

Sustained antidepressant-like effects of psychedelics through 5-HT_{2A}R- β -arrestin signaling

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Supplementary Table 1 | Pharmacology parameters of IHCH-8134 for serotonin and dopamine receptors.

	β-arrestin association		G protein	
	EC ₅₀ / IC ₅₀ (nM) (pEC ₅₀ / pIC ₅₀ ± SEM)	E _{max} (%)	EC ₅₀ / IC ₅₀ (nM) (pEC ₅₀ / pIC ₅₀ ± SEM)	E _{max} (%)
5-HT _{1A} R	Antagonist >10000	-	N. D	N. D
5-HT _{1B} R	agonist 4786.30 (5.32 ± 0.19)	13.31%	agonist 1479.11 (5.83 ± 0.14)	48.89%
5-HT _{1D} R	agonist 851.14 (6.07 ± 0.22)	29.82%	agonist 691.83 (6.16 ± 0.17)	82.76%
5-HT _{1E} R	agonist >10000	-	agonist >10000	-
5-HT _{1F} R	N. D	N. D	agonist >10000	-
5-HT _{2A} R	agonist 169.82 (6.77 ± 0.10)	56.77%	N. D	N. D
5-HT _{2B} R	agonist 83.18 (7.08 ± 0.07)	40.36%	inverse agonist 125.89 (6.90 ± 0.55)	-14.55%
5-HT _{2C} R	N. D	N. D	agonist 707.95 (6.15 ± 0.24)	46.48%
5-HT ₄ R	N. D	N. D	N. D	N. D
5-HT ₅ R	N. D	N. D	N. D	N. D
5-HT ₆ R	antagonist 4466.84 (5.35 ± 0.32)	-	agonist >10000	-
5-HT ₇ R	N. D	N. D	agonist >10000	-
D ₁ R	antagonist >10000	-	antagonist >10000	-
D ₂ R	antagonist >10000	-	antagonist 3235.94 (5.49 ± 0.07)	-
D ₃ R	agonist 1513.56 (5.82 ± 0.72)	41.52%	N. D	N. D

D ₄ R	antagonist >10000	-	agonist 5370.32 (5.27 ± 0.42)	4.46%
D ₅ R	N. D	N. D	N. D	N. D

All data are the mean ± SEM (n = 3-4 independent experiments), and normalized to reference serotonin for serotonin receptors and dopamine for dopamine receptors. N.D means no detectible data. As for G protein, 5-HT_{1A}R, 5-HT_{1B}R, 5-HT_{1D}R, 5-HT_{1E}R, 5-HT_{1F}R, 5-HT₅R, D₂R, and D₄R were measured by BRET Gα_{i1}-Gβ₃Gγ₉ dissociation; D₃R were measured by BRET Gα_{0A}-Gβ₃Gγ₈ dissociation; 5-HT_{2A}R, 5-HT_{2B}R, and 5-HT_{2C}R were measured by BRET G_q bystander; 5-HT₄R, 5-HT₆R, 5-HT₇R, D₁R, and D₅R were measured by BRET miniG_s association.

Supplementary Table 2 | Affinity of IHCH-8134 for serotonin receptors.

	K_i, nM	pK_i ± SEM
5-HT _{2A} R	17.38	7.76 ± 0.02
5-HT _{2B} R	19.95	7.70 ± 0.03
5-HT _{2C} R	41.69	7.38 ± 0.16

Data represent mean K_i (pK_i ± SEM) for competition binding experiments using 0.75 nM [³H]-LSD as the radioligand. All data are the mean ± SEM (n = 3 independent experiments).

Supplementary Table 3 | Crystal data collection, model refinement and validation statistics.

