Pharmacological Mechanism of the Non-Hallucinogenic 5-HT2A agonist Ariadne and Analogs

Michael J. Cunningham[#], Hailey A. Bock[#], Inis C. Serrano[#], Benjamin Bechand, D. J. Vidyadhara, Emma M. Bonniwell, David Lankri, Priscilla Duggan, Antonina L. Nazarova, Andrew B. Cao, Maggie M. Calkins, Prashant Khirsariya, Christopher Hwu, Vsevolod Katritch, Sreeganga S. Chandra, John D. McCorvy*****, Dalibor Sames*

AUTHOR INFORMATION

Corresponding Authors

Dalibor Sames - Department of Chemistry, and Zuckerman Institute of Mind, Brain, Behavior, Columbia University, New York, NY 10027, United States of America; orcid.org/0000-0001-6911-2260 **Email**: ds584@columbia.edu

John D. McCorvy - Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, United States of America; orcid.org/0000-0001-7555-9413 **Email**: jmccorvy@mcw.edu

Other Authors

Michael J. Cunningham - Department of Chemistry, Columbia University, New York, NY 10027, United States of America; orcid.org/0000-0001-5620-2865 **Email:** mjcunnin1@gmail.com

Hailey A. Bock - Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, United States of America; orcid.org/0000-0002-3457-9319 **Email:** hhammen@mcw.edu

Inis C. Serrano - Department of Chemistry, Columbia University, New York, NY 10027, United States of America; orcid.org/0000-0002-3588-821
Email: ics2115@columbia.edu

Benjamin Bechand - Department of Chemistry, Columbia University, New York, NY 10027, United States of America; orcid.org/0000-0001-5237-2373; **Email:** bb2858@columbia.edu

D. J. Vidyadhara - Department of Neuroscience, Department of Neurology, Yale University, New Haven, CT 06510, United States of America; orcid.org/0000-0003-0974-0307 **Email:** vidyadhara.dj@yale.edu

Emma M. Bonniwell - Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, United States of America; orcid.org/0000-0001-5743-852X **Email:** ebonniwell@mcw.edu
David Lankri - Department of Chemistry, Columbia University, New York, NY 10027, United States of America; orcid.org/0000-0002-1210-5673; **Email**: dl3433@columbia.edu

Priscilla Duggan - Department of Neuroscience, Barnard College, New York, NY 10027, United States of America; orcid.org/0000-0002-9728-282X ; **Email:** pcd2122@barnard.edu

Antonina L. Nazarova - Department of Quantitative and Computational Biology, Department of Chemistry, Dornsife Center for New Technologies in Drug Discovery and Development, Bridge Institute, Michelson Center for Convergent Biosciences, University of Southern California, Los Angeles, CA 90089, USA. orcid.org/0000-0002-8480-8861 **Email:** nazarova@usc.edu

Andrew B. Cao - Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, United States of America;
Email: acao@mcw.edu

Maggie M. Calkins - Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, United States of America,
Email: mmcalkins7@gmail.com

Prashant Khirsariya - Department of Chemistry, Columbia University, New York, NY 10027, United States of America; orcid.org/0000-0001-8570-1866
Email: pk2724@columbia.edu

Christopher Hwu - Department of Chemistry, Columbia University, New York, NY 10027, United States of America; **Email:** christopher.hwu@columbia.edu

Vsevolod Katritch - Department of Quantitative and Computational Biology, Department of Chemistry, Dornsife Center for New Technologies in Drug Discovery and Development, Bridge Institute, Michelson Center for Convergent Biosciences, University of Southern California, Los Angeles, CA 90089, USA. orcid.org/0000-0003-3883-4505
Email: katritch@usc.edu

Sreeganga S. Chandra - Department of Neuroscience, Department of Neurology, Yale University, New Haven, CT 06510, United States of America; orcid.org/0000-0001-9035-1733 **Email:** sreeganga.chandra@yale.edu

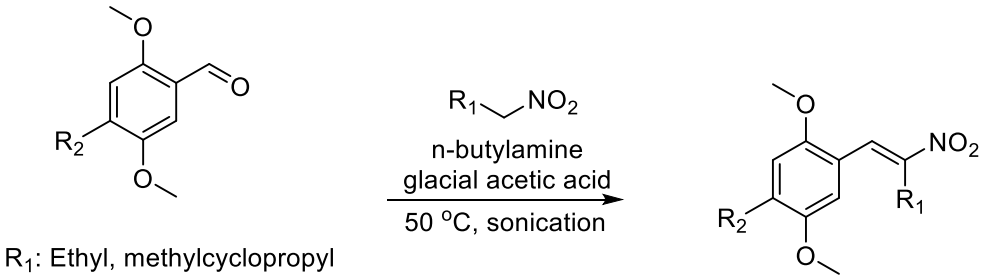
Table of Contents:

Experimental Details

General Considerations. Reagents and solvents were obtained from commercial sources and were used without further purification unless otherwise stated. Reactions were monitored by TLC using solvent mixtures appropriate to each reaction. Column chromatography was performed on silica gel (40 – 63 μm). For basic amines, Et_3N was used in the mobile phase to provide better resolution when using silica gel chromatography. For preparative TLC, glass plates coated with a 1 mm silica layer were used. Nuclear magnetic resonance spectra were recorded on Bruker 400 or 500 MHz instruments, as indicated. Chemical shifts are reported as δ values in ppm referenced to Chloroform-d (^{1}H NMR = 7.26 and ^{13}C NMR = 77.16) or methanol-d_4 (^{1}H NMR = 3.31 and ^{13}C NMR = 49.00). Multiplicity is indicated as follows: s (singlet); d (doublet); t (triplet); dd (doublet of doublets); td (triplet of doublets); dt (doublet of triplets); dq (doublet of quartets); ddd (doublet of doublet of doublets); ddt (doublet of doublet of triplets); m (multiplet); br (broad). Low-resolution mass spectra were recorded on an Advion ExpressIon CMS-L with automated TLC plate reader instrument (ionization mode: APCI+ or ESI+) or by GC-MS (ionization mode: EI). For many aldehyde and nitrostyrene intermediates that poorly ionized, materials were taken forward and characterized as amine products (either as salts or freebases). High-resolution mass spectra (HRMS) were obtained on a Xevo G2 XS Q-ToF mass spectrometer in the positive electrospray ionization mode located in Columbia University chemistry department Mass Spectrometry Core Facility

1. General procedure A: Preparation of nitrostyrenes

Scheme S1.



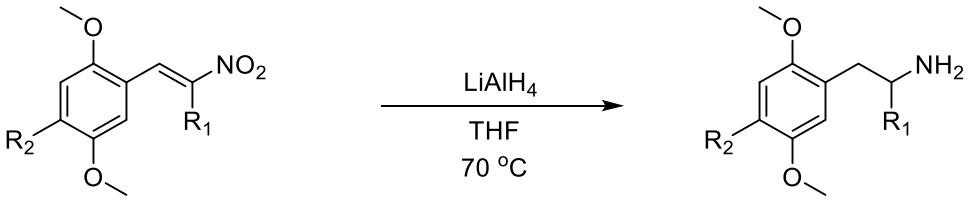
R_1: Ethyl, methylcyclopropyl
R_2: Me, Br, I, CN, CF_3, cyclopropyl, MeS, EtS, PhS

From: Shengkun, L; Kexuan, H.; Xumu, Z.; Enantioselective hydrogenation of α,β-disubstituted nitroalkenes. *Chem. Commun.,* **2014**, 50, 8878-8881. Modification: used without additional heating, vessel temperature reaches ~50 °C over the course of reaction.

