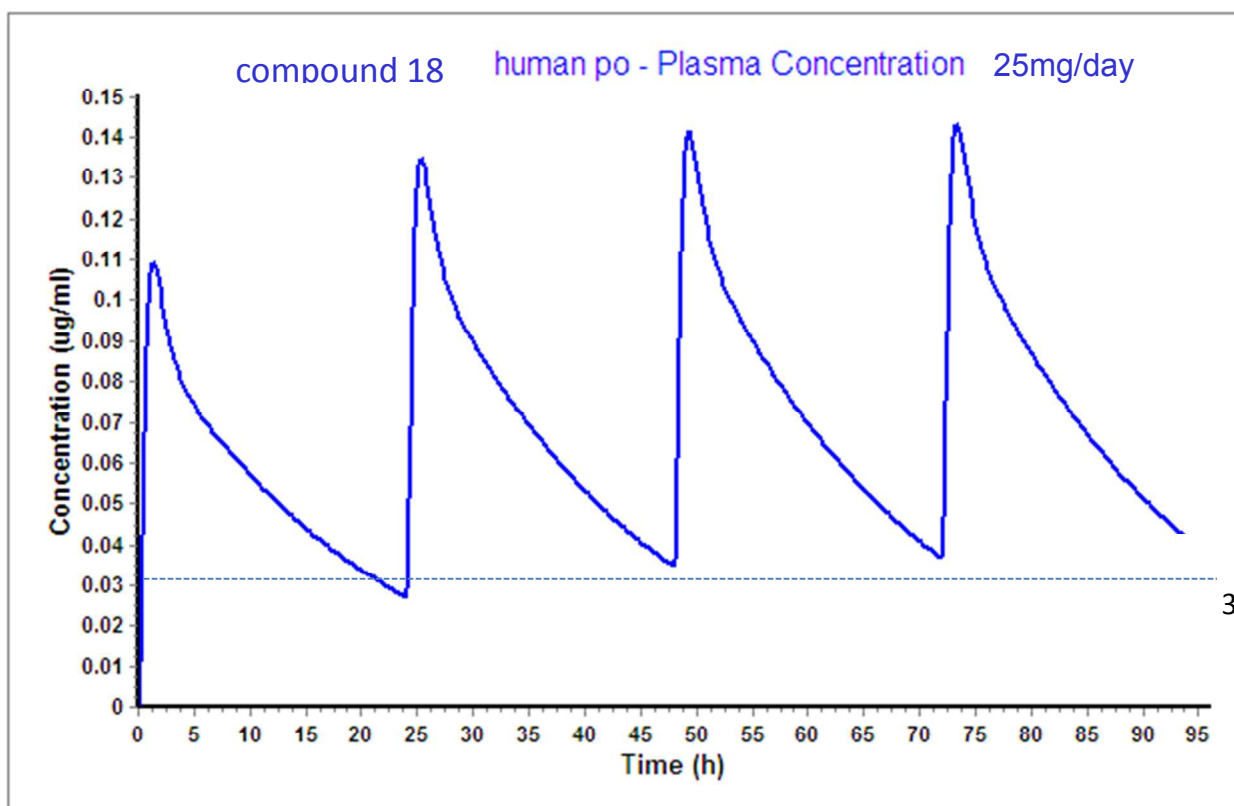
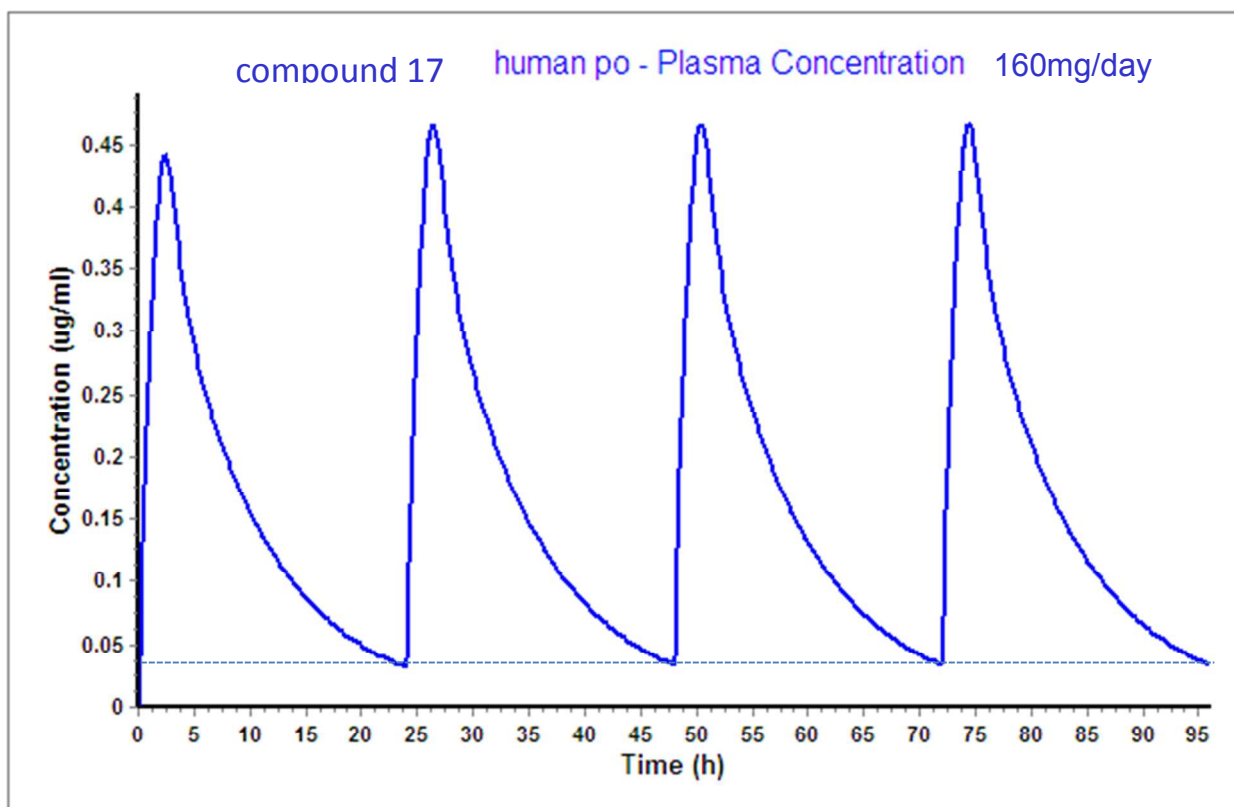
9. Estimating human dose using PBPK modeling with GastroPlus™ software