5-HT_{2A}R / pimavanserin	
Data Collection	Spring8, BL41/45XU 10-μm microfocus beam
Resolution (Å)	49.41-2.60 (2.72-2.60) *
Space group	P2 ₁ 22 ₁
Unit cell dimensions a, b, c (Å)	49.41, 54.70, 179.34
α, β, γ (°)	90, 90, 90
No. total reflections	451863 (14523)
No. unique reflections	14136 (1293)
Multiplicity	32.0 (11.2)
Completeness (%)	90.0 (69.7)
Mean I/σ(I)	9.8 (1.1)
R _{merge} (%)	25.5 (195.2)
R _{pim} (%)	5.0 (73.0)
CC _{1/2} (%)	99.9 (38.1)
Refinement Statistics	
Resolution used in refinement (Å)	47.64-2.60 (2.69-2.60)
No. reflections used in refinement	14088 (1053)
No. reflections used for R-free	776 (57)
R-work (%)	24.2 (37.8)
R-free (%)	29.1 (43.9)
Number of atoms	
5-HT _{2A} R	2209
mbIIIG	585
Ligand	31
Lipids	154
Ion	1
Overall B-factors (Å²)	
5-HT _{2A} R	81.3
mbIIIG	98.1
Ligand	82.7
Lipids	121.6
Ion	30.3
Model Statistics	
RMSD Bond (Å)	0.013
RMSD Angles (°)	1.83
Ramachandran Favored (%)	96.66
Ramachandran Allowed (%)	3.34
Ramachandran Outliers (%)	0

*Values in parentheses are for the highest-resolution shell.

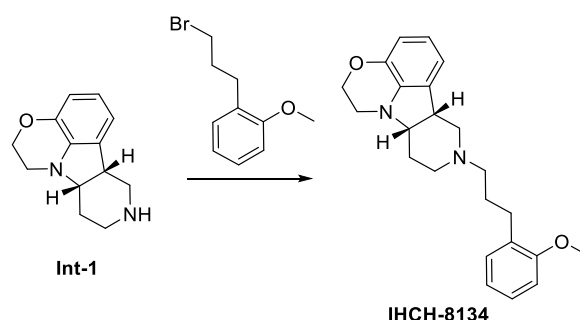
Supplementary Table 4 | Cryo-EM data collection, model refinement and validation statistics.

5-HT _{2A} R / IHCH-8152	
Data collection and processing	
Detector	K3
Magnification	105000
Voltage (kV)	300
Electron exposure (e ⁻ /Å ²)	60
Defocus range (μm)	-1.0~-2.5
Pixel size (Å)	0.832
Symmetry imposed	C1
Initial particle projections (No.)	9885586
Final particle projections (No.)	791492
Map resolution (Å)	3.19
Map resolution range (Å)	3.08-3.50
FSC threshold	0.143
Model Refinement	
Refinement package	Phenix-1.20.1-4487
Real or reciprocal space	Real space
Model-Map CC (mask)	0.78
Model resolution (Å)	3.37
FSC threshold	0.5
B factors (Å², min / max / mean value)	
Protein	15.67/154.06/76.31
Ligand	59.90/99.93/80.63
Model composition	
Non-hydrogen atoms	8221
Protein residues	1117
Ligands	1
RMSD	
Bond lengths (Å)	0.004
Bond angles (°)	0.681
Validation	
MolProbity score	1.74
Clashscore	9.95
Rotamer outliers (%)	0.90
Ramachandran plot (%)	
Favored	96.55
Allowed	3.45
Outliers	0.00

Chemistry and synthetic procedures

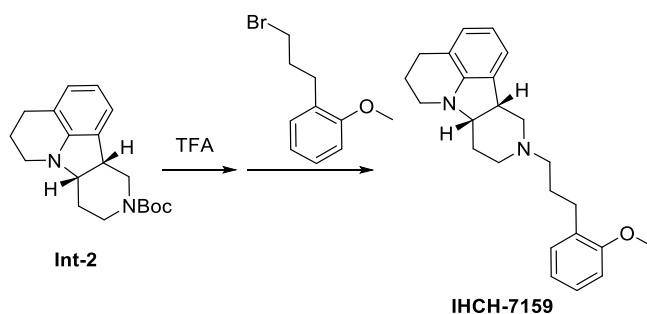
All commercial reagents and solvents were used as obtained without further purification. Synthetic intermediates and final compounds were purified on 230 - 400 mesh silica gel on Teledyne CombiFlash Rf flash chromatography. Compounds were characterized with ^1H NMR and ^{13}C NMR spectra recorded on Bruker AVANCE-III or Bruker AVANCE-III HD spectrometers. NMR chemical shifts were reported in δ (ppm) using residual solvent peaks as standards (CDCl_3 -7.26 (H), 77.16 (C); $\text{MeOH}-d_6$ -3.31 (H), 49.00 (C); $\text{DMSO}-d_6$ -2.50 (H), 39.60 (C)). High-resolution mass spectra (HRMS) were measured using an Agilent 6230 TOF LC/MS spectrometer in ESI mode. Purity of final compounds was determined by analytical HPLC (Shim-pack GIST C18 column (250 x 4.6 mm, particle size 5 μm); 0.05% TFA in H_2O /0.05% TFA in MeOH gradient eluting system; flow rate = 1.0 mL/min, λ = 254 nm). The purity of all final compounds was > 95%.