Benzaldehyde (1 eq) was added to a reaction tube with a magnetic stir bar followed by glacial acetic acid (5.5 eq) and nitropropane (1.5 eq). *n*-Butylamine (2 eq) was then added dropwise with stirring. Reaction mixture was sealed with septum and placed in a sonicating water bath for 16 hr, then diluted with 3x the volume of toluene as the reaction mixture. This solution was transferred immediately to a short silica gel column and purified using 100% toluene as eluent.

2. General procedure B: Preparation of butanamine products using lithium aluminum hydride

Scheme S2.

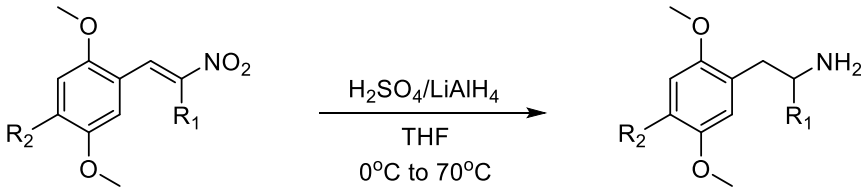


R_1: Ethyl, methylcyclopropyl
R_2: MeS, EtS, PhS

Per 1 mmol of nitrostyrene: A solution of 1 mmol of appropriate nitrostyrene in 2 mL of THF was added dropwise to a suspension of 7 mmol of $LiAlH_4$ in 3.5 mL of THF while stirring under an argon atmosphere. The reaction mixture was heated 18 hr at 70 °C, cooled to 0 °C. Reaction was carefully quenched with isopropanol (200 µL), water (200 µL), aqueous NaOH solution (200 µL, 15% NaOH), and finally more water (600 µL) then stirred vigorously 30 min at room temperature. The suspension was vacuum filtered over Celite pad and filter cake washed 3x with ethyl acetate. Solvent was removed under reduced pressure, and the crude product was purified over with flash chromatography (9:1 ethyl acetate/methanol + 2% triethylamine).

3. General procedure C: Preparation of butanamine products using alane (aluminum hydride)

Scheme S3.



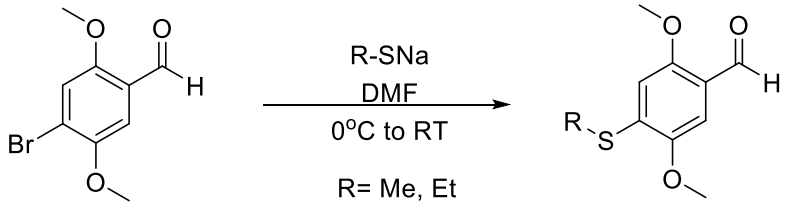
R_1: Ethyl
R_2: Me, Br, I, CF_3, cyclopropyl

Per 1 mmol of nitrostyrene: Under argon atmosphere, 7 mL of anhydrous THF was added to oven dried scintillation vial containing a stir bar followed by lithium aluminum hydride (5 mmol) rapidly added in 3 portions. Reaction mixture was then cooled with ice water

bath 5 min before adding sulfuric acid (2.5 mmol, approx. normality of 36) diluted in 2 mL dry THF in slow, dropwise fashion. This mixture was allowed to stir for 20 min in ice bath. Appropriate nitrosyrene was dissolved in 3 mL of dry THF and added dropwise (followed by 2 x 1 mL washes dry THF). The reaction mixture was then heated to 65 ^{o}C for 2 hr and then cooled to 0 ^{o}C with ice bath. Reaction was diluted with THF (3 mL), isopropanol was added dropwise (200 µL), water was added dropwise (200 µL), aqueous NaOH solution added dropwise (200 µL, 15% NaOH), and more water (600 µL) added dropwise and stirred vigorously 30 min at room temperature. The suspension was vacuum filtered over Celite pad and filter cake washed 3x with ethyl acetate. Solvent was removed under reduced pressure, and the crude product was purified over with flash chromatography (9:1 ethyl acetate/methanol + 2% triethylamine).

4. General procedure D: Preparation of 4-alkylthiobenzaldehydes

Scheme S4.



From: *Synthetic Commun*. **1986,** 16, 565-70. Modification: Commercially available sodium alkylthiolates were used directly instead of generation *in situ*.

11 mmol of appropriate sodium *n*-alkanethiolate was added to 25 mL of anhydrous DMF under argon in one portion. This suspension was cooled in ice-bath for 10 min, then 11 mmol of 4-bromo-2,5- dimethoxybenzaldehyde was added in one portion. Reaction mixture was allowed to reach room temperature naturally and to stir for 16 hr, then it was poured into 400 mL water, off-white precipitate was filtered and allowed to air-dry.

2,5-dimethoxy-4-(trifluoromethyl)benzaldehyde 1 (Procedure A)

From: *Chem. Commun.,* **2013**, 49, 6385.

An oven dried 100 mL flask under argon was charged with 2,5-dimethoxybenzaldehyde (15 mmol), (diacetoxyiodo)benzene (30 mmol), trimethyl(trifluoromethyl)silane (30 mmol) and 50 mL anhydrous DMSO. This mixture was stirred at room temperature for 5 min, then AgF (3.75 mmol) was slowly added portion-wise to the stirring mixture and was stirred under argon at room temperature for 20 hr. The reaction was quenched by pouring contents of flask into 300 mL water and extracted 3 x 100 mL toluene. Pooled organic extracts were washed with brine and dried over $MgSO_4$. Following removal of solvent under reduced pressure, crude material was purified with flash chromatography using silica gel and 1:3 hexanes/DCM yielding 536 mg of product (15% yield).

Alternate preparation of 2,5-dimethoxy-4-(trifluoromethyl)benzaldehyde 1 (Procedure B)

From: Patent WO2022038170A1, "Therapeutic phenethylamine compositions and methods of use".

Note: Separate reactions run in parallel 1g x 10 = 10 g, combined for workup and purification.

In a glass vial with screw teflon cap, methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (1.31 mL, 10.27 mmol) and copper (I) iodide (98 mg, 0.514 mmol) were added to a solution of compound 1 (1 g, 3.42 mmol) in dry DMF (12 mL) at room temperature. The reaction mixture was heated at 75 °C for 16 h. The reaction mixture cooled to room temperature, filtered thorough Celite, washed with ethyl acetate (300 mL) and the solvents were evaporated in vacuo. The residue was purified by column chromatography (hexane/ethyl acetate; 9:1). The product was obtained as a white solid (6.94 g, 87 %).