Distribution properties of compounds 17 and 18 were predicted from physical chemical and blood binding properties using a PBPK method based on tissue partition coefficients calculated from equations of Rodgers & Rowland (2006).²⁹

Blood:plasma ratio was assumed to be 1. Systemic clearance was estimated from scaled in vitro Cl_{int} from HLM. Effective permeability in human intestine and solubility at pH 6.5 were assumed to be $2.0 \times 10^{-4} \text{ cm s}^{-1}$ and 0.1mg/ml respectively.

These inputs were used in GastroPlus™ simulations of plasma concentration vs. time profiles following single daily oral suspension doses that would attain C_{eff} (as determined in the canine PUP model) as a trough concentration, i.e. 70 nM (= 37ng/ml total concentration) for compound 17 and 40nM (= 32ng/ml total concentration) for compound 18.

Examples of simulation outputs are shown below.



10. References

1. Andrews, M. D.; Blagg, J.; Brennan, P. E.; Fish, P. V.; Roberts, L. R.; Storer, R. I.; Whitlock, G. A. Preparation of pyrimido[4,5-d]azepine derivatives as 5-HT_{2C} agonists. WO2008117169, 2008.
2. Sherer, E. C.; Lee, C. H.; Shpungin, J.; Cuff, J. F.; Da, C.; Ball, R.; Bach, R.; Crespo, A.; Gong, X.; Welch, C. J. Systematic Approach to Conformational Sampling for Assigning Absolute Configuration Using Vibrational Circular Dichroism. *J. Med. Chem.* **2014**, *57*, 477-494.
3. Frisch, M. J. T., G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian09, Gaussian Inc, Walingford, CT*, **2009**.
4. Grant, B. J.; Rodrigues, A. P. C.; ElSawy, K. M.; McCammon, J. A.; Caves, L. S. D. Bio3d: an R package for the comparative analysis of protein structures. *Bioinformatics* **2006**, *22*, 2695-2696.
5. Murzyn, K.; Bratek, M.; Pasenkiewicz-Gierula, M. Refined OPLS All-Atom Force Field Parameters for n-Pentadecane, Methyl Acetate, and Dimethyl Phosphate. *J. Phys. Chem. B* **2013**, *117*, 16388-16396.
6. Gao, H.; Yao, L.; Mathieu, H. W.; Zhang, Y.; Maurer, T. S.; Troutman, M. D.; Scott, D. O.; Ruggeri, R. B.; Lin, J. In silico modeling of nonspecific binding to human liver microsomes. *Drug Metab. Dispos.* **2008**, *36*, 2130-2135.
7. (a) Ballesteros, J. A.; Weinstein, H. Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors. *Methods Neurosci.* **1995**, *25*, 366-428; (b) Prioleau, C.; Visiers, I.; Ebersole, B. J.; Weinstein, H.; Sealfon, S. C. Conserved Helix 7 Tyrosine Acts as a Multistate Conformational Switch in the 5HT_{2C} Receptor. Identification of a Novel "Locked-On" Phenotype and Double Revertant Mutations. *J. Biol. Chem.* **2002**, *277*, 36577-36584.
8. (a) Rashid, M.; Manivet, P.; Nishio, H.; Pratuangdejkul, J.; Rajab, M.; Ishiguro, M.; Launay, J.-M.; Nagatomo, T. Identification of the binding sites and selectivity of sarpogrelate, a novel 5-HT₂ antagonist, to human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor subtypes by molecular modeling. *Life Sci.* **2003**, *73*, 193-207; (b) Deraet, M.; Manivet, P.; Janoshazi, A.; Callebort, J.; Guenther, S.; Drouet, L.; Launay, J.-M.; Maroteaux, L. The natural mutation encoding a C terminus-truncated 5-hydroxytryptamine_{2B} receptor is a gain of proliferative functions. *Mol. Pharmacol.* **2005**, *67*, 983-991.
9. Abul Muntasir, H.; Takahashi, J.; Rashid, M.; Ahmed, M.; Komiyama, T.; Hossain, M.; Kawakami, J.; Nashimoto, M.; Nagatomo, T. Site-directed mutagenesis of the serotonin 5-hydroxytryptamine_{2C} receptor: identification of amino acids responsible for sarpogrelate binding. *Biol. Pharm. Bull.* **2006**, *29*, 1645-1650.