Synthesis of compound IHCH-8134:



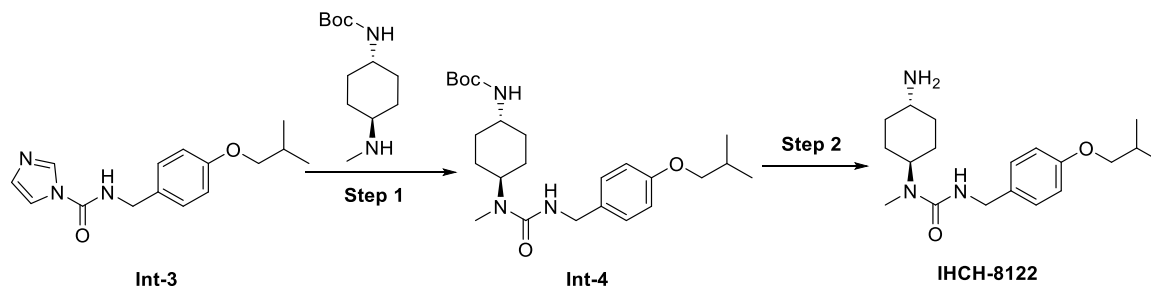
To a solution of **Int-1** (synthesized according to reported procedures¹) (108 mg, 0.5 mmol) and 1-(3-bromopropyl)-2-methoxybenzene (synthesized according to reported methods²) (115 mg, 0.5 mmol) in anhydrous *N,N*-dimethylformamide (DMF) (5 mL) was added *N,N*-diisopropylethylamine (DIPEA) (0.2 mL, 1.5 mmol) at room temperature. The mixture was then heated to 60 °C and stirred overnight. After the reaction was completed, water was added to quench the reaction and the mixture was extracted with ethyl acetate, then the organic phases were combined, washed with brine and dried over anhydrous sodium sulfate. Volatiles were removed and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 50/50). Compound **IHCH-8134** was obtained as yellow oil (83 mg, yield: 51%). ^1H NMR (800 MHz, CDCl_3) δ 7.17 (m, 1H), 7.14 – 7.12 (m, 1H), 6.87 (t, J = 7.3 Hz, 1H), 6.83 (d, J = 8.1 Hz, 1H), 6.69 (d, J = 7.3 Hz, 1H), 6.66 – 6.60 (m, 2H), 4.47 – 4.40 (m, 2H), 3.81 (s, 3H), 3.29 – 3.27 (m, 1H), 3.27 – 3.24 (m, 1H), 3.23 – 3.20 (m, 1H), 2.96 – 2.94 (m, 1H), 2.76 – 2.73 (m, 2H), 2.64 – 2.60 (m, 2H), 2.48 – 2.39 (m, 2H), 2.33 – 2.30 (m, 1H), 2.07 – 2.02 (m, 2H), 2.01 – 1.96 (m, 1H), 1.95 – 1.94 (m, 1H), 1.86 – 1.81 (m, 2H). ^{13}C NMR (201 MHz, CDCl_3) δ 157.53, 143.58, 137.94, 130.42, 129.90, 127.20, 124.11, 120.49, 120.47, 115.89, 113.33, 110.31, 66.87, 65.53, 58.44, 55.34, 48.83, 44.74, 41.28, 31.57, 30.32, 29.85, 28.29. HRMS (ESI) m/z calculated for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{O}_2^+$ ($[\text{M}+\text{H}]^+$): 365.2224, found: 365.2225. $[\alpha]_{\text{D}}^{25} = +49^\circ$ ($c=0.1$, CHCl_3).