1H NMR (500 MHz, Chloroform-d) δ 10.47 (s, 1H), 7.43 (s, 1H), 7.22 (s, 1H), 3.94 (s, 3H), 3.90 (s, 3H). **13C NMR (126 MHz, Chloroform-d)** δ 188.92, 155.52, 151.60 (d, J = 1.8 Hz), 127.59, 124.97 (q, J = 31.1 Hz), 123.00 (q, 1JC-F = 273.3 Hz), 111.70 (q, J = 5.4 Hz), 111.16, 56.74, 56.58. **^{19}F NMR (471 MHz, Chloroform-d)** δ -62.07. **HRMS (ESI+):** calcd. for $C_{10}H_{10}F_3O_3$ $[M+H]^+$ = 235.0582, found [M+H]+ = 235.0612.

***tert*-butyl (1-(4-bromo-2,5-dimethoxyphenyl)butan-2-yl)carbamate 2**

Di-tert-butyl dicarbonate (630 mg, 2.9 mmol, 1.6 eq) was added to a 20 mL scintillation vial followed by 1-(4-bromo-2,5-dimethoxyphenyl)butan-2-amine **21** (520 mg,1.8 mmol, 1 eq), 10 mL DCM and while stirring, 400 µL (2.9 mmol, 1.6 eq) triethylamine was added dropwise and stirred at room temperature 16 hr. The reaction mixture was diluted with 10 mL DCM, then poured into saturated NH_4Cl (70mL), washed with $NaHCO_3$ (70mL), and brine (70mL). DCM solution was dried over Na_2SO_4, dried and purified with flash chromatography using silica gel and 1:2 ethyl acetate/hexanes, yielding 400 mg of product (89% yield).

1H NMR (400 MHz, Chloroform-d) δ 7.02 (s, 1H), 6.74 (s, 1H), 4.47 (s, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.75 (bfs, 1H), 2.74 (m, 2H), 1.60 – 1.55 (m, 1H), 1.36 (s, 9H), 0.94 (t, J = 7.4 Hz, 3H). **LRMS (APCI+)** calcd. For $C_{17}H_{27}BrNO_4$ $[M+H]^+$ = 388.11, found 388.3

tert-butyl (1-(4-cyano-2,5-dimethoxyphenyl)butan-2-yl)carbamate 3

From: *J. Med. Chem*. **2010**, 53, 5656–5666

Polymethylhydrosiloxane (8 mg) was added to a 20 mL oven-dried scintillation vial, followed by tert-butyl (1-(4-bromo-2,5-dimethoxyphenyl)butan-2-yl)carbamate **2** (85mg, 0.22 mmol, 1 eq), followed by zinc cyanide (20mg, 0.17 mmol, 0.8 eq) and tetrakis(triphenylphosphine)palladium (25 mg, 0.22 mmol, 0.1 eq), then sealed with septum and vacuum/argon cycled 3 times. 5 mL anhydrous DMF was then added and a stream of argon was passed through the suspension with a needle while stirring vigorously for 15 min. The reaction was then heated to 75 ^oC for 16 hr. The reaction mixture was cooled, filtered over a pad of Celite, which was rinsed with ethyl acetate (60 mL). The filtrate was washed with 100 mL saturated $NaHCO_3$, followed by 100 mL dionized water and finally with 100 mL brine. Organic fraction was dried over Na_2SO_4 and the concentrated crude material was subjected to flash chromatography using silica gel and 1:2 ethyl acetate/hexanes, yielding 53 mg of product (72% yield).

1H NMR (500 MHz, Chloroform-d) δ 6.95 (s, 1H), 6.82 (s, 1H), 4.42 (d, 1H), 3.88 (s, 3H), 3.80 (s, 3H), 3.78 (m, 1H), 2.82 (dd, 1H), 2.72 (dd, 1H), 1.55 (m, 1H), 1.43 (m, 1H), 1.35 (s, 9H), 0.96 (t, 3H). **LRMS (APCI+)** calcd. For $C_{18}H_{27}N_2O_4$ $[M+H]^+$ = 335.2, found 335.2

4-(2-aminobutyl)-2,5-dimethoxybenzonitrile trifluoroacetate 4

Tert-butyl (1-(4-cyano-2,5-dimethoxyphenyl)butan-2-yl)carbamate (48 mg, 143 umol, 1 eq) was dissolved in 2 mL dry DCM and 66 µL (861 umol, 6 eq) trifluoroacetic acid was added to the stirred solution. The solution was stirred overnight at room temperature before liquids were completely removed by rotary evaporation. This crude material was redissolved in 1 mL water and methanol mixture (3:7) and eluted through C18 cartridge (Thermo-Scientific 50 mg Hypersep column). Solvent was removed under reduced pressure to give 42 mg solidf white trifluoroacetate salt product (72% yield).

1H NMR (500 MHz, DMSO-d6) δ 7.86 (s, 3zzH), 7.36 (s, 1H), 7.13 (s, 1H), 3.87 (s, 3H), 3.79 (s, 3H), 3.36 – 3.29 (m, 1H), 2.92 (dd, J = 13.6, 6.6 Hz, 1H), 2.83 (dd, J = 13.6, 7.2 Hz, 1H), 1.57 – 1.48 (m, 2H), 0.93 (t, J = 7.5 Hz, 3H). **13C NMR (126 MHz, DMSO-d6)** δ 158(m), 155.63, 151.68, 132.91, 116.91, 115.85, 115.42, 99.09, 56.93, 56.75, 52.16, 33.60, 25.63, 9.81. **LRMS (APCI+)** calcd. For $C_{13}H_{18}N_2O_2$ $[M+H]^+$ = 235.1, found 235.2

4-cyclopropyl-2,5-dimethoxybenzaldehyde 5

4-Bromo-2,5-dimethoxybenzaldehyde (1.2 grams, 4.9 mmol, 1 eq), potassium cyclopropyl trifluoroborate salt (797 mg, 5.4 mmol, 1.1eq), RuPhos (91 mg, 0.195 mmol, 0.04 eq), palladium acetate (22 mg, 0.098 mmol, 0.02 eq), and cesium carbonate (479 mg, 14.7 mmol, 3 eq) was added to oven dried vial equipped with stir-bar and sealed with septum. The mixture was vacuum/argon cycled 3 times and toluene (11 mL) and water (4 mL) were added. Reaction mixture was stirred vigorously at 95 °C for 40 hr. The reaction mixture was then cooled, filtered over Celite and rinsed with ethyl acetate. Filtrate was washed with saturated NH_4Cl solution followed by brine and organic fraction was dried over Na_2SO_4 and concentrated under reduced pressure. Crude material was subjected to flash chromatography using silica gel and hexane/DCM gradient to yield 879 mg of off-white solid (87% yield).

1H NMR (500 MHz, Chloroform-d) δ 10.37 (s, 1H), 7.26 (s, 1H), 6.40 (s, 1H), 3.85 (d, J = 2.9 Hz, 6H), 2.28 (tt, J = 8.5, 5.3 Hz, 1H), 1.09 – 1.02 (m, 2H), 0.78 – 0.71 (m, 2H). **13C NMR (101 MHz, Chloroform-d)** δ 189.15, 157.28, 152.55, 142.48, 122.43, 108.39, 108.23, 56.27, 56.21, 10.50, 9.46 (2C).

