

Supporting Information

Table of contents

1. <i>In-vitro</i> Plasma Stability Study	2
2. <i>In-vitro</i> Microsomal Stability Study	3
3. Organic Synthesis	4
4. Biological Assays	20
5. Physicochemical Properties	22
6. Kinetic Solubility	24
7. Notes and references	25

1. *In-vitro* Plasma Stability Study

10 mM DMSO stock solution of test compound was diluted 20 fold with DMSO-H₂O (1:1) and incubated at 37°C for 2h with rat plasma added 5% DMSO (pre-heated at 37 °C for 10 min). The final concentration was 2 µM. At each time point (0, 5, 15, 30, 60, 120 min), 50 µL of incubation mixture was diluted with 200 µL cold CH₃CN spiked with 200 nM internal standard, followed by centrifugation at 3500 g for 20 min. The supernatant was further diluted with H₂O (1:1) for analysis. The concentration of test compound was quantified by LC/MS-MS. The percentage of test compound remaining at each time point relative to t = 0 was calculated. The half-lives ($t_{1/2}$) were determined by the more appropriate equation using a non-linear regression of compound concentration versus time, and were reported as mean values along with their standard deviations (n = 3).

The analyses were performed on a Waters ACQUITY UPLC/MS TQD system consisting of a TQD (Triple Quadrupole Detector) Mass Spectrometer equipped with an Electrospray Ionization interface and a Photodiode Array eλ Detector. The analyses were run on an ACQUITY UPLC BEH C₁₈ (50 x 2.1 mmID, particle size 1.7 µm) with a VanGuard BEH C₁₈ pre-column (5 x 2.1 mmID, particle size 1.7 µm) at 40 °C, using 0.1% HCOOH in H₂O (A) and 0.1% HCOOH in CH₃CN (B) as mobile phase.

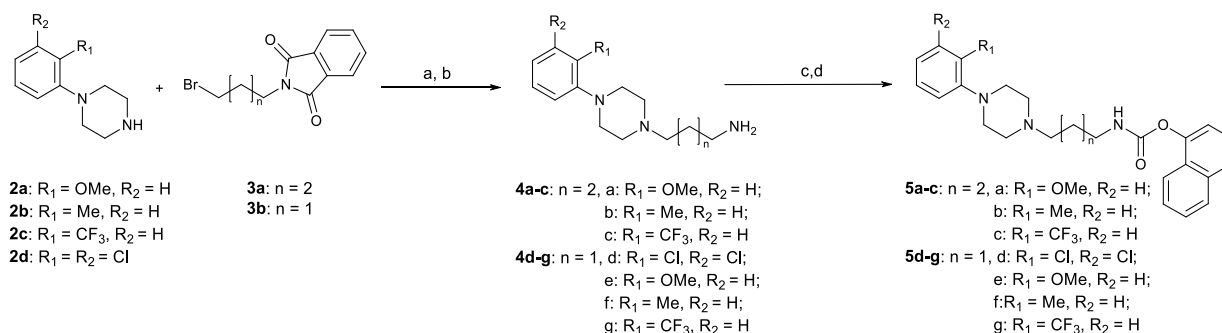
2. *In-vitro* Microsomal Stability Study

10 mM DMSO stock solution of test compound was pre-incubated at 37°C for 15 min with rat liver microsomes added 0.1M Tris-HCl buffer (pH 7.4) with 10% DMSO. The final concentration was 4.6 µM. After pre-incubation, the cofactors (NADPH, G6P, G6PDH and MgCl₂ pre-dissolved in 0.1M Tris-HCl) were added to the incubation mixture and the incubation was continued at 37 °C for 1 h. At each time point (0, 5, 15, 30, 60 min), 30 µL of incubation mixture was diluted with 200 µL cold CH₃CN spiked with 200 nM internal standard, followed by centrifugation at 3500 g for 20 min. The supernatant was further diluted with H₂O (1:1) for analysis. A reference incubation mixture (microsomes without cofactors) was prepared for each test compound and analysed at t = 0 and t = 60 min in order to verify the compound stability in the matrix. The concentration of test compound was quantified by LC/MS-MS. The percentage of test compound remaining at each time point relative to t = 0 was calculated. The half-lives ($t_{1/2}$) were determined by a one-phase decay equation using a non-linear regression of compound concentration versus time and were reported as mean values along with their standard deviations ($n = 3$).

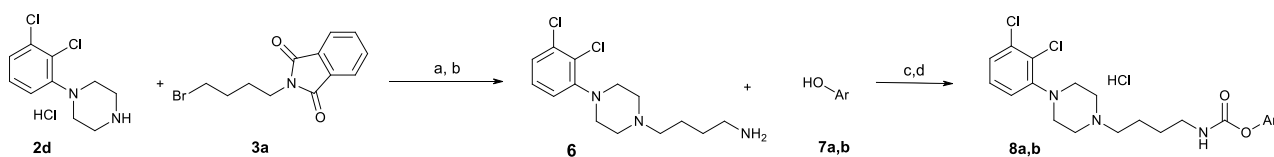
The analyses were performed on a Waters ACQUITY UPLC/MS TQD system consisting of a TQD (Triple Quadrupole Detector) Mass Spectrometer equipped with an Electrospray Ionization interface and a Photodiode Array eλ Detector. The analyses were run on an ACQUITY UPLC BEH C₁₈ (50 x 2.1 mmID, particle size 1.7 µm) with a VanGuard BEH C₁₈ pre-column (5 x 2.1 mmID, particle size 1.7 µm) at 40 °C, using 0.1% HCOOH in H₂O (A) and 0.1% HCOOH in CH₃CN (B) as mobile phase.

3. Organic Synthesis

Synthetic scheme

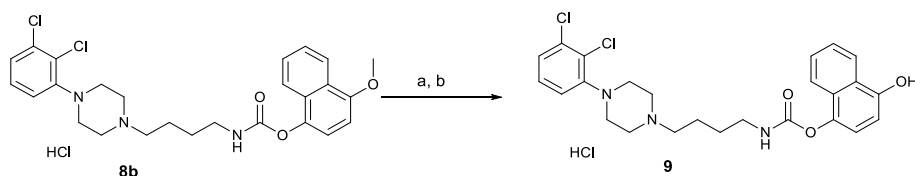


Scheme S1. a) CH₃CN, K₂CO₃, reflux, 5 h or CH₃CN, NEt₃, 100 °C, MW, 30 min; b) Hydrazine monohydrate, Methanol, reflux, 2 h, then 2 N HCl, reflux, 1 h; c) 1-Naphtol, *p*-nitrophenylchloroformate, DIPEA, DMA : DCM (1 : 1), r.t., 72 h or 1-Naphtol, (Boc)₂O, DMAP, CH₃CN, r.t., 24 h; d) 4 M HCl in 1,4-dioxane, r.t., 1 h for compound **5b**, **c** and **5f**.

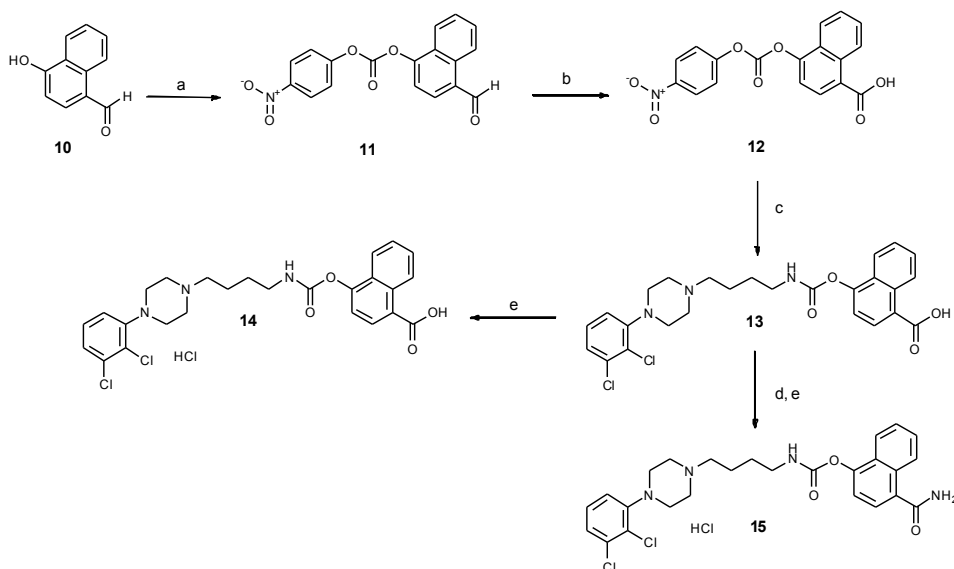


Compounds	Ar
7a/8a	
7b/8b	

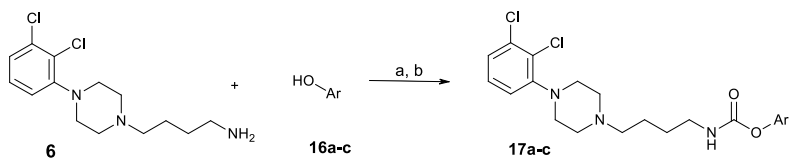
Scheme S2. a) CH₃CN, K₂CO₃, reflux, 5 h; b) Hydrazine monohydrate, Methanol, reflux, 2 h, then HCl 2N, reflux, 1 h; c) (Boc)₂O, DMAP, CH₃CN, r.t., 24 h; d) 4 M HCl in 1,4-dioxane or 1.25 M in MeOH, r.t., 1 h.



Scheme S3. a) BBr₃, DCM, r.t., overnight; b) 1.25 M HCl in MeOH, r.t., 1 h.