Synthesis of compound IHCH-7159:



The intermediate **Int-2** was obtained according to reported procedures.¹ To the solution of **Int-2** (72 mg, 0.2 mmol) in dichloromethane (3 mL), trifluoroacetic acid (TFA) (0.5 mL) was added. One hour later, the solvent was removed and the residue was dissolved in dimethyl sulfoxide (DMSO) (3 mL). Then 1-(3-bromopropyl)-2-methoxybenzene (61 mg, 0.27 mmol), and DIPEA (2 mL) were added. The resulted mixture was stirred at 60 °C for 3 hours. Water (50 mL) was added and the reaction mixture was extracted with ethyl acetate. The combined extracts were washed with brine and concentrated *in vacuo*. The resulting residue was purified on flash chromatography (methanol/dichloromethane = 5/95) to give **IHCH-7159** (45 mg, 60%) as brown oil. ¹H NMR (600 MHz, CDCl₃) δ 7.19 – 7.15 (m, 1H), 7.13 (d, *J* = 7.4 Hz, 1H), 6.91 (d, *J* = 7.2 Hz, 1H), 6.89 – 6.82 (m, 3H), 6.63 (t, *J* = 7.4 Hz, 1H), 3.81 (s, 1H), 3.29 – 3.24 (m, 2H), 3.19 – 3.15 (m, 1H), 2.92 – 2.84 (m, 1H), 2.72 – 2.68 (m, 3H), 2.63 – 2.62 (m, 2H), 2.55 – 2.51 (m, 1H), 2.43 – 2.35 (m, 2H), 2.29 – 2.24 (m, 1H), 2.16 – 2.05 (m, 2H), 1.99 – 1.93 (m, 3H), 1.85 – 1.79 (m, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 157.55, 149.77, 131.16, 130.68, 129.90, 127.10, 126.79, 120.98, 120.52, 120.44, 118.62, 110.28, 64.18, 58.68, 56.11, 55.33, 49.03, 44.62, 41.16, 28.35, 27.07, 25.01, 24.28, 23.17. HRMS (ESI) *m/z* calculated for C₂₄H₃₁N₂O⁺ ([M+H]⁺): 363.2431, found: 363.2438.

Synthesis of compound **IHCH-8122**:

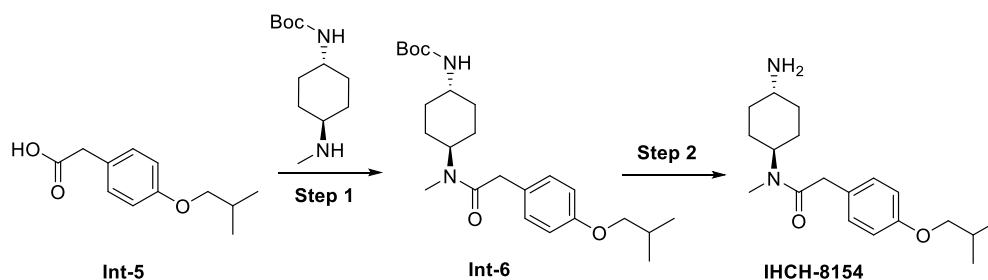


Step 1: To a mixture of **Int-3** (synthesized according to reported procedures³) (273.3 mg, 1.0 mmol) and potassium carbonate (207.3 mg, 1.5 mmol) in DMF (10 mL), *tert*-butyl (*trans*-4-(methylamino)cyclohexyl)carbamate (228.3 mg, 1.0 mmol) was added, and the mixture was

stirred at room temperature overnight. After the reaction was completed, water was added and the mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by silica chromatography (methanol/dichloromethane = 5/95) to give **Int-4** as white solid (301 mg, 69.5% yield). ^1H NMR (800 MHz, CDCl_3) δ 7.23 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 4.35 (s, 2H), 4.15 (s, 1H), 3.70 (d, J = 6.6 Hz, 2H), 3.37 (s, 1H), 2.69 (s, 3H), 2.10 – 2.03 (m, 3H), 1.74 – 1.69 (m, 2H), 1.53 – 1.47 (m, 2H), 1.43 (s, 9H), 1.29 – 1.22 (m, 2H), 1.01 (d, J = 6.7 Hz, 6H). HRMS (ESI) m/z calculated for $\text{C}_{24}\text{H}_{40}\text{N}_3\text{O}_4^+$ ($[\text{M}+\text{H}]^+$): 434.3013, found: 434.3019.