4-iodo-2,5-dimethoxybenzaldehyde 6

From: *Org. Biomol. Chem.*; **2014**,12, 6105-6113

A mixture of 2,5-dimethoxybenzaldehyde (5.1 g, 31 mmol, 1 eq), silver nitrate (5.21 g, 31 mmol, 1 eq), and iodine (8.1 g, 31.9 mmol, 1.04 eq) in 125 mL of methanol was stirred under argon overnight. The yellow precipitate was filtered and washed with cool methanol. The remaining iodine was reduced with saturated sodium bisulfite solution by addition until the disappearance of yellow color. The solvent was removed on a rotary evaporator and the residue recrystallized from 95% ethanol to yield 8.3 grams of fluffy beige solid (93% yield).

1H NMR (500 MHz, Chloroform-d) δ 10.40 (s, 1H), 7.47 (s, 1H), 7.22 (s, 1H), 3.90 (s, 3H), 3.87 (s, 3H). **HRMS (ESI+):** calcd. for $C_9H_{10}IO_3$ $[M+H]^+$ = 292.9675, found 292.9693.

2,5-dimethoxy-4-propylbenzaldehyde 7

To an oven-dried flask was added 4-bromo-2,5-dimethoxy-benzaldehyde (7.5 g, 30.6 mmol, 1 eq), XPhos (4.38, 9.18 mmol, 0.30 eq), $Pd_2(dba)_3$ (2.8 g, 3.06 mmol, 0.10 eq), followed by toluene (300 mL), propyl-boronic acid (4.04 g, 45.9 mmol, 1.5 eq) and K_3PO_4 (19.49 g, 91.81 mmol, 3 eq). The solution was stirred under argon at 110 °C for 18 hr. After cooling, the reaction mixture was filtered over Celite and rinsed with ethyl acetate and concentrated under reduced pressure. The crude material was subjected to flash chromatography using silica gel and ether/hexane gradient to yield 2.55 grams of pale yellow oil (40% yield).

1H NMR (300 MHz, Chloroform-d) δ 10.47 – 10.30 (m, 1H), 7.26 (s, 1H), 6.78 (s, 1H), 3.87 (s, 3H), 3.80 (s, 3H), 2.72 – 2.53 (m, 2H), 1.74 – 1.49 (m, 2H), 1.05 – 0.87 (m, 3H). **^{13}C NMR (75 MHz, Chloroform-d)** δ 189.17, 156.69, 151.77, 140.92, 122.89, 113.91, 108.08, 56.16, 55.76, 33.07, 22.72, 13.99.

5-ethoxy-2-methoxybenzaldehyde 8

5-hydroxy-2-methoxybenzaldehyde (1.5 g, 9.86 mmol, 1 eq) and anhydrous K_2CO_3 (2.73 g, 19.72 mmol, 2 eq), were added to acetonitrile (15 mL) in a 50-mL flask. The reaction

mixture was heated gently at 65 °C for 30 min, iodoethane (6.34 mL, 78.87 mmol, 8 eq) was added

dropwise, and was heated at 65 °C for 16 hr. After completion of the reaction, the reaction mixture concentrated partitioned between toluene and water, organics washed with brine. The crude material was subjected to flash chromatography using silica gel and hexane/DCM gradient to yield 1.27 grams of amber oil (71% yield). Taken forward without further characterization.

5-ethoxy-2-methoxy-4-(trifluoromethyl)benzaldehyde 9

Prepared in the same fashion as compound **1** using 5-ethoxy-2-methoxybenzaldehyde **8** as starting material with a yield of 12%

^{1}H NMR (300 MHz, Chloroform-d) δ 10.47 (s, 1H), 7.43 (s, 1H), 7.22 (s, 1H), 4.13 (q, *J* = 7.0 Hz, 2H), 3.94 (s, 3H), 1.42 (t, *J* = 9.7, 4.2 Hz, 3H).

(4-bromo-2,5-dimethoxyphenyl)methanol 10

To an oven-dried flask was added 4-bromo-2,5-dimethoxybenzaldehyde (3.2 g 13 mmol, 1 eq), 40mL MeOH, 10mL diethyl ether, 10mL THF. Afer cooling with ice water bath, sodium borohydride (553 mg, 14.6 mmol, 1.12 eq) was added in 2 portions spaced 5 min. Reaction allowed to reach rt naturally and stirred 16 hr. Reaction was poured into 10% HCl and extracted with DCM. Dried over magnesium sulfate, concentrated and used without further purification with yield of 99%.

1-bromo-2,5-dimethoxy-4-(methoxymethyl)benzene 11

A suspension of (4-bromo-2,5-dimethoxyphenyl)methanol (3.22 g; 13 mmol) in anhydrous dimethylformamide (90 mL) was cooled to 0 °C. Then, NaH (60% dispersion in mineral oil; 1.2 mg; 30 mmol; 2.3 eq) was added. The reaction mixture was stirred for 1 h at room temperature. Then methyl iodide (4.46 mL, 71.7 mmol; 5.5 eq) was added and the stirring was continued for further 3 h. The mixture was added to water (250 mL) and extracted with dichloromethane (3x 50 mL). The organic layer was dried over anhydrous Na_2SO_4,

filtered, and evaporated in vacuo. The crude material was eluted through a silica gel pad with DCM to give a crude solid that was used without further purification with yield of 62%.

2,5-dimethoxy-4-(methoxymethyl)benzaldehyde 12

To a solution of 1-bromo-2,5-dimethoxy-4-(methoxymethyl)benzene (2.07 g, 7.93 mmol, 1 eq) in anhydrous diethyl ether (125 mL) at 0°C was added n-butyllithium (2M cyclohexanes, 4.44 mL, 8.88 mmol, 1.12 eq) dropwise over five min. The solution was stirred at 0°C for 10 min, and DMF (1.84 mL, 23.78 mmol, 3 eq) was added. The reaction was stirred at 0°C for 5 min, rt 10 min, and heated to 40°C for 10 min. The reaction was poured into 10% HCl, was extracted with DCM, and was dried over Mg_2SO_4, filtered, and concentrated to a yellow solid. Purification by silica gel chromatography provided product in 49% yield.

^{1}H NMR (400 MHz, Chloroform-d) δ 10.43 (s, 1H), 7.28 (s, 1H), 7.12 (s, 1H), 4.52 (d, *J* = 0.8 Hz, 2H), 3.92 (s, 3Hz), 3.83 (s, 3H), 3.49 (s, 3H)

<u>Preparation of Nitrostyrenes</u>

(E)-1,4-dimethoxy-2-methyl-5-(2-nitrobut-1-en-1-yl)benzene 13

Prepared according to general procedure A using 2,5-dimethoxy-4-methylbenzaldehyde and nitropropane with yield of 80%.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.23 (s, 1H), 6.80 – 6.74 (m, 2H), 3.82 (d, J = 8.3 Hz, 6H), 2.85 (q, J = 7.4 Hz, 2H), 2.27 (s, 3H), 1.28 (t, J = 7.4 Hz, 3H).