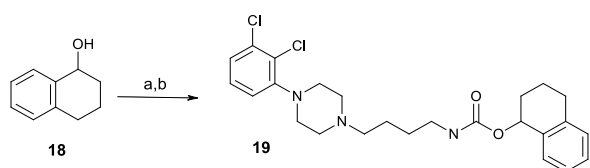


Scheme S4. a) *p*-nitrophenylchloroformate, DIPEA, DCM, r.t., 1 h; b) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, *t*-BuOH, H₂O, r.t., overnight; c) **6**, NEt₃, DCM, DMF, r.t., overnight; d) (Boc)₂O, NH₄HCO₃, Pyridine, 1,4-dioxane, 40 °C, 5 h; e). 1.25 M HCl in MeOH, r.t., 1 h.

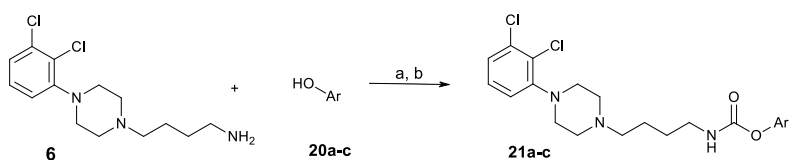


Compounds	Ar
16a/17a	
16b/17b	
16c/17c	

Scheme S5. a) (Boc)₂O, DMAP, CH₃CN, r.t., 24 h; b) 1.25 M HCl MeOH, r.t., 1 h for compound **17c**.

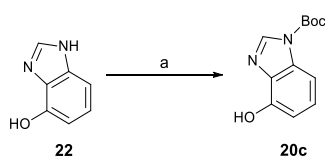


Scheme S6. a) *p*-nitrophenylchloroformate, Pyridine, DCM, r.t, 20 h; b) **6**, NEt₃, DCM, r.t., 2 h.

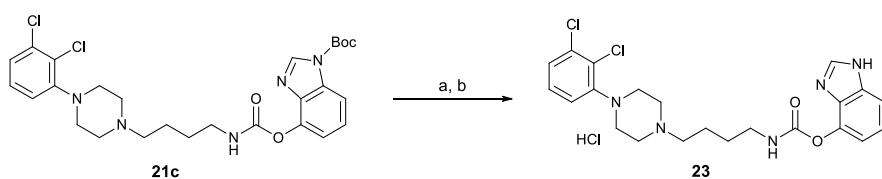


Compounds	Ar
20a/21a	
20b/21b	
20c/21c	

Scheme S7. a) $(\text{Boc})_2\text{O}$, DMAP, CH_3CN , r.t., 24 h; b) 1.25 M HCl MeOH, r.t., 1 h for compound **21a**.



Scheme S8. a) $(\text{Boc})_2\text{O}$, DMAP, NEt_3 , DMF, r.t., 2 h.



Scheme S9. a) 4 M HCl in 1,4-dioxane, r.t., 5 h, b) 1.25 M HCl in MeOH, r.t., 1 h.

General reagents and materials

Automated column chromatography purifications were performed on a Teledyne ISCO apparatus (CombiFlashTM Rf) with pre-packed silica gel columns of different sizes (from 4 g to 120 g). Mixtures of increasing polarity of cyclohexane and ethyl acetate or dichloromethane and methanol were used as eluents. NMR experiments were run on a Bruker Avance III 400 system (400.13 MHz for ^1H , and 100.62 MHz for ^{13}C), equipped with a BBI probe and Z-gradients. Spectra were acquired at 300 K, using deuterated dimethylsulfoxide ($\text{DMSO-}d_6$) or deuterated chloroform ($\text{Chloroform-}d$) as solvents. Chemical shifts for ^1H and ^{13}C spectra were recorded in parts per million using the residual non-deuterated solvent as the internal standard (for $\text{Chloroform-}d$: 7.26 ppm, ^1H and 77.16 ppm, ^{13}C ; for $\text{DMSO-}d_6$: 2.50 ppm, ^1H ; 39.52 ppm, ^{13}C).

Signals were attributed based on DEPT 135, COSY, HSQC and HMBC experiments. UPLC/MS analyses were run on a Waters ACQUITY UPLC/MS system consisting of a SQD (Single Quadrupole Detector) Mass Spectrometer equipped with an Electrospray Ionization interface and a Photodiode Array Detector. PDA range was 210-400 nm. Electrospray ionization in positive and negative mode was applied. UPLC mobile phases were: (A) 10 mM NH₄OAc in H₂O, pH 5; (B) 10 mM NH₄OAc in MeCN/H₂O (95 : 5) pH 5. Analyses were performed with method A and B.

Method A (generic):

Gradient: 5 to 95% B over 3 min. Flow rate 0.5 mL/min. Temp. 40 °C

Pre column: Vanguard BEH C18 (1.7 μm 2.1x5 mm). Column: BEH C18 (1.7 μm 2.1x50 mm)

Accurate mass measurement was performed on a quadrupole time-of-flight instrument (Synapt G2 QTof, Waters, USA), equipped with an ESI ion source. Compounds were diluted to 20 μM in water/acetonitrile and analyzed. Leucine Enkephalin (2 ng/mL) was used as lockmass reference compound for spectra calibration.

Microwave-assisted reactions were carried out in a CEM Discover reactor.

General procedure for the preparation of compounds 4a-f

A mixture of appropriate aryl piperazine (**2a-d**) (1 eq.), *N*-(bromoalkyl)phthalimide (**3a-b**) (1-1.1 eq.) and K₂CO₃ (2.5-3 eq.) in CH₃CN was heated to reflux for 5 h. The hot suspension was filtered and the residue was washed with acetone several time. The filtrates were concentrated under reduced pressure to afford the phthalimide intermediate, which was dissolved in MeOH, and heated to reflux in presence of hydrazine hydrate (1.2 eq.) for 2 h. To the hot solution 2 N HCl was added and reflux was continued for further 1 h. After cooling at room temperature the mixture was filtered, the residue was washed with methanol and the filtrates were concentrated under reduced pressure. The residue was suspended in water, alkalinized with 2 N NaOH until pH 10 and the aqueous solution was extracted with ethyl acetate. The organic phase was dried over Na₂SO₄ anhydrous, filtered and evaporated to dryness to give the product which was pure enough for the next step.

Synthesis of 4-[4-(2-methoxyphenyl)piperazin-1-yl]butan-1-amine (4a)

The title compound was synthesized using 1-(2-methoxyphenyl)piperazine hydrochloride (**2a**) (0.6 g, 2.62 mmol), *N*-(4-bromobutyl)phthalimide (**3a**) (0.814 g, 2.89 mmol) and K₂CO₃ (1.088 g, 7.87 mmol) in CH₃CN (7 mL). Treating of phthalimide intermediate with hydrazine hydrate (0.14 mL, 2.91 mmol) in MeOH (3 mL) afforded **5a** (0.388 g, 1.28 mmol, 49% yield over two steps) as a yellow oil. UPLC-MS (method A): Rt 1.07 min; m/z 264 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.07 – 6.90 (m, 3H), 6.87 (dt, *J* = 7.9, 1.5 Hz, 1H), 3.88 (d, *J* = 1.6 Hz, 3H), 3.12 (br. s, 4H), 2.74 (td, *J* = 6.9, 1.5 Hz, 2H), 2.67 (br. s, 4H), 2.44 (td, *J* = 7.3, 1.4 Hz, 2H), 1.65 – 1.55 (m, 2H), 1.54 – 1.46 (m, 2H).

Synthesis of 4-[4-(*o*-tolyl)piperazin-1-yl]butan-1-amine (**4b**)

The title compound was synthesized using 1-(2-methylphenyl)piperazine (**2b**) (0.350 g, 1.99 mmol), *N*-(4-bromobutyl)phthalimide (**3a**) (0.560 g, 1.99 mmol) and K₂CO₃ (0.686 g, 4.97 mmol) in CH₃CN (6 mL). Treating of phthalimide intermediate with hydrazine hydrate (0.12 mL, 2.38 mmol) in MeOH (4 mL) afforded **5b** (0.384 g, 1.55 mmol, 78% yield over two steps) as a yellow oil. UPLC-MS (method A): Rt 1.26 min; m/z 248 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.19 – 7.12 (m, 2H), 7.05 – 7.00 (m, 1H), 6.99 – 6.93 (m, 1H), 2.94 (t, *J* = 4.8 Hz, 4H), 2.72 (t, *J* = 6.8 Hz, 2H), 2.60 (br. s, 4H), 2.46 – 2.36 (m, 2H), 2.29 (s, 3H), 1.63 – 1.43 (m, 4H).

Synthesis of 4-[4-[2-(trifluoromethyl)phenyl]piperazin-1-yl]butan-1-amine (**4c**)

The title compound was synthesized using 1-(2-trifluoromethylphenyl)piperazine (**2c**) (0.350 g, 1.52 mmol), *N*-(4-bromobutyl)phthalimide (**3a**) (0.429 g, 1.52 mmol) and K₂CO₃ (0.525 g, 3.8 mmol) in CH₃CN (6 mL). Treating of phthalimide intermediate with hydrazine hydrate (0.08 mL, 1.67 mmol) in MeOH (4 mL) afforded **5c** (0.312 g, 1.03 mmol, 68% yield over two steps) as a yellow oil. UPLC-MS (method A): Rt 1.45 min; m/z 302 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.59 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.48 (td, *J* = 7.7, 1.5 Hz, 1H), 7.38 – 7.32 (m, 1H), 7.22 – 7.15 (m, 1H), 2.98 – 2.91 (m, 4H), 2.71 (t, *J* = 6.8 Hz, 2H), 2.58 (br. s, 4H), 2.45 – 2.35 (m, 2H), 1.59 – 1.41 (m, 4H).