Step 2: To a solution of **Int-4** (300 mg, 0.72 mmol) in ethyl acetate was added HCl (g) in 1,4-dioxane (2M, 1 mL), and the mixture was stirred at room temperature for 4 hours. Volatiles were then removed under vacuum and the crude product was purified by silica chromatography (methanol/dichloromethane = 5/95) to give compound **IHCH-8122** as a white solid (212 mg, 89% yield). ^1H NMR (800 MHz, $\text{MeOH}-d_4$) δ 7.19 (d, J = 8.6 Hz, 2H), 6.83 (d, J = 8.1 Hz, 2H), 4.28 (s, 2H), 4.12 – 4.03 (m, 1H), 3.70 (d, J = 6.4 Hz, 2H), 3.11 – 3.04 (m, 1H), 2.79 – 2.75 (m, 3H), 2.09 (d, J = 11.5 Hz, 2H), 2.06 – 1.99 (m, 1H), 1.76 – 1.71 (m, 2H), 1.71 – 1.62 (m, 2H), 1.57 – 1.50 (m, 2H), 1.01 (d, J = 6.7 Hz, 6H). ^{13}C NMR (201 MHz, $\text{MeOH}-d_4$) δ 159.12, 158.31, 132.19, 128.06, 113.98, 74.12, 52.29, 49.12, 43.38, 29.60, 28.14, 27.35, 27.25, 18.17, 18.15. HRMS (ESI) m/z calculated for $\text{C}_{19}\text{H}_{32}\text{N}_3\text{O}_2^+$ ($[\text{M}+\text{H}]^+$): 334.2489; found: 334.2493.

Synthesis of compound **IHCH-8154**:

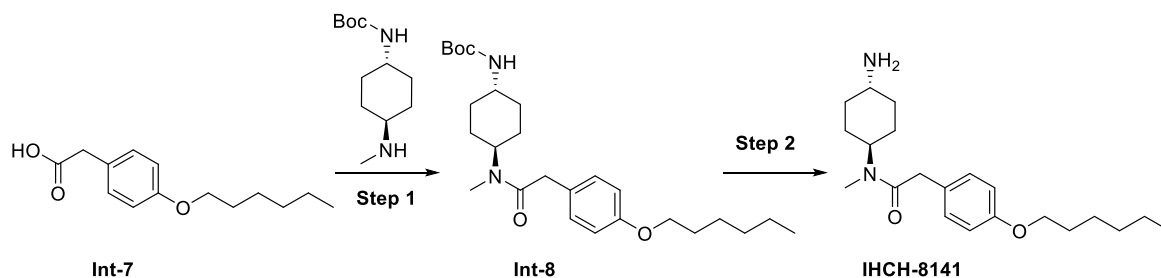


Step 1: A mixture of intermediate **Int-5** (synthesized according to reported procedures⁴) (500 mg, 2.0 mmol), *tert*-butyl (*trans*-4-(methylamino)cyclohexyl)carbamate (460 mg, 2.0 mmol), 2-(7-azabenzotriazol-1-yl)-*N,N,N,N*-tetramethyluronium hexafluorophosphate (HATU) (1.1 g, 3.0 mmol) and DIPEA (1 mL, 6 mmol) in anhydrous DMF (10 mL) was stirred at room temperature overnight. Water was added to quench the reaction and the mixture was then extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by silica chromatography (ethyl acetate/petroleum ether = 50/50) to give **Int-6** as yellow oil (322 mg, 70% yield). ^1H NMR (800 MHz, CDCl_3) δ 7.14 (dd, J = 22.6, 8.3 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 4.54 – 4.34 (m, 1H), 3.69 – 3.63 (m, 2H), 3.68 – 3.62 (m, 2H), 3.41 – 3.31 (m, 1H), 2.78 (s, 3H), 2.11 – 1.96 (m, 3H), 1.69 – 1.62 (m, 2H), 1.60 – 1.48 (m, 3H), 1.43 (s, 9H), 1.30 – 1.24 (m, 1H), 1.01 (d, J = 6.7 Hz, 6H). HRMS (ESI) m/z calculated for $\text{C}_{24}\text{H}_{39}\text{N}_2\text{O}_4^+$ ($[\text{M}+\text{H}]^+$): 419.2904, found: 419.2912.