(E)-1-bromo-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene 14

Prepared according to general procedure A using 4-bromo-2,5-dimethoxybenzaldehyde and nitropropane with yield of 83% and taken forward without further characterization.

(E)-1-iodo-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene 15

Prepared according to general procedure A using 4-iodo-2,5-dimethoxybenzaldehyde and nitropropane with yield of 66%.

^{1}H NMR (500 MHz, Chloroform-d) δ 8.10 (d, J = 0.6 Hz, 1H), 7.35 (s, 1H), 6.74 (s, 1H), 3.84 (d, J = 10.3 Hz, 6H), 2.80 (q, J = 7.4 Hz, 2H), 1.27 (t, J = 7.4 Hz, 3H).

(E)-1-cyclopropyl-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene 16

Prepared according to general procedure A using 4-cyclopropyl-2,5-dimethoxybenzaldehyde and nitropropane with yield of 72%.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.23 (s, 1H), 6.80 (s, 1H), 6.40 (s, 1H), 3.82 (d, J = 17.0 Hz, 6H), 2.85 (q, J = 7.4 Hz, 2H), 2.24 (tt, J = 8.5, 5.3 Hz, 1H), 1.29 (t, J = 7.4 Hz, 3H), 1.07 – 0.98 (m, 2H), 0.76 – 0.68 (m, 2H).

(E)-1,4-dimethoxy-2-(2-nitrobut-1-en-1-yl)-5-(trifluoromethyl) benzene 17

Prepared according to general procedure A using 2,5-dimethoxy-4-(trifluoromethyl) benzaldehyde and nitropropane with yield of 89%.

^{1}H NMR (500 MHz, Chloroform-d) δ 8.09 (s, 1H), 7.13 (s, 1H), 6.91 (s, 1H), 3.87 (d, J = 8.9 Hz, 6H), 2.78 (q, J = 7.3 Hz, 2H), 1.26 (t, J = 7.4 Hz, 3H).

(E)-(2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)phenyl)(methyl)sulfane 18

Prepared according to general procedure A using 2,5-dimethoxy-4-(methylthio)benzaldehyde and nitropropane with yield of 79%.

^{1}H NMR (500 MHz, Chloroform-d) δ 8.26 (s, 1H), 6.81 (s, 1H), 6.74 (s, 1H), 3.89 (d, J = 1.7 Hz, 6H), 2.87 (q, J = 7.4 Hz, 2H), 2.51 (s, 3H), 1.31 (t, J = 7.4 Hz, 3H).

(E)-(2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)phenyl)(ethyl)sulfane 19

Prepared according to general procedure A using 4-(ethylthio)-2,5-dimethoxybenzaldehyde and nitropropane with yield of 69%.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.24 – 8.20 (m, 1H), 6.81 (d, J = 6.9 Hz, 2H), 3.86 (d, J = 4.2 Hz, 6H), 2.98 (q, J = 7.4 Hz, 2H), 2.85 (q, J = 7.4 Hz, 2H), 1.38 (t, J = 7.4 Hz, 3H), 1.29 (t, J = 7.4 Hz, 3H).

(E)-1-ethyl-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene 20

Prepared according to general procedure A using commercially available 4-ethyl-2,5-dimethoxybenzaldehyde and nitropropane with yield of 66% and taken forward without further characterization.

(E)-1-ethoxy-4-methoxy-5-(2-nitrobut-1-en-1-yl)-2-(trifluoromethyl)benzene 21

Prepared from 5-ethoxy-2-methoxy-4-(trifluoromethyl)benzaldehyde using general procedure A with a yield of 50% and taken forward without further characterization.

(E)-1,4-dimethoxy-2-(methoxymethyl)-5-(2-nitrobut-1-en-1-yl)benzene 22

Prepared from 2,5-dimethoxy-4-(methoxymethyl)benzaldehyde **12** using general procedure A with a yield of 93%.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.22 (s, 2H), 7.04 (s, 2H), 6.79 (s, 2H), 4.52 (s, 4H), 3.86 (s, 3H), 3.81 (s, 3H), 3.48 (s, 3H), 2.83 (q, *J* = 7.4 Hz, 2H), 1.27 (t, *J* = 7.4 Hz, 3H).

(E)-1-(2-cyclopropyl-2-nitrovinyl)-2,5-dimethoxy-4-methylbenzene 23

Prepared according to general procedure A from 2,5-dimethoxy-4-methylbenzaldehyde and (nitromethyl)cyclopropane with a yield of 80% and taken forward without further characterization.

(E)-1-chloro-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene 24

Prepared according to general procedure A using 4-chloro-2,5-dimethoxybenzaldehyde and nitropropane with yield of 92% and taken forward without further characterization.

(E)-1,4-dimethoxy-2-(2-nitrobut-1-en-1-yl)-5-propylbenzene 25

Prepared according to general procedure A using 2,5-dimethoxy-4-propylbenzaldehyde and nitropropane with yield of 73%.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.27 (s, 1H), 6.80 (d, *J* = 15.4 Hz, 2H), 3.95 – 3.72 (m, 6H), 2.97 – 2.78 (m, 2H), 2.75 – 2.49 (m, 2H), 1.72 – 1.52 (m, 2H), 1.31 (t, *J* = 7.3 Hz, 3H), 1.00 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR (101 MHz, Chloroform-d)** δ 152.51, 152.13, 151.29, 135.64, 129.58, 118.85, 113.23, 111.36, 56.13, 55.91, 32.70, 22.99, 21.10, 14.05, 12.47.

(E)-1,4-dimethoxy-2-(2-nitropent-1-en-1-yl)-5-propylbenzene 26

Prepared according to general procedure A using 2,5-dimethoxy-4-propylbenzaldehyde and nitrobutane with yield of 79%.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.29 (s, 1H), 6.79 (d, *J* = 15.6 Hz, 2H), 3.95 – 3.74 (m, 6H), 2.99 – 2.75 (m, 2H), 2.73 – 2.54 (m, 2H), 1.79 – 1.50 (m, 4H), 1.13 – 0.92 (m, 6H). **^{13}C NMR (101 MHz, Chloroform-d)** δ 152.59, 151.27, 151.02, 135.62, 129.74, 118.88, 113.24, 111.24, 56.15, 55.87, 32.71, 29.57, 22.98, 21.49, 14.06 (2C).

Preparation of Ariadne (32) and Analogs

1-(4-(ethylthio)-2,5-dimethoxyphenyl)butan-2-amine 27

Prepared from (E)-(2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)phenyl)(ethyl)sulfane using general procedure B with yield of 23%.