Synthesis of 3-[4-(2,3-dichlorophenyl)piperazin-1-yl]propan-1-amine (**4d**)

The title compound was synthesized using 1-(2,3-dichlorophenyl)piperazine hydrochloride (**2d**) (0.400 g, 1.49 mmol), *N*-(3-bromopropyl)phthalimide (**3b**) (0.441 g, 1.64 mmol) and K₂CO₃ (0.516 g, 3.74 mmol) in CH₃CN (7 mL). Treating of phthalimide intermediate with hydrazine hydrate (0.07 mL, 1.53 mmol) in MeOH (4 mL) afforded **5d** (0.233 g, 0.81 mmol, 54% yield over two steps) as a yellow oil. UPLC-MS (method A): Rt 1.55 min; m/z 288 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.18 – 7.08 (m, 2H), 6.94 (dd, *J* = 6.7, 2.9 Hz, 1H), 3.14 – 2.95 (m, 4H), 2.79 (t, *J* = 6.7 Hz, 2H), 2.71 – 2.53 (m, 4H), 2.49 (t, *J* = 7.3 Hz, 2H), 1.68 (p, *J* = 6.9 Hz, 2H).

Synthesis of 3-[4-(2-methoxyphenyl)piperazin-1-yl]propan-1-amine (**4e**)

The title compound was synthesized using 1-(2-methoxyphenyl)piperazine hydrochloride (**2a**) (1.00 g, 4.37 mmol), *N*-(3-bromopropyl)phthalimide (**3b**) (1.289 g, 4.81 mmol) and K₂CO₃ (1.511 g, 10.93 mmol) in CH₃CN (7 mL). Treating of phthalimide intermediate with hydrazine hydrate (0.24 mL, 4.90 mmol) in MeOH (8 mL) afforded **5e** (0.631 g, 2.53 mmol, 58% yield over two steps) as a yellow oil. UPLC-MS (method A): Rt 1.10 min; m/z 250 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.01 (ddd, *J* = 7.9, 6.4, 2.6 Hz, 1H), 6.98 – 6.90 (m, 2H), 6.87 (dd, *J* = 8.0, 1.3 Hz, 1H), 3.87 (s, 3H), 3.11 (br. s, 4H), 2.99 (t, *J* = 6.2 Hz, 2H), 2.27 (br. s, 4H), 2.59 (t, *J* = 6.5 Hz, 2H), 1.82 (p, *J* = 6.4 Hz, 2H).

Synthesis of 3-[4-(*o*-tolyl)piperazin-1-yl]propan-1-amine (4f)

The title compound was synthesized using 1-(2-methylphenyl)piperazine (**2b**) (0.350 g, 1.99 mmol), *N*-(3-bromopropyl)phthalimide (**3b**) (0.587 g, 2.19 mmol) and K₂CO₃ (0.687 g, 4.97 mmol) in CH₃CN (6 mL). Treating of phthalimide intermediate with hydrazine hydrate (0.175 mL, 2.32 mmol) in MeOH (4 mL) afforded **5f** (0.349 g, 1.49 mmol, 50% yield over two steps) as a yellow oil. UPLC-MS (method A): Rt 1.21 min; m/z 234 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.19 – 7.12 (m, 2H), 7.05 – 6.94 (m, 2H), 2.94 (t, *J* = 4.8 Hz, 4H), 2.78 (t, *J* = 6.8 Hz, 2H), 2.61 (br. s, 4H), 2.51 – 2.43 (m, 2H), 2.30 (s, 3H), 1.68 (p, *J* = 7.0 Hz, 2H).

Synthesis of Synthesis of 3-[4-[2-(trifluoromethyl)phenyl]piperazin-1-yl]propan-1-amine (4g)

A microwave vial was loaded with 1-(2-trifluoromethylphenyl)piperazine (**2c**) (0.230 g, 1.00 mmol), to the vial *N*-(3-bromopropyl)phthalimide (**3b**) (0.281 g, 1.05 mmol) was added, followed by CH₃CN (2 mL) and NEt₃ (0.348 mL, 2.50 mmol). The vial was heated at 100 °C under microwave irradiation for 30 min. The solvent was removed under reduced pressure and the crude residue was portioned between water and DCM. The organic layer was dried over Na₂SO₄, filtered and evaporated to dryness to give a crude which was purified by flash chromatography on silica gel (Eluent: from DCM to DCM : MeOH = 95 : 5) to afford the phthalimide intermediate. The phthalimide intermediate was dissolved in MeOH (5 mL), and heated to reflux in presence of hydrazine hydrate (0.061 mL, 1.26 mmol) for 2 h. To the hot solution 2 N HCl (2 mL) was added and reflux was continued for further 1h. After cooling at room temperature the mixture was filtered, the residue was washed with methanol and the filtrates were concentrated under reduced pressure. The residue was suspended in water, alkalinized with 2 N NaOH until pH 10 and the aqueous solution was extracted with ethyl acetate. The organic phase was dried over Na₂SO₄ anhydrous, filtered and evaporated to dryness to give the product **5g** (0.225 g, 0.78 mmol, 78% yield over two steps) as a yellow oil. UPLC-MS (method A): Rt 1.54 min; m/z 288 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.61 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.49 (td, *J* = 7.6, 1.5 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.25 – 7.15 (m, 1H), 2.95 (t, *J* = 4.8 Hz, 4H), 2.77 (t, *J* = 6.8 Hz, 2H), 2.59 (br. s, 4H), 2.50 – 2.42 (m, 2H), 1.74 – 1.62 (m, 2H).

General procedure for the preparation of compounds 5a and 5e

The amine derivative **4a** and **4e** (1 eq.) were treated with *p*-nitrophenylchloroformate (1.1 eq.) and DIPEA (1.1 eq.) in a mixture of DMA : DCM 1 : 1. The reaction mixture was stirred at room temperature for 30 min. To the resulting *p*-nitrophenyl carbamate solution, the 1-Naphtol (1.15-1.25 eq.) and DIPEA (1.1 eq.) were added and the resulting mixture was stirred at room temperature for 72h. The reaction solution was washed several time with brine and water, the united organic layers were dried over Na₂SO₄ anhydrous, filtered and evaporated to dryness. The crude was purified by flash chromatography (Eluent: DCM : MeOH).

General procedure for the preparation of compounds **5b-d** and **5f-g**

To a solution of di-*tert*-butyl dicarbonate (1.1-1.4 eq.) in CH₃CN, a solution of DMAP (1.1-1.2 eq.) in CH₃CN and a solution of **4b-d** and **4f-g** (1 eq.) in CH₃CN were added in sequence and the resulting mixture was stirred at room temperature for 1h. Then 1-naphtol (1.1-1.2 eq.) was added and the mixture was stirred at the same temperature overnight. The solvent was evaporated under reduced pressure, the residue was dissolved in AcOEt and washed with a saturated solution of NH₄Cl; the united organic layers were dried over Na₂SO₄ anhydrous, filtered and evaporated to dryness. The crude was purified by flash chromatography (Eluent: DCM : MeOH) on silica gel. Compounds **5b**, **c** and **5f** were obtained as hydrochloride salt by treating with 4 M HCl in 1,4-dioxane.

Synthesis of 1-naphthyl *N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]carbamate (**5a**)

The title compound was synthesized starting from *p*-nitrophenylchloroformate (0.084 g, 0.42 mmol), DIPEA (0.15 mL, 0.84 mmol), **4a** (0.100 g, 0.38 mmol) and 1-naphtol (0.054 g, 0.47 mmol) in a mixture of DMA : DCM 1 : 1 (4 mL). Eluting with DCM : MeOH (100 : 0 – 95 : 5) afforded **5a** (0.106 g, 0.24 mmol, 64% yield) as a white solid. UPLC-MS (method A): Rt 2.26 min; m/z 434 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.06 (t, *J* = 5.6 Hz, 1H), 8.01 – 7.96 (m, 1H), 7.94 – 7.88 (m, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 7.60 – 7.54 (m, 2H), 7.54 – 7.48 (m, 1H), 7.29 (dd, *J* = 7.5, 1.1 Hz, 1H), 6.95 – 6.91 (m, 2H), 6.91 – 6.81 (m, 2H), 3.78 (s, 3H), 3.16 (q, *J* = 6.1 Hz, 2H), 2.97 (br. s, 4H), 2.55 – 2.49 (m, 4H), 2.43 – 2.34 (m, 2H), 1.65 – 1.48 (m, 4H). ¹³C NMR (101 MHz, DMSO) δ 154.43, 151.94, 146.72, 141.29, 134.09, 127.85, 127.28, 126.33, 126.28, 125.68, 124.97, 122.25, 121.01, 120.79, 118.40, 117.82, 111.88, 57.57, 55.26, 53.01 (2C), 50.08 (2C), 40.92, 27.26, 23.56. UPLC-MS Purity (UV @ 215 nm): 98%.

Synthesis of 1-naphthyl *N*-[4-[4-(*o*-tolyl)piperazin-1-yl]butyl]carbamate hydrochloride (**5b**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.148 g, 0.68 mmol), DMAP (0.071 g, 0.58 mmol), **4b** (0.120 g, 0.49 mmol) and 1-naphtol (0.077 g, 0.53 mmol) in CH₃CN (3 mL). The crude was purified by flash chromatography with DCM : MeOH (100 : 0 – 95 : 5) to afford the desired compound, which was treated with 4 M HCl in 1,4-dioxane. Evaporation of the solvent produced the title compound **5b** (0.111 g, 0.24 mmol, 50% yield over two steps) as a white solid. UPLC-MS (method A): Rt 2.52 min; m/z 418 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.22 (br. s, 1H), 8.15 (t, *J* = 5.7 Hz, 1H), 7.98 (dd, *J* = 7.5, 1.8 Hz, 1H), 7.93 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 7.65 – 7.55 (m, 2H), 7.52 (t, *J* = 7.9 Hz, 1H), 7.32 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.23 – 7.16 (m, 2H), 7.07 – 6.97 (m, 2H), 3.53 (d, *J* = 9.2 Hz, 2H), 3.25 – 3.08 (m, 10H), 2.26 (s, 3H), 1.93 – 1.80 (m, 2H), 1.60 (p, *J* = 7.2 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 154.55, 149.75, 146.70, 134.09, 131.93, 130.99, 127.88, 127.23, 126.68, 126.44, 126.39, 125.70, 125.08, 123.71, 121.08, 118.90, 118.45, 55.04 (2C), 51.31 (2C), 48.04, 40.20, 26.49, 20.42, 17.42. UPLC-MS Purity (UV @ 215 nm): 95%.