Step 2: To a solution of **Int-6** (210 mg, 0.5 mmol) in ethyl acetate (2 mL) was added HCl (g) in 1,4-dioxane (2M, 1 mL), and the mixture was stirred at room temperature for 4 hours. Volatiles

were then removed under vacuum and the crude product was purified by silica chromatography (methanol/dichloromethane = 5/95) to give compound **IHCH-8154** as a white solid (123 mg, 77.3% yield). ^1H NMR (800 MHz, DMSO- d_6) δ 7.12 – 7.10 (m, 2H), 6.85 (t, J = 8.3 Hz, 2H), 4.26 – 4.18 & 3.67 – 3.64 (m, 1H), 3.72 – 3.69 (m, 2H), 3.67 (s, 1H), 3.58 (s, 1H), 2.97 – 2.87 (m, 1H), 2.83 – 2.62 (m, 3H), 2.02 – 1.96 (m, 2H), 1.94 – 1.88 (m, 1H), 1.62 – 1.47 (m, 3H), 1.45 – 1.40 (m, 1H), 1.39 – 1.33 (m, 2H), 0.97 (d, J = 6.7 Hz, 6H). ^{13}C NMR (201 MHz, DMSO- d_6) δ 170.76, 157.78, 130.27, 130.17, 127.97, 114.85, 114.77, 74.15, 55.03, 51.05, 48.79, 30.03, 29.82, 29.71, 28.18, 27.92, 27.39, 26.94, 19.54. HRMS (ESI) m/z calculated for $\text{C}_{19}\text{H}_{31}\text{N}_2\text{O}_2^+$ ($[\text{M}+\text{H}]^+$): 319.2380, found: 319.2385.

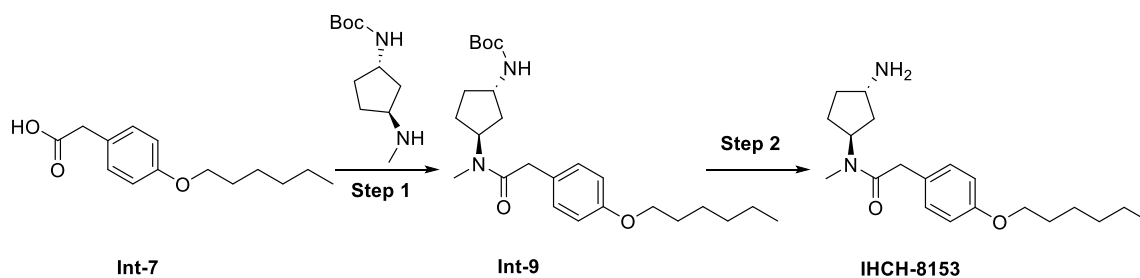
Synthesis of compound **IHCH-8141**:



Step 1: A mixture of intermediate **Int-7** (synthesized according to reported procedures⁴) (472 mg, 2.0 mmol), *tert*-butyl (*trans*-4-(methylamino)cyclohexyl)carbamate (460 mg, 2.0 mmol), HATU (1.1 g, 3.0 mmol) and DIPEA (1 mL, 6 mmol) in anhydrous DMF (10 mL) was stirred at room temperature overnight. Water was added and the mixture was then extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by silica chromatography (ethyl acetate/petroleum ether = 50/50) to give **Int-8** as yellow oil (560 mg, 67% yield). ^1H NMR (800 MHz, CDCl_3) δ 7.13 (t, J = 7.9 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 4.48 (s, 1H), 4.37 (d, J = 39.9 Hz, 1H), 3.92 (t, J = 6.6 Hz, 2H), 3.71 – 3.61 (m, 2H), 2.78 (s, 3H), 2.06 – 1.98 (m, 2H), 1.78 – 1.74 (m, 2H), 1.71 – 1.64 (m, 6H), 1.61 – 1.52 (m, 1H), 1.44 (d, J = 18.8 Hz, 11H), 1.35 – 1.31 (m, 4H), 0.95 – 0.87 (m, 3H). HRMS (ESI) m/z calculated for $\text{C}_{26}\text{H}_{43}\text{N}_2\text{O}_4^+$ ($[\text{M}+\text{H}]^+$): 447.3217, found: 447.3221.