10. Canal, C. E.; Cordova-Sintjago, T. C.; Villa, N. Y.; Fang, L.-J.; Booth, R. G. Drug discovery targeting human 5-HT_{2C} receptors: Residues S3.36 and Y7.43 impact ligand-Binding pocket structure via hydrogen bond formation. *Eur. J. Pharmacol.* **2011**, *673*, 1-12.
11. Janoshazi, A.; Deraet, M.; Callebert, J.; Setola, V.; Guenther, S.; Saubamea, B.; Manivet, P.; Launay, J.-M.; Maroteaux, L. Modified receptor internalization upon coexpression of 5-HT_{1B} receptor and 5-HT_{2B} receptors. *Mol. Pharmacol.* **2007**, *71*, 1463-1474.
12. Rasmussen, S. G. F.; DeVree, B. T.; Zou, Y.-Z.; Kruse, A. C.; Chung, K.-Y.; Kobilka, T.-S.; Thian, F.-S.; Chae, P.-S.; Pardon, E.; Calinski, D.; Mathiesen, J. M.; Shah, S. T. A.; Lyons, J. A.; Caffrey, M.; Gellman, S. H.; Steyaert, J.; Skinotis, G.; Weis, W. I.; Sunahara, R. K.; Kobilka, B. K. Crystal structure of the β_2 adrenergic receptor-Gs protein complex. *Nature (London, U. K.)* **2011**, *477*, 549-555.
13. Rasmussen, S. G. F.; Choi, H.-J.; Rosenbaum, D. M.; Kobilka, T. S.; Thian, F. S.; Edwards, P. C.; Burghammer, M.; Ratnala, V. R. P.; Sanishvili, R.; Fischetti, R. F.; Schertler, G. F. X.; Weis, W. I.; Kobilka, B. K. Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. *Nature (London, U. K.)* **2007**, *450*, 383-387.
14. Fechteler, T.; Dengler, U.; Schomburg, D. Prediction of protein three-dimensional structures in insertion and deletion regions: a procedure for searching databases of representative protein fragments using geometric scoring criteria. *J. Mol. Biol.* **1995**, *253*, 114-131.
15. (a) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids. *J. Am. Chem. Soc.* **1996**, *118*, 11225-11236; (b) Kaminski, G. A.; Friesner, R. A.; Tirado-Rives, J.; Jorgensen, W. L. Evaluation and Reparametrization of the OPLS-AA Force Field for Proteins via Comparison with Accurate Quantum Chemical Calculations on Peptides. *J. Phys. Chem. B* **2001**, *105*, 6474-6487.
16. Courcot, B.; Bridgeman, A. J. Modeling the interactions between polyoxometalates and their environment. *J. Comput. Chem.* **2011**, *32*, 3143-3153.
17. Liebeschuetz, J. W.; Cole, J. C.; Korb, O. Pose prediction and virtual screening performance of GOLD scoring functions in a standardized test. *J. Comput.-Aided Mol. Des.* **2012**, *26*, 737-748.
18. (a) Kollman, P. A.; Massova, I.; Reyes, C.; Kuhn, B.; Huo, S.; Chong, L.; Lee, M.; Lee, T.; Duan, Y.; Wang, W.; Donini, O.; Cieplak, P.; Srinivasan, J.; Case, D. A.; Cheatham, T. E., III Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models. *Acc. Chem. Res.* **2000**, *33*, 889-897; (b) Heifetz, A.; Barker, O.; Verquin, G.; Wimmer, N.; Meutermans, W.; Pal, S.; Law, R. J.; Whittaker, M. Fighting Obesity with a Sugar-Based Library: Discovery of Novel MCH-1R Antagonists by a New Computational-VAST Approach for Exploration of GPCR Binding Sites. *J. Chem. Inf. Model.* **2013**, *53*, 1084-1099; (c) Hoffer, L.; Horvath, D. S4MPLE - Sampler For Multiple Protein-Ligand Entities: Simultaneous Docking of Several Entities. *J. Chem. Inf. Model.* **2013**, *53*, 88-102.
19. Homeyer, N.; Gohlke, H. FEW: A workflow tool for free energy calculations of ligand binding. *J. Comput. Chem.* **2013**, *34*, 965-973.
20. Labute, P. LowModeMD - Implicit Low-Mode Velocity Filtering Applied to Conformational Search of Macrocycles and Protein Loops. *J. Chem. Inf. Model.* **2010**, *50*, 792-800.
21. Berendsen, H. J. C.; Postma, J. P. M.; Van Gunsteren, W. F.; DiNola, A.; Haak, J. R. Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* **1984**, *81*, 3684-3690.