Synthesis of 1-naphthyl *N*-[4-[4-[2-(trifluoromethyl)phenyl]piperazin-1-yl]butyl]carbamate hydrochloride (**5c**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.080 g, 0.36 mmol), DMAP (0.045 g, 0.36 mmol), **4c** (0.100 g, 0.33 mmol) and 1-naphtol (0.053 g, 0.36 mmol) in CH₃CN (3 mL). The crude was purified by flash chromatography with DCM : MeOH (100 : 0 – 95 : 5) to afford the desired compound, which was treated with 4 M HCl in 1,4-dioxane. Evaporation of the solvent produced the title compound **5c** (0.041 g, 0.080 mmol, 24% yield over two steps) as a white solid. UPLC-MS (method A): Rt 2.70 min; m/z 472 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.83 (br. s, 1H), 8.11 (t, *J* = 5.7 Hz, 1H), 8.01 – 7.95 (m, 1H), 7.94 – 7.88 (m, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.76 – 7.68 (m, 2H), 7.63 – 7.47 (m, 4H), 7.42 (t, *J* = 7.5 Hz, 1H), 7.30 (dd, *J* = 7.5, 1.1 Hz, 1H), 3.62 – 3.49 (m, 2H), 3.30 – 3.26 (m, 2H), 3.25 – 3.15 (m, 4H), 3.14 – 3.01 (m, 4H), 1.91 – 1.75 (m, 2H), 1.59 (p, *J* = 7.2 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 154.55, 150.49, 146.69, 134.10, 133.91, 127.89, 127.23, 127.11, 127.06, 126.43, 126.39, 126.12, 125.70, 125.09, 124.45, 122.56, 121.07, 118.45, 54.96, 51.42 (2C), 49.68 (2C), 39.62, 26.45, 20.50. UPLC-MS Purity (UV @ 215 nm): 99%.

Synthesis of 1-naphthyl *N*-[3-[4-(2,3-dichlorophenyl)piperazin-1-yl]propyl]carbamate (**5d**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.127 g, 0.58 mmol), DMAP (0.061 g, 0.50 mmol), **4d** (0.120 g, 0.42 mmol) and 1-naphtol (0.066 g, 0.46 mmol) in CH₃CN (3 mL). Eluting with DCM : MeOH (100 : 0 – 95 : 5) afforded **5d** (0.101 g, 0.22 mmol, 52% yield) as a yellow solid. UPLC-MS (method A): Rt 2.92 min; m/z 458 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.03 (t, *J* = 5.6 Hz, 1H), 8.01 – 7.95 (m, 1H), 7.93 – 7.87 (m, 1H), 7.80 (d, *J* = 8.3 Hz, 1H), 7.62 – 7.53 (m, 2H), 7.51 (dd, *J* = 8.3, 7.4 Hz, 1H), 7.32 – 7.26 (m, 3H), 7.14 (dd, *J* = 6.4, 3.2 Hz, 1H), 3.18 (q, *J* = 6.6 Hz, 2H), 3.00 (br. s, 4H), 2.57 (br. s, 4H), 2.45 (t, *J* = 7.1 Hz, 2H), 1.72 (p, *J* = 7.0 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 154.44, 151.17, 146.73, 134.08, 132.58, 128.40, 127.87, 127.23, 126.37, 126.33, 125.97, 125.70, 125.00, 124.28, 121.03, 119.52, 118.41, 55.15, 52.81(2C), 50.96 (2C), 39.38, 26.48. UPLC-MS Purity (UV @ 215 nm): 97%.

Synthesis of 1-naphthyl *N*-[3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl]carbamate (**5e**)

The title compound was synthesized starting from *p*-nitrophenylchloroformate (0.177 g, 0.88 mmol), DIPEA (0.31 mL, 1.76 mmol), **4e** (0.200 g, 0.80 mmol) and 1-naphtol (0.144 g, 1.00 mmol) in a mixture of DMA : DCM 1 : 1 (4 mL). Eluting with DCM : MeOH (100 : 0 – 95 : 5) afforded **5e** (0.111 g, 0.26 mmol, 33% yield) as a yellow solid. UPLC-MS (method A): Rt 1.98 min; m/z 420 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.04 (t, *J* = 5.6 Hz, 1H), 8.00 – 7.95 (m, 1H), 7.93 – 7.87 (m, 1H), 7.80 (d, *J* = 8.2 Hz, 1H), 7.62 – 7.54 (m, 2H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.32 – 7.26 (m, 1H), 6.97 – 6.89 (m, 2H), 6.89 – 6.82 (m, 2H), 3.77 (s, 3H), 3.18 (q, *J* = 6.6 Hz, 2H), 2.97 (br. s, 4H), 2.54 (br. s, 4H), 2.43 (t, *J* = 7.1 Hz, 2H), 1.72 (p, *J* = 7.1 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 154.43, 151.96, 146.73, 141.26, 134.08, 127.88, 127.23, 126.38, 126.33, 125.71, 125.01, 122.30,

121.03, 120.82, 118.42, 117.86, 111.92, 55.35, 55.30, 53.05 (2C), 50.09 (2C), 39.42, 26.48. UPLC-MS Purity (UV @ 215 nm): 97%.

Synthesis of 1-naphthyl *N*-[3-[4-(*o*-tolyl)piperazin-1-yl]propyl]carbamate hydrochloride (**5f**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.455 g, 2.08 mmol), DMAP (0.218 g, 1.79 mmol), **4f** (0.349 g, 1.49 mmol) and 1-naphthol (0.258 g, 1.79 mmol) in CH₃CN (9 mL). The crude was purified by flash chromatography with DCM : MeOH (100 : 0 – 95 : 5) to afford the desired compound, which was treated with 4 M HCl in 1,4-dioxane. Evaporation of the solvent produced the title compound **5f** (0.198 g, 0.45 mmol, 30% yield over two steps) as a white solid. UPLC-MS (method A): Rt 2.28 min; m/z 404 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.08 (br. s, 1H), 8.21 (t, *J* = 5.8 Hz, 1H), 8.01 – 7.96 (m, 1H), 7.95 – 7.90 (m, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 7.63 – 7.54 (m, 2H), 7.52 (t, *J* = 7.9 Hz, 1H), 7.32 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.23 – 7.15 (m, 2H), 7.07 – 6.99 (m, 2H), 3.63 – 3.50 (m, 2H), 3.32 – 3.08 (m, 10H), 2.27 (s, 3H), 2.11 – 1.98 (m, 2H). ¹³C NMR (101 MHz, DMSO) δ 154.55, 149.73, 146.63, 134.09, 131.95, 131.01, 127.88, 127.16, 126.70, 126.45, 126.42, 125.69, 125.14, 123.73, 121.10, 118.91, 118.42, 53.35, 51.47 (2C), 48.11 (2C), 38.02, 23.81, 17.44. UPLC-MS Purity (UV @ 215 nm): 99%.

Synthesis of 1-naphthyl *N*-[3-[4-[2-(trifluoromethyl)phenyl]piperazin-1-yl]propyl]carbamate (**5g**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.128 g, 0.59 mmol), DMAP (0.061 g, 0.50 mmol), **4g** (0.120 g, 0.42 mmol) and 1-naphthol (0.066 g, 0.46 mmol) in CH₃CN (3 mL). Eluting with DCM : MeOH (100 : 0 – 95 : 5) afforded **5g** (0.093 g, 0.20 mmol, 48% yield) as a yellow solid. UPLC-MS (method A): Rt 2.70 min; m/z 458 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.03 (t, *J* = 5.6 Hz, 1H), 8.00 – 7.95 (m, 1H), 7.94 – 7.87 (m, 1H), 7.80 (d, *J* = 8.2 Hz, 1H), 7.68 – 7.61 (m, 2H), 7.60 – 7.52 (m, 3H), 7.52 – 7.47 (m, 1H), 7.35 – 7.27 (m, 2H), 3.19 (q, *J* = 6.6 Hz, 2H), 2.93 – 2.82 (m, 4H), 2.53 (br. s, 4H), 2.44 (t, *J* = 7.1 Hz, 2H), 1.72 (p, *J* = 7.0 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 154.45, 152.42, 146.74, 134.09, 133.54, 127.89, 127.24, 126.92, 126.87, 126.38, 126.33, 125.71, 125.67, 125.02 (2C), 124.36, 121.04, 118.43, 55.25, 53.19 (2C), 53.16 (2C), 39.38, 26.50. UPLC-MS Purity (UV @ 215 nm): 94%.

Synthesis of 4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butan-1-amine (**6**)

A mixture of 1-(2,3-dichlorophenyl)piperazine hydrochloride (**2d**) (1.000 g, 3.74 mmol), *N*-(4-bromobutyl)phthalimide (**3a**) (1.160 g, 4.11 mmol) and K₂CO₃ (1.292 g, 9.35 mmol) in CH₃CN (7 mL) was heated to reflux for 5h. The hot suspension was filtered and the residue was washed with acetone several times. The filtrates were concentrated under reduced pressure to afford the phthalimide intermediate, which was dissolved in MeOH (8 mL), and heated to reflux in presence of hydrazine hydrate (0.215 mL, 4.44 mmol) for 2h. To the hot solution 2N HCl (3 mL) was added and reflux was continued for further 1h. After cooling at room temperature the mixture was filtered, the residue was washed with methanol and the

filtrates were concentrated under reduced pressure. The residue was suspended in water, alkalized with 2 N NaOH until pH 10 and the aqueous solution was extracted with ethyl acetate. The organic phase was dried over Na₂SO₄, filtered and evaporated to dryness to afford **6** (1.07 g, 3.5 mmol, 93% yield over two steps) as a yellow oil, which was pure enough for the next step. UPLC-MS (method A): Rt 1.41 min; m/z 302 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.18 – 7.10 (m, 2H), 6.96 (dd, *J* = 6.7, 2.9 Hz, 1H), 3.16 – 2.99 (m, 4H), 2.74 (t, *J* = 6.6 Hz, 2H), 2.70 – 2.55 (m, 4H), 2.44 (t, *J* = 7.2 Hz, 2H), 1.68 – 1.44 (m, 4H).