^{1}H NMR (400 MHz, Chloroform-d) δ 6.84 (s, 1H), 6.69 (s, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 2.91 (q, J = 7.4 Hz, 3H), 2.79 (dd, J = 13.1, 4.6 Hz, 1H), 2.43 (dd, J = 13.1, 8.6 Hz, 1H), 1.57 – 1.45 (m, 1H), 1.36 (dd, J = 14.2, 6.9 Hz, 2H), 1.29 (t, J = 7.4 Hz, 4H), 0.97 (t, J = 7.4 Hz, 3H). **^{13}C NMR (101 MHz, Chloroform-d)** δ 151.86, 151.81, 127.46, 122.07, 114.17, 113.88, 56.46, 56.15, 52.92, 38.77, 30.57, 26.93, 14.29, 10.63. **LRMS (APCI+)** calcd. For $C_{14}H_{24}NO_2S$ $[M+H]^+$ = 270.1, found 270.1.

1-(2,5-dimethoxy-4-(methylthio)phenyl)butan-2-amine 28

Prepared from (E)-(2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)phenyl)(methyl)sulfane using general procedure B with yield of 70%.

^{1}H NMR (400 MHz, Methanol-d) δ 6.77 (s, 1H), 6.68 (s, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 2.92 (m, J = 8.5, 7.4, 4.9 Hz, 1H), 2.84 – 2.75 (m, 1H), 2.44 (s, 4H), 1.52 (m, 1H), 1.36 (m, 1H), 0.98 (t, J = 7.4 Hz, 3H). **LRMS (APCI+)** calcd. For $C_{13}H_{22}NO_2S$ $[M+H]^+$ = 256.1, found 256.1.

1-(2,5-dimethoxy-4-(trifluoromethyl)phenyl)butan-2-amine 29

Prepared from (E)-1,4-dimethoxy-2-(2-nitrobut-1-en-1-yl)-5-(trifluoromethyl)benzene using general procedure C with yield of 24%.

^{1}H NMR (500 MHz, Chloroform-d) δ 7.03 (s, 1H), 6.84 (s, 1H), 3.86 (s, 3H), 3.80 (s, 3H), 3.00 – 2.92 (m, 1H), 2.85 (dd, J = 13.0, 4.7 Hz, 1H), 2.49 (dd, J = 13.0, 8.6 Hz, 1H), 1.59 – 1.46 (m, 1H), 1.44 – 1.30 (m, 1H), 0.98 (t, J = 7.4 Hz, 3H). **^{13}C NMR (126 MHz, Chloroform-d)** δ 151.42, 151.38, 133.98, 123.89 (q, $^1J_{C\text{-}F}$ = 272.1 Hz), 117.09 (q, J = 31.1 Hz), 116.08, 109.49 (q, J = 5.4 Hz), 56.89, 56.22, 52.94, 39.12, 30.85, 10.73. **^{19}F NMR** (471 MHz, Chloroform-d) δ -60.85. **HRMS (ESI):** calcd. for $C_{13}H_{19}F_3NO_2$ $[M+H]^+$ = 278.1368, found 278.1374.

1-(4-cyclopropyl-2,5-dimethoxyphenyl)butan-2-amine 30

Prepared from (E)-1-cyclopropyl-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene using general procedure C in yield of 44%.

^{1}H NMR (400 MHz, Methanol-d4) δ 6.71 (s, 1H), 6.43 (s, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 2.89 (ddt, J = 8.1, 7.1, 5.3 Hz, 1H), 2.77 (dd, J = 13.1, 5.2 Hz, 1H), 2.45 (dd, J = 13.1, 8.1 Hz, 1H), 2.12 (tt, J = 8.6, 5.4 Hz, 1H), 1.56 – 1.30 (m, 2H), 0.97 (t, J = 7.5 Hz, 3H), 0.91 – 0.85 (m, 2H), 0.67 – 0.58 (m, 2H). **HRMS (ES+)** calcd. For $C_{15}H_{23}NO_2$ $[M+H]^+$ = 250.1807, found 250.1817.

1-(4-bromo-2,5-dimethoxyphenyl)butan-2-amine 31

Prepared from (E)-1-bromo-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene using general procedure C with yield of 90%.

^{1}H NMR (500 MHz, Chloroform-d) δ 7.03 (s, 1H), 6.75 (s, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 2.92 (s, 1H), 2.78 (dd, J = 13.1, 4.6 Hz, 1H), 2.42 (dd, J = 13.1, 8.6 Hz, 1H), 1.55 – 1.45 (m, 1H), 1.34 (dt, J = 13.5, 7.3 Hz, 1H), 0.97 (t, J = 7.5 Hz, 3H). **^{13}C NMR (126 MHz, Chloroform-d)** δ 152.22, 149.78, 128.60, 115.89, 115.41, 108.90, 56.97, 56.09, 52.69, 38.84, 10.61. **LRMS (APCI+)** calcd. For $C_{12}H_{19}BrNO_2$ $[M+H]^+$ = 288.1, found 288.1.

1-(2,5-dimethoxy-4-methylphenyl)butan-2-amine 32 (Ariadne)

Prepared from (E)-1,4-dimethoxy-2-methyl-5-(2-nitrobut-1-en-1-yl)benzene using general procedure C with yield of 92%.

^{1}H NMR (400 MHz, Chloroform-d) δ 6.67 (d, J = 11.3 Hz, 2H), 3.77 (d, J = 8.0 Hz, 6H), 3.00 – 2.90 (m, 1H), 2.81 (dd, J = 13.2, 4.6 Hz, 1H), 2.46 (dd, J = 13.2, 8.6 Hz, 1H), 2.26 (s, 2H), 2.21 (s, 3H), 1.61 – 1.46 (m, 1H), 1.39 (dt, J = 13.6, 7.3 Hz, 1H), 0.98 (t, J = 7.4 Hz, 3H). **^{13}C NMR (126 MHz, Chloroform-d** δ 151.48, 151.43, 125.94, 125.03, 113.96, 113.79, 56.11, 56.06, 53.06, 38.65, 30.49, 16.17, 10.66. **LRMS (APCI+)** calcd. For $C_{13}H_{22}NO_2$ $[M+H]^+$ = 224.2, found 224.4. **Optical rotation:** Enantiomers were resolved from racemic freebase using chiral supercritical fluid chromatography (see details below, **example 19**) and values collected on Jasco P-2000 Digital Polarimeter. (S)-Ariadne: +30.5, (R)-Ariadne: -31.5. Averaged values of three runs each, freebases dissolved in anhydrous methanol at a concentration of 50% (w/v), average temperature of 27.7°C and path length of 100mm.

1-(2,5-dimethoxy-4-(methylsulfonyl)phenyl)butan-2-amine trifluoroacetate 33

1-(2,5-dimethoxy-4-(methylthio)phenyl)butan-2-amine (25 mg, 98 umol, 1eq) was dissolved in 2mL ethyl acetate. Vial was cooled in dry ice/acetonitrile bath and mCPBA was added in one portion (71mg, 411umol, 4.2 eq). Reaction reached room temperature and stirred 20 hr. Reaction mixture was diluted with ethyl acetate, washed with sodium sulfite (sat., 2 × 5 mL), sodium bicarbonate (sat., 1 × 5 mL),and water (1 × 5 mL). The extract was dried over Na2SO4, filtered and evaporated to dryness. The resulting oily residue was purified by addition of trifluoroacetic acid and methanol and elutied through Thermo Scientific™ HyperSep™ C18 cartridge and evaporated to give product in 46% yield.