Step 2: To a solution of **Int-7** (223 mg, 0.5 mmol) in ethyl acetate (2 mL), HCl (g) in 1,4-dioxane (2M, 1 mL) was added, the mixture was stirred at room temperature for 4 hours. Volatiles were then removed under vacuum and the crude product was purified by silica chromatography (methanol/dichloromethane = 5/95) to give compound **IHCH-8141** as a white solid (143 mg, 83% yield). ^1H NMR (800 MHz, DMSO- d_6) δ 7.12 – 7.09 (m, 2H), 6.85 (t, J = 8.0 Hz, 2H), 4.27 – 4.18 & 3.67 – 3.64 (m, 1H), 3.94 – 3.88 (m, 2H), 3.69 – 3.67 (m, 1H), 3.58 (s, 1H), 2.97 – 2.88 (m, 1H), 2.79 – 2.65 (m, 3H), 1.99 (d, J = 11.8 Hz, 1H), 1.95 – 1.88 (m, 1H), 1.71 – 1.66 (m, 2H), 1.59 – 1.47 (m, 3H), 1.44 – 1.34 (m, 5H), 1.31 – 1.28 (m, 4H), 0.87 (t, J = 7.0 Hz, 3H). ^{13}C NMR (201 MHz, DMSO- d_6) δ 170.30, 157.20, 129.81, 129.69, 127.46, 114.31, 114.24, 67.31, 54.54, 50.56, 48.32, 48.10, 31.01, 29.37, 28.69, 26.46, 25.22, 22.08, 13.92. HRMS (ESI) m/z calculated for $\text{C}_{21}\text{H}_{35}\text{N}_2\text{O}_2^+$ ($[\text{M}+\text{H}]^+$): 347.2693, found: 347.2693.

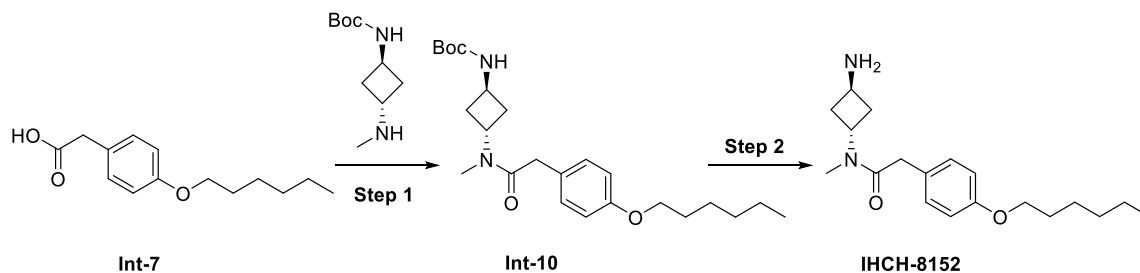
Synthesis of compound **IHCH-8153**:



Step 1: A mixture of intermediate **Int-7** (472 mg, 2.0 mmol), *tert*-butyl ((1*s*, 3*s*)-3-(methylamino)cyclopentyl)carbamate (432 mg, 2.0 mmol), HATU (1.1 g, 3.0 mmol) and DIPEA (1 mL, 6 mmol) in anhydrous DMF (10 mL) was stirred at room temperature overnight. Water was added and the mixture was then extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by silica chromatography (ethyl acetate/petroleum ether = 50/50) to give **Int-9** as yellow oil (524 mg, 61% yield). ¹H NMR (800 MHz, CDCl₃) δ 7.11 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 5.23 – 5.09 & 4.59 – 4.56 (m, 1H), 4.45 – 4.35 & 4.04 – 4.02 (m, 1H), 3.92 (t, *J* = 6.6 Hz, 2H), 3.73 – 3.58 (m, 2H), 2.80 (s, 3H), 2.78 (s, 1H), 2.15 – 2.04 (m, 1H), 1.94 – 1.86 (m, 1H), 1.86 – 1.82 (m, 1H), 1.80 – 1.72 (m, 4H), 1.72 – 1.62 (m, 2H), 1.50 – 1.39 (m, 13H), 1.34 – 1.31 (m, 4H), 0.89 (t, *J* = 6.9 Hz, 3H). HRMS (ESI) *m/z* calculated for C₂₅H₄₁N₂O₄⁺ ([M+H]⁺): 433.3061, found: 433.3066.