General procedure for the preparation of compounds **8a,b, **17a-c** and **21a-c**.**

To a solution of di-*tert*-butyl dicarbonate (1.1-1.4 eq.) in CH₃CN, a solution of DMAP (1.1-1.2 eq.) in CH₃CN and a solution of **6** (1 eq.) in CH₃CN were added in sequence and the resulting mixture was stirred at room temperature for 1 h. Then the appropriate alcohol derivative **7a,b**, **17a-c** and **21a-c** (1.1-1.2 eq.) was added and the mixture was stirred at the same temperature overnight. The solvent was evaporated under reduced pressure, the residue was dissolved in AcOEt and washed with a saturated solution of NH₄Cl; the united organic layers were dried over Na₂SO₄ anhydrous, filtered and evaporated to dryness. The crude was purified by flash chromatography (Eluent: DCM : MeOH) on silica gel. Compounds **8a,b**, **17c** and **21a** were obtained as hydrochloride salt by treating with 4 M HCl in 1,4-dioxane or 1.25 M HCl in MeOH.

Synthesis of (4-fluoro-1-naphthyl) *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate hydrochloride (8a**)**

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.254 g, 1.16 mmol), DMAP (0.122 g, 0.99 mmol), **6** (0.250 g, 0.83 mmol) and 4-fluoronaphthalen-1-ol (**7a**) (0.162 g, 0.99 mmol) in CH₃CN (5 mL). The crude was purified by flash chromatography with DCM : MeOH (100 : 0 – 95 : 5) to afford the desired compound, which was treated with 4 M HCl in 1,4-dioxane. Evaporation of the solvent produced the title compound **8a** (0.182 g, 0.34 mmol, 41% yield over two steps) as a white solid. UPLC-MS (method A): Rt 2.63 min; m/z 490 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.91 (br. s, 1H), 8.14 (t, *J* = 5.7 Hz, 1H), 8.11 – 8.04 (m, 1H), 7.99 – 7.90 (m, 1H), 7.76 – 7.64 (m, 2H), 7.43 – 7.28 (m, 4H), 7.21 (dd, *J* = 7.2, 2.4 Hz, 1H), 3.64 – 3.52 (m, 2H), 3.50 – 3.38 (m, 2H), 3.27 – 3.09 (m, 8H), 1.94 – 1.72 (m, 2H), 1.58 (p, *J* = 7.2 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 154.48, 149.51, 142.81, 132.72, 128.64, 127.66, 127.18, 126.04, 125.29, 121.49, 120.26, 120.22, 119.80, 118.40, 118.31, 109.38, 109.17, 55.06, 51.05 (2C), 47.68 (2C), 40.35, 26.43, 20.47. UPLC-MS Purity (UV @ 215 nm): 99%.

(4-methoxy-1-naphthyl) *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate hydrochloride (8b**)**

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.254 g, 1.16 mmol), DMAP (0.122 g, 0.99 mmol), **6** (0.250 g, 0.83 mmol) and 4-methoxynaphthalen-1-ol (**7b**) (0.173 g, 0.99 mmol) in CH₃CN (5 mL). The crude was purified by flash chromatography with DCM : MeOH (100 : 0 – 95 : 5) to afford

the desired compound, which was treated with 1.25 M HCl in MeOH. Evaporation of the solvent produced the title compound **8b** (0.169 g, 0.31 mmol, 37% yield over two steps) as beige solid. UPLC-MS (method A): Rt 2.60 min; m/z 502 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.90 (br. s, 1H), 8.21 – 8.13 (m, 1H), 8.02 (t, *J* = 5.7 Hz, 1H), 7.86 – 7.78 (m, 1H), 7.61 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.2, 6.8, 1.3 Hz, 1H), 7.41 – 7.31 (m, 2H), 7.25 – 7.15 (m, 2H), 6.93 (d, *J* = 8.4 Hz, 1H), 3.97 (s, 3H), 3.58 (d, *J* = 10.6 Hz, 2H), 3.43 (d, *J* = 11.1 Hz, 2H), 3.28 – 3.08 (m, 8H), 1.93 – 1.74 (m, 2H), 1.64 – 1.50 (m, 2H). ¹³C NMR (101 MHz, DMSO) δ 154.94, 152.23, 149.50, 139.91, 132.72, 128.63, 127.89, 126.87, 126.04, 125.69, 125.29, 125.23, 121.79, 121.02, 119.80, 118.38, 103.63, 55.77, 55.06, 51.04 (2C), 47.67 (2C), 40.17, 26.48, 20.46. UPLC-MS Purity (UV @ 215 nm): 99%.

Synthesis of (4-hydroxy-1-naphthyl) *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate hydrochloride (**9**)

8b (0.050 g, 0.09 mmol) was dissolved in DCM (1.5 mL), 1 M BBr₃ solution in DCM (0.315 mL, 0.315 mmol) was added and the reaction was stirred at room temperature overnight. The reaction was quenched by addition of H₂O, the resulting mixture was diluted with DCM and H₂O and extracted with DCM. The united organic layers were dried over anhydrous Na₂SO₄, filtered and evaporated to dryness. The crude was purified by flash chromatography on silica gel (Eluent: from DMC to DCM : MeOH = 95 : 5) to afford the desired compound, which was treated with 1.25 M HCl in MeOH. Evaporation of the solvent produced the title compound **9** (0.032 g, 0.061 mmol, 68% yield over two steps) as a yellow solid. UPLC-MS (method A): Rt 2.19 min; m/z 488 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.20 – 10.05 (m, 2H), 8.14 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.95 (t, *J* = 5.7 Hz, 1H), 7.80 – 7.70 (m, 1H), 7.59 – 7.45 (m, 2H), 7.42 – 7.32 (m, 2H), 7.22 (dd, *J* = 7.4, 2.2 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 3.70 – 3.54 (m, 2H), 3.52 – 3.37 (m, 2H), 3.27 – 3.06 (m, 8H), 1.91 – 1.71 (m, 2H), 1.57 (p, *J* = 7.3 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 155.14, 150.68, 149.45, 138.65, 132.73, 128.65, 128.09, 126.48, 126.06, 125.33, 124.92, 124.89, 122.38, 120.87, 119.85, 118.74, 106.99, 55.15, 51.15 (2C), 47.76 (2C), 40.24, 26.49, 20.57. UPLC-MS Purity (UV @ 215 nm): 96%.

Synthesis of (4-formyl-1-naphthyl) (4-nitrophenyl) carbonate (**11**)

To a solution of 4-hydroxy-1-naphthaldehyde (**10**) (0.200 g, 1.16 mmol) and 4-nitrophenyl chloroformate (0.234 g, 1.16 mmol) in dry DCM (3.5 mL) cooled at 0 °C, DIPEA (0.200 mL, 1.16 mmol) was added dropwise over a period of 5 minutes. The cooling bath was removed, and the reaction mixture stirred at room temperature for 1 h. The reaction mixture was diluted with DCM and washed with a 0.1 M HCl solution; the organic layer was passed through a PS column and concentrated in vacuum to afford **11** (0.391 g of crude, 1.16 mmol, 100% yield) as yellow solid. ¹H NMR (400 MHz, Chloroform-*d*) δ 10.41 (s, 1H), 9.41 – 9.30 (m, 1H), 8.40 – 8.32 (m, 2H), 8.24 – 8.16 (m, 1H), 8.11 – 8.04 (m, 1H), 7.83 – 7.66 (m, 3H), 7.59 – 7.52 (m, 2H).

Synthesis of 4-(4-nitrophenoxy)carbonyloxynaphthalene-1-carboxylic acid (**12**)

To a solution of **11** (0.391 g, 1.16 mmol) in *tert*-BuOH (1.26 mL) 2-methyl-2-butene (0.84 mL) was added. To this a solution containing NaClO₂ (0.136 g, 1.51 mmol) and NaH₂PO₄ (0.235 g, 1.51 mmol) in H₂O (1.16 mL) was added dropwise. The resulting solution was stirred at room temperature overnight. The reaction solution was concentrated in vacuum and diluted with water; the aqueous phase was acidified with 2 N HCl (until pH 2), saturated with sodium chloride and extracted with EtOAc. The combined organic layers were dried over Na₂SO₄ and evaporated. The brown solid was triturated with DCM, filtered and dried reduce pressure to afford **12** (0.162 g, 0.46 mmol, 39% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.30 (s, 1H), 9.00 – 8.92 (m, 1H), 8.44 – 8.21 (m, 4H), 7.90 – 7.70 (m, 5H).