22. Dunlop, J.; Zhang, Y.; Ring, R.; Kowal, D. Measuring calcium mobilization with Gq-coupled GPCRs using the fluorometric imaging plate reader (FLIPR). *Drug Discovery Ser.* **2006**, *5*, 293-303.
23. (a) Gustafsson, B. I.; Tommeras, K.; Nordrum, I.; Loennechen, J. P.; Brunsvik, A.; Solligard, E.; Fossmark, R.; Bakke, I.; Syversen, U.; Waldum, H. Long-Term Serotonin Administration Induces Heart Valve Disease in Rats. *Circulation* **2005**, *111*, 1517-1522; (b) Adams, J. W.; Ramirez, J.; Ortuno, D.; Shi, Y.; Thomsen, W.; Richman, J. G.; Morgan, M.; Dosa, P.; Teegarden, B. R.; Al-Shamma, H.; Behan, D. P.; Connolly, D. T. Anti-thrombotic and vascular effects of AR246686, a novel 5-HT_{2A} receptor antagonist. *Eur. J. Pharmacol.* **2008**, *586*, 234-243.
24. Wakenhut, F.; Allan, G. A.; Fish, P. V.; Jonathan Fray, M.; Harrison, A. C.; McCoy, R.; Phillips, S. C.; Stobie, A.; Westbrook, D.; Westbrook, S. L.; Whitlock, G. A. N-[(3S)-Pyrrolidin-3-yl]benzamides as novel dual serotonin and noradrenaline reuptake inhibitors: Impact of small structural modifications on P-gp recognition and CNS penetration. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5078-5081.
25. (a) Stolzenburg, J.-U.; Neuhaus, J.; Liatsikos Evangelos, N.; Schwalenberg, T.; Ludewig, E.; Ganzer, R. Histomorphology of canine urethral sphincter systems, including three-dimensional reconstruction and magnetic resonance imaging. *Urology* **2006**, *67*, 624-630; (b) Sajadi, K. P.; Gill, B., C.; Damaser, M. S. Neurogenic aspects of stress urinary incontinence. *Curr Opin Obstet Gynecol* **2010**, *22*, 425-429.
26. Janssens, L. A.; Peeters, S. Comparisons between stress incontinence in women and sphincter mechanism incompetence in the female dog. *Vet Rec* **1997**, *141*, 620-625.
27. (a) Noel, S.; Massart, L.; Hamaide, A. Urodynamic investigation by telemetry in Beagle dogs: validation and effects of oral administration of current urological drugs: a pilot study. *BMC Vet Res* **2013**, *9*, 197; (b) Conlon, K.; Christy, C.; Westbrook, S.; Whitlock, G.; Roberts, L.; Stobie, A.; McMurray, G. Pharmacological properties of 2-((R-5-chloro-4-methoxymethyl-indan-1-yl)-1H-imidazole (PF-3774076), a novel and selective α 1A-adrenergic partial agonist, in in vitro and in vivo models of urethral function. *J. Pharmacol. Exp. Ther.* **2009**, *330*, 892-901; (c) Klarskov, N.; Scholfield, D.; Soma, K.; Darekar, A.; Mills, I.; Lose, G. Evaluation of the sensitivity of urethral pressure reflectometry (UPR) and urethral pressure profilometry (UPP) to detect pharmacological augmentation of urethral pressure, using [S,S]-Reboxetine. *J. Urol.* **2008**, *179*, 521-523; (d) Athanasiou, S.; Chaliha, C.; Digesu, G. A.; Sotiropoulou, M.; Georgoulis, N.; Khullar, V.; Antsaklis, A. The effects of duloxetine on urethral function and sphincter morphology. *Int Urogynecol J Pelvic Floor Dysfunct* **2007**, *18*, 763-767; (e) Musselman, D. M.; Ford, A. P. D. W.; Gennevois, D. J.; Harbison, M. L.; Laurent, A. L.; Mokattrin, A. S.; Stoltz, R. R.; Blue, D. R. A randomized crossover study to evaluate Ro 115-1240, a selective α 1A/1L-adrenoceptor partial agonist in women with stress urinary incontinence. *BJU Int.* **2004**, *93*, 78-83; (f) Fish, P. V.; Harrison, A.; Wakenhut, F.; Whitlock, G. A. A case history on the challenges of central nervous system and dual pharmacology drug discovery. *RSC Drug Discovery Ser.* **2011**, *4*, 267-286; (g) Conlon, K.; Miner, W.; McCleary, S.; McMurray, G. Identification of 5-HT_{2C} mediated mechanisms involved in urethral sphincter reflexes in a guinea-pig model of urethral function. *BJU Int.* **2012**, *110*, e113-e117; (h) Brennan, P. E.; Whitlock, G. A.; Ho, D. K. H.; Conlon, K.; McMurray, G. Discovery of a novel azepine series of potent and selective 5-HT_{2C} agonists as potential treatments for urinary incontinence. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4999-5003; (i) Andrews, M. D.; Green, M. P.; Allerton, C. M. N.; Batchelor, D. V.; Blagg, J.; Brown, A. D.; Gordon, D. W.; McMurray, G.; Millns, D. J.; Nichols, C. L.; Watson, L.

Design and synthesis of piperazinympyrimidinones as novel selective 5-HT_{2C} agonists. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5346-5350; (j) Andrews, M. D.; Fish, P. V.; Blagg, J.; Brabham, T. K.; Brennan, P. E.; Bridgeland, A.; Brown, A. D.; Bungay, P. J.; Conlon, K. M.; Edmunds, N. J.; af Forselles, K.; Gibbons, C. P.; Green, M. P.; Hanton, G.; Holbrook, M.; Jessiman, A. S.; McIntosh, K.; McMurray, G.; Nichols, C. L.; Root, J. A.; Storer, R. I.; Sutton, M. R.; Ward, R. V.; Westbrook, D.; Whitlock, G. A. Pyrimido[4,5-d]azepines as potent and selective 5-HT_{2C} receptor agonists: Design, synthesis, and evaluation of PF-3246799 as a treatment for urinary incontinence. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2715-2720.

28. Bishop, M. J.; Nilsson, B. M. New 5-HT_{2C} receptor agonists. *Expert Opin. Ther. Pat.* **2003**, *13*, 1691-1705.

29. Rodgers, T.; Rowland, M. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. *J. Pharm. Sci.* **2006**, *95*, 1238-1257.