^{1}H NMR (400 MHz, Methanol-d4) δ 7.47 (s, 2H), 7.16 (s, 2H), 3.97 (s, 6H), 3.89 (s, 6H), 3.52 – 3.41 (m, 2H), 3.23 (s, 6H), 3.07 (d, *J* = 6.0 Hz, 2H), 2.95 (dd, *J* = 13.7, 7.6 Hz, 2H), 1.76 – 1.60 (m, 4H), 1.09 – 1.04 **^{13}C NMR (101 MHz, Methanol-d4)** δ 151.32, 132.32 (s), 127.52, 116.37, 110.53, 56.01, 55.19, 52.88, 41.82, 33.23, 25.45, 8.46. **LRMS (APCI+)** calcd. For $C_{13}H_{22}NO_4S$ $[M+H]^+$ = 288.1, found 288.5.

1-(4-iodo-2,5-dimethoxyphenyl)butan-2-amine 34

Prepared from (E)-1-iodo-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene using general procedure C with a yield of 82%.

^{1}H NMR (400 MHz, Chloroform-d) δ 6.68 (d, *J* = 5.6 Hz, 4H), 3.78 (d, *J* = 6.1 Hz, 11H), 3.05 (ddd, *J* = 13.0, 7.6, 5.4 Hz, 2H), 2.84 (dd, *J* = 13.3, 5.2 Hz, 2H), 2.57 (dd, *J* = 13.3, 8.2 Hz, 3H), 1.61 – 1.43 (m, 4H), 1.00 (t, *J* = 7.5 Hz, 6H). **LRMS (APCI+)** calcd. For $C_{12}H_{19}INO_2$ $[M+H]^+$ = 336.0, found 336.2

1-(4-ethyl-2,5-dimethoxyphenyl)butan-2-amine 35

Prepared from (E)-1-ethyl-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene using general procedure B. Freebase was dissolved in anhydrous diethyl ether and treated with 2N HCl in ether and filtered to produce hydrochloride salt in 55% total yield.

^{1}H NMR (300 MHz, MeOD) δ 6.77 (d, *J* = 15.1 Hz, 2H), 3.78 (d, *J* = 6.9 Hz, 6H), 3.37 (dd, *J* = 13.2, 6.5 Hz, 1H), 2.88 (ddd, *J* = 21.2, 13.8, 6.7 Hz, 2H), 2.59 (q, *J* = 7.5 Hz, 2H), 1.74 – 1.60 (m, 2H), 1.14 (t, *J* = 7.5 Hz, 3H), 1.03 (t, *J* = 7.5 Hz, 3H) **LRMS (APCI+)** calcd. For $C_{14}H_{23}NO_2$ $[M+H]^+$ = 238.2, found 238.4

1-(5-ethoxy-2-methoxy-4-(trifluoromethyl)phenyl)butan-2-amine 36

Prepared from (E)-1-ethoxy-4-methoxy-5-(2-nitrobut-1-en-1-yl)-2-(trifluoromethyl)benzene, following general procedure C in a yield of 56%

^{13}C NMR (101 MHz, Chloroform-d) δ 151.12 (s), 150.59 (s), 133.44 (s), 125.04 (s), 122.33 (s), 117.24 (s), 109.16 (dd), 65.44 (s), 55.97 (s), 52.72 (s), 38.69 (s), 30.37 (s), 14.76 (s), 10.50 (s). **LRMS (APCI+)** calcd. For $C_{14}H_{21}F_3NO_2$ $[M+H]^+$ = 292.2, found 292.4.

1-(2,5-dimethoxy-4-(methoxymethyl)phenyl)butan-2-amine 37

Prepared from (E)-1,4-dimethoxy-2-(methoxymethyl)-5-(2-nitrobut-1-en-1-yl)benzene according to general procedure B with yield of 87%.

^{1}H NMR (400 MHz, Chloroform-d) δ 6.90 (s, 1H), 6.70 (s, 1H), 4.47 (s, 2H), 3.79 (s, 6H), 3.43 (s, 3H), 3.01 – 2.88 (m, 1H), 2.82 (dd, 1H), 2.46 (dd, 1H), 1.67 (bs, 2H), 1.57 – 1.44 (m, 1H), 1.42 – 1.26 (m, 1H), 0.97 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR (101 MHz, Chloroform-**

d) δ 151.93, 150.95, 128.27, 125.25, 114.20, 112.03, 69.52, 58.58, 56.34, 56.13, 53.08, 38.93, 30.60, 10.74. **HRMS (ESI+)** calcd. For $C_{14}H_{23}NO_3$ $[M+H]^+$ 254.1756, found 254.1764

1-cyclopropyl-2-(2,5-dimethoxy-4-methylphenyl)ethan-1-amine 38

Prepared from ((E)-1-(2-cyclopropyl-2-nitrovinyl)-2,5-dimethoxy-4-methylbenzene using general procedure B with yield of 41% (as HCl salt).

^{1}H NMR (400 MHz, MeOD) 6.82 (s, 1H), 6.79 (s, 1H), 3.90 – 3.73 (m, 6H), 3.39 – 3.28 (m, 4H), 3.12 – 2.93 (m, 2H), 2.72 – 2.60 (m, 1H), 2.21 (s, 3H), 1.08 – 0.91 (m, 1H), 0.72 – 0.54 (m, 1H), 0.47 – 0.36 (m, 1H), 0.23 – 0.13 (m, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 151.71, 151.31, 126.40, 121.40, 113.69, 113.46, 57.87, 55.12, 54.88, 34.25, 14.89, 13.37, 3.38, 2.75. **HRMS (ES+)** calcd. For $C_{14}H_{21}NO_2$ $[M+Na]^+$ 258.147, found 258.1465

1-(2,5-dimethoxy-4-methylphenyl)pentan-2-amine 39

Prepared from 2,5-dimethoxy-4-methylbenzaldehyde using general procedures A directly followed by B with yield of 2% (over 2 steps).

^{1}H NMR (500 MHz, MeOD) δ 6.77 (s, 1H), 6.70 (s, 1H), 3.83 – 3.72 (m, 6H), 3.04 – 2.97 (m, 1H), 2.81 (dd, *J* = 13.1, 5.2 Hz, 1H), 2.46 (dd, *J* = 13.1, 8.2 Hz, 1H), 1.54 – 1.28 (m, 4H), 1.00 – 0.91 (m, 3H). **^{13}C NMR (126 MHz, MeOD)** δ 151.48, 151.42, 125.03, 124.97, 113.50, 113.46, 61.42, 55.04, 50.78, 38.79, 37.76, 28.78, 14.84, 13.11. **LRMS (APCI+)** calcd. For $C_{16}H_{28}NO_2$ $[M+H]^+$ = 266.2 found 266.2

1-(2,6-dimethoxy-4-methylphenyl)butan-2-amine 40

Prepared from 2,6-dimethoxy-4-methylbenzaldehyde using general procedures A directly followed by B with yield of 1.3% (over 2 steps).