Synthesis of 4-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butylcarbamoxyloxy]naphthalene-1-carboxylic acid (**13**)

Compound **12** (0.254 g, 0.72 mmol) was dissolved in dry DCM (1.5 mL) and stirred at 0 °C, then a solution of **6** (0.198 g, 0.65 mmol) and NEt₃ (0.25 mL, 1.81 mmol) in dry DMF (1.5 mL) was added dropwise. The resulting mixture was stirred at room temperature overnight. The solution was diluted with DCM and washed with water. The organic layer was passed through a PS column and concentrated under vacuum. The crude was purified by flash chromatography on silica gel (Eluent: from DMC to DCM : MeOH = 80 : 20) to afford the desired compound **13** (0.162 g, 0.31 mmol, 48% yield) as a white solid. UPLC-MS (method A): Rt 1.71 min; m/z 516 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.01 – 8.92 (m, 1H), 8.25 – 8.14 (m, 2H), 8.07 – 8.00 (m, 1H), 7.75 – 7.59 (m, 2H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.34 – 7.25 (m, 2H), 7.18 – 7.10 (m, 1H), 3.16 (q, *J* = 6.0 Hz, 2H), 3.06 – 2.91 (m, 4H), 2.57 (br. s, 4H), 2.45 – 2.35 (m, 2H), 1.64 – 1.49 (m, 4H).

Synthesis of 4-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butylcarbamoxyloxy]naphthalene-1-carboxylic acid hydrochloride (**14**)

Compound **13** (0.062 g, 0.12 mmol) was treated with 1.25 M HCl in MeOH. Evaporation of the solvent produced the title compound **14** (0.060 g, 0.11 mmol, 91% yield) as a white solid. UPLC-MS (method A): Rt 1.73 min; m/z 516 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.15 (br. s, 1H), 10.69 (br. s, 1H), 9.01 – 8.92 (m, 1H), 8.25 (t, *J* = 5.7 Hz, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.05 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.76 – 7.62 (m, 2H), 7.46 – 7.32 (m, 3H), 7.21 (dd, *J* = 7.2, 2.4 Hz, 1H), 3.67 – 3.54 (m, 2H), 3.43 (s, 2H), 3.25 – 3.10 (m, 8H), 1.91 – 1.76 (m, 2H), 1.60 (p, *J* = 7.2 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 168.06, 153.93, 150.09, 149.51, 132.73, 132.07, 130.41, 128.64, 128.00, 127.32, 126.63, 126.05, 125.85, 125.30, 124.65, 121.59, 119.82, 117.20, 55.11, 51.11 (2C), 47.74 (2C), 40.25, 26.39, 20.55. UPLC-MS Purity (UV @ 215 nm): 97%.

Synthesis of (4-carbamoyl-1-naphthyl) N-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate hydrochloride (**15**)

Compound **13** (0.162 g, 0.31 mmol) was dissolved in 1,4-dioxane (12 mL), then di-*tert*-butyl dicarbonate (0.088 g, 0.40 mmol) and Pyridine (0.12 mL, 1.5 mmol) were added followed by ammonium bicarbonate (0.031 g, 0.39 mmol). The resulting mixture was stirred at 40 °C for 5 h. The solution was diluted with EtOAc and washed with water and brine, the organic layer was dried over Na₂SO₄, filtered and concentrated under vacuum. The crude was purified by flash chromatography on silica gel (Eluent: from DMC to DCM : MeOH = 80 : 20) to afford the desired compound, which was treated with 1.25 M HCl in MeOH. Evaporation of the solvent produced the title compound **15** (0.059 g, 0.10 mmol, 25% yield over two steps) as a white solid. UPLC-MS (method A): Rt 1.93 min; m/z 515 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.96 (br. s, 1H), 8.40 – 8.33 (m, 1H), 8.18 (t, *J* = 5.7 Hz, 1H), 8.01 (br. s, 1H), 7.99 – 7.94 (m, 1H), 7.68 – 7.60 (m, 3H), 7.58 (br. s, 1H), 7.41 – 7.31 (m, 3H), 7.21 (dd, *J* = 7.1, 2.5 Hz, 1H), 3.64 – 3.54 (m, 2H), 3.49 – 3.41 (m, 2H), 3.28 – 3.11 (m, 8H), 1.93 – 1.79 (m, 2H), 1.59 (p, *J* = 7.2 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 170.10, 159.85, 154.26, 149.51, 132.73, 131.90, 130.98, 128.65, 127.26, 127.00, 126.58, 126.04, 125.91, 125.29, 125.10, 121.26, 119.81, 117.32, 55.07, 51.05 (2C), 47.68 (2C), 40.32, 26.43, 20.48. UPLC-MS Purity (UV @ 215 nm): 98%.

Synthesis of phenyl N-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate (**17a**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.144 g, 0.66 mmol), DMAP (0.068 g, 0.56 mmol), **6** (0.141 g, 0.47 mmol) and phenol (**16a**) (0.043 g, 0.56 mmol) in CH₃CN (4 mL). Eluting with DCM : MeOH (100 : 0 – 95 : 5) afforded **17a** (0.085 g, 0.20 mmol, 43% yield) as a white solid. UPLC-MS (method A): Rt 2.21 min; m/z 422 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 77.77 (t, *J* = 5.7 Hz, 1H), 7.40 – 7.34 (m, 2H), 7.33 – 7.26 (m, 2H), 7.22 – 7.15 (m, 1H), 7.14 (dd, *J* = 6.4, 3.2 Hz, 1H), 7.11 – 7.06 (m, 2H), 3.15 – 3.05 (m, 2H), 3.04 – 2.93 (m, 4H), 2.61 – 2.51 (m, 4H), 2.43 – 2.30 (m, 2H), 1.58 – 1.44 (m, 4H). ¹³C NMR (101 MHz, DMSO) δ 154.29, 151.20, 151.12, 132.58, 129.18 (2C), 128.41, 125.98, 124.78, 124.29, 121.70 (2C), 119.52, 57.38, 52.77 (2C), 50.94 (2C), 40.24, 27.18, 23.54. UPLC-MS Purity (UV @ 215 nm): 99%.

Synthesis of tetralin-5-yl N-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate (**17b**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.080 g, 0.37 mmol), DMAP (0.045 g, 0.37 mmol), compound **6** (0.100 g, 0.33 mmol) and tetralin-5-ol (**16b**) (0.054 g, 0.37 mmol) in CH₃CN (3 mL). Eluting with DCM : MeOH (100 : 0 – 90 : 10) afforded **17b** (0.040 g, 0.08 mmol, 25% yield) as a yellow sticky solid. UPLC-MS (method A): Rt 3.05 min; m/z 476 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.71 (t, *J* = 5.7 Hz, 1H), 7.34 – 7.27 (m, 2H), 7.14 (dd, *J* = 6.4, 3.2 Hz, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 6.95 – 6.89 (m, 1H), 6.84 – 6.78 (m, 1H), 3.08 (q, *J* = 6.4, 5.8 Hz, 2H), 3.02 – 2.92 (m, 4H), 2.77 – 2.69 (m, 2H), 2.59 – 2.50 (m, 6H), 2.37 (d, *J* = 6.3 Hz, 2H), 1.75 – 1.65 (m, 4H), 1.56 – 1.45 (m, 4H). ¹³C NMR (101 MHz, DMSO) δ 151.20, 149.25, 138.27, 134.69, 132.60, 130.20, 129.63, 128.44, 125.83, 125.67, 124.31, 119.52, 119.46,

57.39, 52.76 (2C), 50.96 (2C), 40.79, 28.74, 27.24, 23.45, 22.76, 22.29 (2C). UPLC-MS Purity (UV @ 215 nm): 99%.

Synthesis of 2,3-dihydro-1,4-benzodioxin-5-yl *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate hydrochloride (**17c**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.162 g, 0.74 mmol), DMAP (0.078 g, 0.63 mmol), **6** (0.160 g, 0.53 mmol) and 2,3-Dihydro-1,4-benzodioxin-5-ol (**16c**) (0.096 g, 0.63 mmol) in CH₃CN (4 mL). The crude was purified by flash chromatography with DCM : MeOH (100 : 0 – 95 : 5) to afford the desired compound, which was treated with 1.25 M HCl in MeOH. Evaporation of the solvent produced the title compound **17c** (0.148 g, 0.29 mmol, 39% yield over two steps) as a white solid. UPLC-MS (method A): Rt 2.20 min; m/z 480 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.87 (br. s, 1H), 7.76 (t, *J* = 5.7 Hz, 1H), 7.42 – 7.32 (m, 2H), 7.21 (dd, *J* = 7.2, 2.4 Hz, 1H), 6.82 – 6.70 (m, 2H), 6.65 (dd, *J* = 7.7, 1.9 Hz, 1H), 4.23 (s, 4H), 3.56 (d, *J* = 10.6 Hz, 2H), 3.43 (d, *J* = 11.2 Hz, 2H), 3.27 – 3.04 (m, 8H), 1.87 – 1.70 (m, 2H), 1.52 (p, *J* = 7.2 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 153.86, 149.51, 144.34, 139.70, 136.59, 132.72, 128.64, 126.04, 125.29, 119.81, 119.58, 115.45, 113.81, 63.94 (2C), 55.05, 51.04 (2C), 47.68 (2C), 40.93, 26.44, 20.45. UPLC-MS Purity (UV @ 215 nm): 97%.