Step 2: To a solution of **Int-9** (201 mg, 0.5 mmol) in ethyl acetate (2 mL), HCl (g) in 1,4-dioxane (2M, 1 mL) was added, the mixture was stirred at room temperature for 4 hours. Volatiles were then removed under vacuum and the crude product was purified by silica chromatography (methanol/dichloromethane = 5/95) to give compound **IHCH-8153** as a white solid (92 mg, 75% yield). ¹H NMR (800 MHz, DMSO-*d*₆) δ 7.19 (d, *J* = 5.8 Hz, 1H), 7.09 (d, *J* = 8.2 Hz, 1H), 6.88 – 6.80 (m, 2H), 5.05 – 4.96 & 4.79 – 4.74 (m, 1H), 3.92 (t, *J* = 6.4 Hz, 2H), 3.74 – 3.63 (m, 1H), 3.60 – 3.56 (m, 2H), 2.99 (d, *J* = 7.0 Hz, 1H), 2.84 – 2.62 (m, 3H), 2.10 – 2.01 (m, 1H), 1.93 – 1.83 (m, 1H), 1.82 – 1.74 (m, 2H), 1.71 – 1.67 (m, 2H), 1.62 – 1.53 (m, 2H), 1.43 – 1.36 (m, 2H), 1.29 (t, *J* = 16.2 Hz, 4H), 0.88 (t, *J* = 6.3 Hz, 3H). ¹³C NMR (201 MHz, DMSO-*d*₆) δ 171.24, 157.69, 130.45, 130.40, 128.31, 114.68, 114.65, 67.78, 56.19, 52.55, 50.06, 49.55, 49.04, 45.77, 31.48, 29.16, 27.29, 25.69, 22.55, 14.38. HRMS (ESI) *m/z* calculated for C₂₀H₃₃N₂O₂⁺ ([M+H]⁺): 333.2537, found: 333.2554.

Synthesis of compound IHCH-8152:



Step 1: A mixture of intermediate **Int-7** (472 mg, 2.0 mmol), *tert*-butyl (*trans*-3-(methylamino)cyclobutyl)carbamate (404 mg, 2.0 mmol), HATU (1.1g, 3.0 mmol) and DIPEA (1 mL, 6 mmol) in anhydrous DMF (10 mL) was stirred at room temperature overnight. Water was added and

the mixture was then extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by silica chromatography (ethyl acetate/petroleum ether = 50/50) to give **Int-10** as yellow oil (568 mg, 68% yield). ¹H NMR (800 MHz, CDCl₃) δ 7.11 (d, *J* = 8.0 Hz, 2H), 6.87 – 6.81 (m, 2H), 4.68 - 4.62 & 4.07 – 3.96 (m, 1H), 3.92 (t, *J* = 6.6 Hz, 2H), 3.66 (s, 2H), 2.94 (s, 3H), 2.51 – 2.37 (m, 2H), 1.78 – 1.74 (m, 2H), 1.48 – 1.43 (m, 11H), 1.35 – 1.31 (m, 4H), 0.90 (t, *J* = 6.5 Hz, 3H). HRMS (ESI) *m/z* calculated for C₂₄H₃₉N₂O₄⁺ ([M+H]⁺): 419.2904, found: 419.2911.

Step 2: To a solution of **Int-10** (209 mg, 0.5 mmol) in ethyl acetate (2 mL) was added HCl (*g*) in 1,4-dioxane (2M, 1 mL), and the mixture was stirred at room temperature for 4 hours. Volatiles were then removed under vacuum and the crude product was purified by silica chromatography (methanol/dichloromethane = 5/95) to give compound **IHCH-8152** as a white solid (88 mg, 81% yield). ¹H NMR (800 MHz, DMSO-*d*₆) δ 7.15 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 6.84 (t, *J* = 8.0 Hz, 2H), 3.91 (t, *J* = 6.3 Hz, 2H), 3.65 – 3.61 (m, 1H), 3.59 – 3.57 (m, 1H), 3.55 – 3.51 (m, 1H), 3.02 – 2.73 (m, 3H), 2.55 – 2.46 (m, 2H), 2.16 – 2.05 (m, 2H), 1.71 – 1.65 (m, 2H), 1.43 – 1.36 (m, 2H), 1.33 – 1.26 (m, 4H), 0.90 – 0.84 (m, 3H). ¹³C NMR (201 MHz, DMSO-*d*₆) δ 170.79, 157.25, 130.08, 130.05, 127.35, 114.22, 114.15, 67.33, 48.17, 45.37, 41.13, 40.68, 31.55, 31.03, 28.70, 27.22, 25.23, 22.10, 13.94. HRMS (ESI) *m/z* calculated for C₁₉H₃₁N₂O₂⁺ ([M+H]⁺): 319.2380, found: 319.2381.

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¹H NMR and ¹³C NMR spectra for all final compounds