Synthesis of tetralin-1-yl *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate (**19**)

Tetralin-1-ol (**18**) (0.400 g, 2.70 mmol) was dissolved in dry DCM (20 mL), then *p*-nitrophenylchloroformate (0.489 g, 2.43 mmol) and pyridine (0.223 mL, 2.75 mmol) were added and the resulting mixture was stirred at room temperature for 20 h. The mixture was diluted with DCM and washed with a saturated water solution of NaHCO₃. The organic layer was passed through a PS column and concentrated in vacuum. The crude was purified by flash chromatography on silica gel (Eluent: from Cyclohexane to Cyclohexane : Ethyl Acetate = 50 : 50) to afford the desired *p*-nitrophenyl carbamate intermediate (0.300 g, 0.96 mmol, 39% yield). This latter intermediate (0.136 g, 0.43 mmol) was dissolved in dry DCM (10 mL), then **6** (0.131 g, 0.43 mmol) and NEt₃ (0.062 mL, 0.44 mmol) were added and the resulting mixture was stirred at room temperature for 2h. The mixture was diluted with DCM and washed with a saturated water solution of NaHCO₃ and with brine. The organic layer was passed through a PS column and concentrated in vacuum. The crude was purified by flash chromatography on silica gel (Eluent: from DMC to DCM : MeOH = 98 : 2) to afford the title compound **19** (0.145 g, 0.30 mmol, 70% yield) as a yellow sticky solid. UPLC-MS (method A): Rt 1.71 min; m/z 476 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.33 – 7.27 (m, 2H), 7.25 – 7.08 (m, 6H), 5.71 (t, *J* = 4.7 Hz, 1H), 3.00 (dt, *J* = 26.3, 5.7 Hz, 6H), 2.83 – 2.63 (m, 2H), 2.57 – 2.43 (m, 4H), 2.39 – 2.28 (m, 2H), 1.95 – 1.70 (m, 4H), 1.52 – 1.34 (m, 4H). ¹³C NMR (101 MHz, DMSO) δ 156.08, 151.19, 137.35, 135.35, 132.57, 128.93, 128.72, 128.40, 127.63, 125.95, 125.77, 124.27, 119.49, 68.65, 57.42, 52.74 (2C), 50.91(2C), 39.83, 29.00, 28.37, 27.36, 23.53, 18.50. UPLC-MS Purity (UV @ 215 nm): 99%.

Synthesis of benzothiophen-4-yl *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate hydrochloride (**21a**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.162 g, 0.74 mmol), DMAP (0.078 g, 0.63 mmol), **6** (0.160 g, 0.53 mmol) and benzothiophen-4-ol (**20a**) (0.095 g, 0.63 mmol) in CH₃CN (4 mL). The crude was purified by flash chromatography with DCM : MeOH (100 : 0 – 95 : 5) to afford the desired compound, which was treated with 1.25 M HCl in MeOH. Evaporation of the solvent produced the title compound **21a** (0.118 g, 0.23 mmol, 31% yield over two steps) as a white solid. UPLC-MS (method A): Rt 2.50 min; m/z 478 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.83 (br. s, 1H), 8.03 (t, *J* = 5.7 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.78 (d, *J* = 5.5 Hz, 1H), 7.43 – 7.28 (m, 4H), 7.21 (dd, *J* = 7.2, 2.4 Hz, 1H), 7.15 (d, *J* = 7.7 Hz, 1H), 3.63 – 3.54 (m, 2H), 3.48 – 3.40 (m, 2H), 3.27 – 3.11 (m, 8H), 1.89 – 1.76 (m, 2H), 1.63 – 1.50 (m, 2H). ¹³C NMR (101 MHz, DMSO) δ 154.25, 149.52, 145.87, 140.69, 133.34, 132.74, 128.66, 127.92, 126.07, 125.32, 124.90, 119.83, 119.77, 119.67, 117.03, 55.09, 51.08 (2C), 47.71 (2C), 40.35, 26.44, 20.51. UPLC-MS Purity (UV @ 215 nm): 95%.

Synthesis of 1H-indol-4-yl *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate (**21b**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.162 g, 0.74 mmol), DMAP (0.078 g, 0.63 mmol), **6** (0.160 g, 0.53 mmol) and 4-hydroxyindole (**20b**) (0.084 g, 0.63 mmol) in CH₃CN (4 mL). Eluting with DCM : MeOH (100 : 0 – 95 : 5) afforded **21b** (0.114 g, 0.25 mmol, 47% yield) as a white solid. UPLC-MS (method A): Rt 2.12 min; m/z 461 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.20 (s, 1H), 7.78 (t, *J* = 5.7 Hz, 1H), 7.35 – 7.26 (m, 3H), 7.24 (d, *J* = 8.1 Hz, 1H), 7.18 – 7.10 (m, 1H), 7.03 (t, *J* = 7.9 Hz, 1H), 6.70 (d, *J* = 7.6 Hz, 1H), 6.31 – 6.24 (m, 1H), 3.18 – 3.06 (m, 2H), 3.05 – 2.90 (m, 4H), 2.64 – 2.52 (m, 4H), 2.44 – 2.32 (m, 2H), 1.61 – 1.46 (m, 4H). ¹³C NMR (101 MHz, DMSO) δ 154.45, 151.21, 143.88, 137.69, 132.58, 128.41, 125.98, 125.13, 124.29, 121.51, 120.97, 119.53, 111.03, 108.53, 97.87, 57.42, 52.78 (2C), 50.95 (2C), 40.85, 27.27, 23.53. UPLC-MS Purity (UV @ 215 nm): 99%.

Synthesis of *tert*-butyl 4-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamoyloxy]benzimidazole-1-carboxylate (**21c**)

The title compound was synthesized starting from di-*tert*-butyl dicarbonate (0.144 g, 0.66 mmol), DMAP (0.068 g, 0.56 mmol), **6** (0.141 g, 0.47 mmol) and **20c** (0.132 g, 0.56 mmol) in CH₃CN (4 mL). Eluting with DCM : MeOH (100 : 0 – 95 : 5) afforded **21c** (0.113 g, 0.20 mmol, 43% yield) as a white solid. UPLC-MS (method A): Rt 2.48 min; m/z 562 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.58 (s, 1H), 7.94 (t, *J* = 5.7 Hz, 1H), 7.78 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.39 (t, *J* = 8.1 Hz, 1H), 7.33 – 7.26 (m, 2H), 7.18 – 7.08 (m, 2H), 3.19 – 3.07 (m, 2H), 2.99 (br. s, 4H), 2.55 (br. s, 4H), 2.43 – 2.31 (m, 2H), 1.65 (s, 9H), 1.58 – 1.46 (m, 4H).

Synthesis of *tert*-butyl 4-hydroxybenzimidazole-1-carboxylate (**20c**)

A mixture of 1H-Benzoimidazol-4-ol (**22**) (0.200 g, 1.49 mmol), di-*tert*-butyl dicarbonate (0.325 g, 1.49 mmol), DMAP (0.001 g, 0.0149 mmol) and NEt₃ (0.050 mL, 3.43 mmol) in DMF (1 mL) was stirred at room temperature for 2h. Then the mixture was diluted with EtOAc, and washed with saturated solution of NH₄Cl. The organic layer was dried over Na₂SO₄ anhydrous, and concentrated in vacuum. The residue was purified by flash chromatography on silica gel (Eluent: from Cyclohexane to Cyclohexane : Ethyl Acetate = 50 : 50) to afford **20c** (0.132 g, 0.56 mmol, 38%) as a white solid. UPLC-MS (method A): Rt 1.98 min; m/z 235 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.09 (br. s, 1H), 8.48 (s, 1H), 7.41 – 7.34 (m, 1H), 7.19 (t, *J* = 8.1 Hz, 1H), 6.73 (dd, *J* = 8.0, 1.0 Hz, 1H), 1.64 (s, 9H).

Synthesis of 1H-benzimidazol-4-yl *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]carbamate hydrochloride (**23**)

A mixture of **21c** (0.113 g, 0.20 mmol) and 4 M HCl in 1,4-dioxane (1.8 mL, 7.2 mmol) was stirred at room temperature for 5 h. The solvent was removed under reduce pressure, the white solid was dissolved and neutralized with a saturated water solution of NaHCO₃ and extracted with DCM. The united organic layers were dried over Na₂SO₄ anhydrous, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel (Eluent: from DCM to DCM : MeOH = 80 : 20) to afford the desired compound, which was treated with 1.25 M HCl in MeOH. Evaporation of the solvent produced the title compound **23** (0.034 g, 0.069 mmol, 35% yield over two steps) as a white solid. UPLC-MS (method A): Rt 1.85 min; m/z 462 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 15.19 ((br. s, 1H), 11.29 (br. s, 1H), 9.42 (br. s, 1H), 8.12 (t, *J* = 5.7 Hz, 1H), 7.67 (d, *J* = 8.2 Hz, 1H), 7.49 (t, *J* = 8.1 Hz, 1H), 7.41 – 7.32 (m, 2H), 7.29 (d, *J* = 7.9 Hz, 1H), 7.21 (dd, *J* = 7.1, 2.6 Hz, 1H), 3.65 – 3.36 (m, 4H), 3.32 – 3.09 (m, 8H), 1.94 – 1.77 (m, 2H), 1.59 (p, *J* = 7.1 Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 153.41, 149.55, 141.05, 138.58, 133.35, 132.72, 128.65, 126.03, 125.75, 125.28, 119.77, 118.07, 111.41, 99.49, 55.03, 51.00 (2C), 47.63 (2C), 40.55, 26.29, 20.43. UPLC-MS Purity (UV @ 215 nm): 99%.

4. Biological Assays

Cell culture conditions

Hek293 cells stably transfected with human FAAH-1 were used as enzyme source (membrane enrichment) to evaluate hFAAH-1 activity. ValiScreen Dopamine D3 (human) CHO-K1 (ES-173-C, Perkin Elmer) were used to perform cell-based cAMP assay to determine D3R activation. Cells were maintained in DMEM or Ham's F-12, respectively, both supplemented with 10% FBS. 500 µg/mL or 400 µg/mL G418 respectively were added to culture medium to maintain selective pressure.

Activities on D2R and CB-1 were assayed on a DRD2 short-stably transfected Hek-293 cell line and human CB-1 expressing CHO cells.

Preparation of hFAAH-1 membrane-enriched lysate

Cells were grown in 150 mm dishes and scraped off with cold PBS 1x pH 7.4 at 80% confluency. Cell suspensions were centrifuged at 300xg for 7 minutes at 4°C. Cell pellets were re-suspended in homogenizing buffer (20 mM Tris-HCl pH 7.4, 0.32 M sucrose), disrupted by sonication (10 pulses, 2 times) and centrifuged at 1000xg for 10 minutes at 4°C. Supernatants were then centrifuged at 12000xg for 10 minutes and then at 105,000xg for 1 hour at 4°C. Membranes pellets were re-suspended in PBS to obtain h-FAAH1 preparation and protein concentration was measured by Bradford Protein Assay (Bio Rad) and samples aliquoted and stored at -80°C until use.

Human recombinant Fatty Acid Amide Hydrolase (FAAH-1) fluorescent assay

The assay was run in 96 well microplates (Black OptiPlate™-96 F; PerkinElmer, Massachusetts, USA) in a total reaction volume of 180 µL. hFAAH-1 protein preparation (2,5 µg) was pre-incubated for 50 minutes with various concentrations of test compounds or vehicle control (2,5% DMSO) in assay buffer (50mM Tris-HCl pH 7.4, 0.05% Fatty acid-free BSA). AMC Arachidonyl Amide (A6855, Sigma) was used as a substrate (1 µM) and the reaction carried for 4 hours at 37°C. The substrate is prepared in DMSO in order to achieve the final percentage of 5%. Fluorescence was measured with EnVision 2014 Multilabel Reader (PerkinElmer, Massachusetts, USA) using an excitation wavelength of 355 nm and an emission of 460 nm. The compounds were tested at 8 different concentrations ranging from 10 pM up to 250 µM in triplicates. The results are expressed as a percent of the total enzymatic activity (protein preparation incubated with the vehicle control).

With this setup, the FAAH assay performed in the current study returned the following activity data on know FAAH inhibitors:

PF-04457845 (FAAH Inhibitor) IC₅₀: 0.13 nM ± 0.01, n= 16

URB597 (FAAH Inhibitor) IC₅₀: 3.15 nM ± 0.54, n=3

D3R, D2R-short Dopamine receptors and CB-1 cellular assay

D3R assay was run in 384 well microplates (384 Well Small Volume™ HiBase Polystyrene Microplates, Greiner) in a total reaction volume of 20 µl. Activities on D3R were tested with an HTRF-cAMP functional assay (cAMP dynamic 2, CISBIO) on stably transfected human-DRD3 expressing CHO-K1 cells. The cells are suspended in HBSS buffer (Life Technologies) complemented with 20 mM Hepes/NaOH (pH 7.4), 0.1% BSA and 200 µM IBMX. Cells are then seeded in 384 multiwell microplates at a density of 10⁴ cells/well in the presence of either of the HBSS (basal control), the reference agonist (stimulated control) or various concentrations of the test compounds. After 10 minutes of pre-incubation at room temperature (RT), the adenylyl cyclase activator NKH 477 (N3290, Sigma) is added at a final concentration of 0.5 µM. Following 45 minutes incubation at RT, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) are added. After 1 hour at room temperature, the fluorescence transfer is measured at λ_{ex}=320 nm and λ_{em}=620 and 665 nm using EnVision 2014 Multilabel Reader (PerkinElmer, Massachusetts, USA). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The compounds were tested at 7 different concentrations ranging from 1 nM up to 1 µM in triplicates. The results are expressed as a percent of the control response to 300 nM dopamine.

With this setup, the D3R assay performed in the current study returned the following activity data on known D3R modulators:

Dopamine EC₅₀: 3,46nM ± 0.86, n=15

PD128907 (D3R full agonist) EC₅₀: 2.64 nM ± 0.21, n = 3

CJB090 (D3R partial agonist) EC₅₀: 17.27 nM ± 4.66, n = 3, efficiency: 45.23% ± 6.79%

D2R and CB-1 assays were run by CEREP (Le Bois l'Évêque, FR), as previously described by De Simone et al.¹














Analysis of the Biological Data








Dose-response curves were run at least in two independent experiments, performed in three technical replicates. For compounds assayed on D3R and hFAAH-1, concentrations were corrected by NMR determinations. EC₅₀ or IC₅₀ values (concentrations causing half-maximal response or inhibition of control agonist response) were determined by non-linear regression analysis of the Log [concentration]/response curves generated with mean replicate values using a four parameter Hill equation curve fitting with GraphPad Prism 5 (GraphPad Software Inc., CA – USA).

5. Physicochemical Properties

Physicochemical properties play a key role in optimizing drugs for the central nervous system (CNS), as these need to penetrate the blood-brain barrier (BBB). In this study, our efforts were focused on modifying different structural features to improve solubility. In fact, an improved solubility is usually a fundamental prerequisite to better absorption. To this aim, attempts were made to improve the solubility of starting compound **1**, by adding ionizable or polar group, adding groups capable of establishing hydrogen bonds, introducing out-of-plane substitution to reduce crystal packing (reducing sp^2/sp^3 carbon ratio) and reducing logP. In each synthesized compound, we monitored molecular weight (MW), number of hydrogen bond donor (HBD), the calculated logarithm of the partition coefficient (cLogP), polar surface area (PSA) and the sp^2/sp^3 carbon atom ratio of each synthesized compound (Table S1).

Table S1. Physicochemical properties of reported compounds

Compound	Structure				HCl salt	MW ^a	HBD	cLogP ^b	PSA (Å ²) ^b	Sp ² /sp ³ Carbon Ratio
	R ₁	R ₂	n	Ar						
1	Cl	Cl	2		no	471.1	1	6.20	39.23	2.13
5a	OMe	H	2		no	433.2	1	4.98	46.86	1.89
5b	Me	H	2		yes	417.2	1	5.29	39.23	1.89
5c	CF ₃	H	2		yes	471.2	1	6.13	39.23	1.89
5d	Cl	Cl	1		no	457.1	1	5.70	39.23	2.43
5e	OMe	H	1		no	419.2	1	4.49	46.86	2.13
5f	Me	H	1		yes	403.2	1	4.81	39.23	2.13
5g	CF ₃	H	1		no	457.2	1	5.65	39.23	2.13
8a	Cl	Cl	2		yes	489.1	1	6.35	39.23	2.13
8b	Cl	Cl	2		yes	501.1	1	6.17	46.86	1.89
9	Cl	Cl	2		yes	487.1	2	5.82	55.78	2.13
14	Cl	Cl	2		yes	515.1	2	5.78	67.64	2.25
15	Cl	Cl	2		yes	514.1	3	5.02	73.29	2.25

17a	Cl	Cl	2		no	421.1	1	4.87	39.41	1.63
17b	Cl	Cl	2		no	475.2	1	6.06	39.42	1.08
17c	Cl	Cl	2		yes	479.1	1	4.65	55.01	1.30
19	Cl	Cl	2		no	475.2	1	5.85	38.79	1.08
21a	Cl	Cl	2		yes	477.1	1	5.82	40.25	1.88
21b	Cl	Cl	2		no	460.1	2	5.09	49.28	1.88
23	Cl	Cl	2		yes	461.1	2	4.30	60.80	1.75

^aMW of parent; ^bR. Abagyan, A. Orry, E. Raush, M. Totrov. ICM Manual 3.7; Molsoft LCC: San Diego, CA, 2013.

6. Kinetic Solubility

The aqueous solubility is one of the key parameters in order to evaluate the preliminary physico-chemical properties of pharmacological active compounds. In the present study, the aqueous *kinetic* solubility was measured in high throughput as one of the routine assay in each project screening cascade. The kinetic solubility study in aqueous buffer mimics the conditions for biological assays, in which compounds are predissolved in DMSO prior to addition to an in vitro assay. In fact, the purpose of this study was to determine the aqueous kinetic solubility of compounds from a 10 mM DMSO stock solution in Phosphate Buffered Saline (PBS) at pH 7.4. The study was performed incubating an aliquot of 10 mM DMSO stock solution in PBS (pH 7.4) at 25 °C for 24h followed by centrifugation and quantification of dissolved compound in the supernatant by UPLC/MS. The target concentration was 250 µM resulting in a final concentration of 2.5% DMSO. Depending on their kinetic solubility values, compounds can be arbitrarily grouped into low- (0-10 µM), medium/moderate- (10-100 µM), and high-soluble compounds (> 100 µM). These ranges are in line with those reported by Bevan² (i.e., sparingly soluble < 10 µg/mL; partially soluble 10-100 µg/mL; soluble > 100 µg/mL). These ranges can be used as a guideline for addressing potential solubility issues in early stage drug discovery projects. The final aqueous solubility (S_{Kinetic}) was determined by UV quantification at a specific wavelength (215 nm). S_{Kinetic} was calculated by dividing the peak area of the supernatant by the peak area of the reference and multiply by the reference concentration (µM) and dilution factor. For compounds with poor UV absorbance, the aqueous solubility was verified by MS quantification using the extracted IC trace. The analytical samples were further diluted in order to be within the linearity range for quantification. In the present study, the reference concentration was 250 µM and the final dilution factor was 1.25 giving the following equation:

$$\text{Aqueous Solubility } (\mu\text{M}) = \frac{\text{Peak Area at 215 nm (Supernatant)}}{\text{Peak Area at 215 nm (Reference)}} \times 250 \times 1.25$$

In this study, three reference compounds representative of lower, medium/moderate, and higher aqueous kinetic solubility, respectively, were also tested in parallel according to the same protocol (caffeine >250 µM, terfenadine 98 ± 5 µM, β-estradiol 11 ± 3 µM).

Tested in according to the same protocol, known FAAH inhibitors and D3R modulators were assigned the following aqueous kinetic solubility values:

URB597: 2 µM;

PF-04457845: <1 µM;

NGB-2904 (D3R antagonist): <1 µM;

CJB090 (D3R partial agonist): <1 µM.

7. Notes and references

- 1 A. De Simone, G. F. Ruda, C. Albani, G. Tarozzo, T. Bandiera, D. Piomelli, A. Cavalli, G. Bottegoni, *Chem. Commun.*, 2014, **50**, 4904.
- 2 C. D. Bevan, *Anal. Chem.*, 2000, **72**, 1781.