^{1}H NMR (500 MHz, MeOD) δ 6.47 (s, 2H), 3.80 (s, 6H), 2.98 – 2.90 (m, 1H), 2.76 (dd, *J* = 13.0, 5.2 Hz, 1H), 2.61 (dd, *J* = 13.0, 8.1 Hz, 1H), 2.34 (s, 3H), 1.50 – 1.34 (m, 4H), 0.97 – 0.90 (m, 3H). **^{13}C NMR (126 MHz, MeOD)** δ 158.36(2C), 137.52, 112.07, 104.22(2C), 54.56(2C), 50.90, 38.81, 29.78, 20.66, 18.96, 13.09. **LRMS (APCI+)** calcd. For $C_{13}H_{22}NO_2$ $[M+H]^+$ = 224.2, found 224.5.

1-(4-chloro-2,5-dimethoxyphenyl)butan-2-amine 41

Prepared from (E)-1-chloro-2,5-dimethoxy-4-(2-nitrobut-1-en-1-yl)benzene using general procedures A with yield of 51%.

^{1}H NMR (400 MHz, MeOD) δ 7.06 (s, 1H), 7.00 (s, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.46 – 3.37 (m, 1H), 3.01 (dd, *J* = 13.9, 6.2 Hz, 1H), 2.89 (dd, *J* = 13.8, 7.4 Hz, 1H), 1.76 – 1.61 (m, 2H), 1.07 (t, *J* = 7.5 Hz, 3H). **^{13}C NMR (101 MHz, MeOD)** δ 151.86, 149.26, 123.46, 121.57, 115.91, 113.05, 56.03, 55.23, 53.25, 32.89, 25.29, 8.57. **LRMS (APCI+)** calcd. For $C_{12}H_{19}ClNO_2$ $[M+H]^+$ = 244.1, found 244.1

1-(2,5-dimethoxy-4-propylphenyl)butan-2-amine 42

Prepared from corresponding styrene using general procedures A with yield of 54% (as the HCl salt). **^{1}H NMR (400 MHz, MeOD)** δ 6.82 (s, 1H), 6.78 (s, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 3.57 (h, *J* = 6.7 Hz, 1H), 2.95 (dd, *J* = 13.5, 6.7 Hz, 1H), 2.85 (dd, *J* = 13.5, 7.0 Hz, 1H), 2.63 – 2.56 (m, 2H), 1.68 – 1.54 (m, 2H), 1.29 (d, *J* = 6.6 Hz, 3H), 0.96 (t, *J* = 7.4 Hz, 3H).**^{13}C NMR (101 MHz, MeOD)** δ 151.54, 151.36, 131.13, 121.57, 113.84, 112.96, 55.17, 54.92, 48.08, 35.32, 32.04, 22.98, 17.23, 12.92. **LRMS (APCI+)** calcd. For $C_{15}H_{26}NO_2$ $[M+H]^+$ = 252.2, found 252.2.

1-(2,5-dimethoxy-4-propylphenyl)pentan-2-amine 43

Prepared from corresponding styrene using general procedures A with yield of 41% (as the HCl salt). **^{1}H NMR (400 MHz, MeOD)** δ 6.82 (s, 1H), 6.79 (s, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.51 – 3.42 (m, 1H), 2.99 (dd, *J* = 13.9, 6.0 Hz, 1H), 2.85 (dd, *J* = 13.8, 7.4 Hz, 1H), 2.63 – 2.55 (m, 2H), 1.68 – 1.56 (m, 4H), 1.54 – 1.40 (m, 2H), 1.03 – 0.92 (m, 6H).**^{13}C NMR (101 MHz, MeOD)** δ 151.57, 151.41, 131.16, 121.49, 113.88, 112.99, 55.18, 54.93, 51.94, 34.47, 33.49, 32.04, 22.98, 18.19, 12.92, 12.65. **LRMS (APCI+)** calcd. For $C_{16}H_{28}NO_2$ $[M+H]^+$ = 266.2, found 266.2

1-(2,5-dimethoxy-4-propylphenyl)propan-2-amine 44 (DOPR)

Prepared from corresponding styrene using general procedures A with yield of 41% (as the HCl salt). **^{1}H NMR (400 MHz, MeOD)** δ 6.82 (s, 1H), 6.78 (s, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 3.57 (h, *J* = 6.7 Hz, 1H), 2.95 (dd, *J* = 13.5, 6.7 Hz, 1H), 2.85 (dd, *J* = 13.5, 7.0 Hz, 1H), 2.63 – 2.56 (m, 2H), 1.68 – 1.54 (m, 2H), 1.29 (d, *J* = 6.6 Hz, 3H), 0.96 (t, *J* = 7.4 Hz, 3H).**^{13}C NMR (101 MHz, MeOD)** δ 151.54, 151.36, 131.13, 121.57, 113.84, 112.96, 55.17, 54.92, 48.08, 35.32, 32.04, 22.98, 17.23, 12.92. **LRMS (APCI+)** calcd. For $C_{14}H_{24}NO_2$ $[M+H]^+$ = 238.3, found 238.4

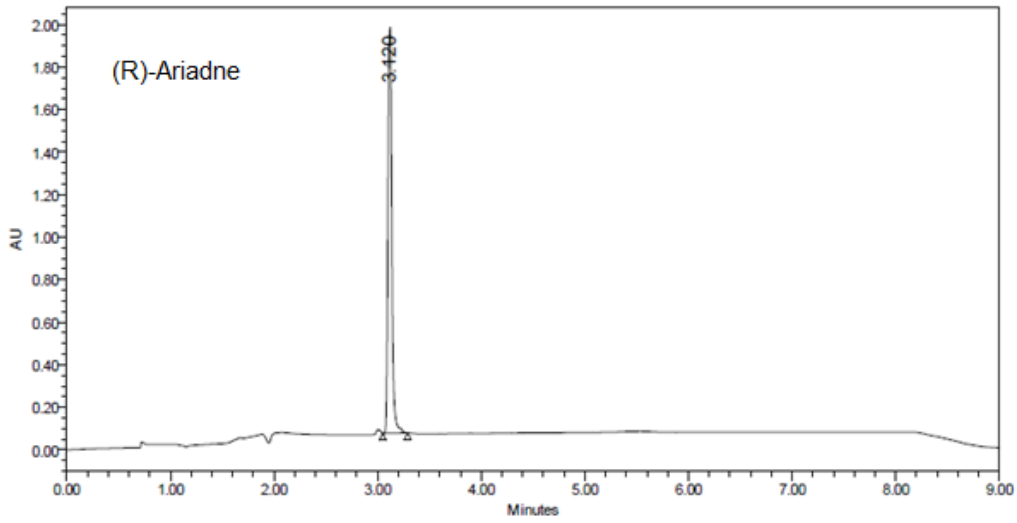
Separation of Enantiomers

Enantiomers of Ariadne were separated at WuXi Apptec (Shanghai) using the following methods. Compound was dissolved in 100mL methanol/DCM and injected in 0.8mL portions. Following separations, the fractions were dried off via rotary evaporator at bath temperature 40 °C. Waters UPCC with PDA Detector was used for chiral SFC trace.

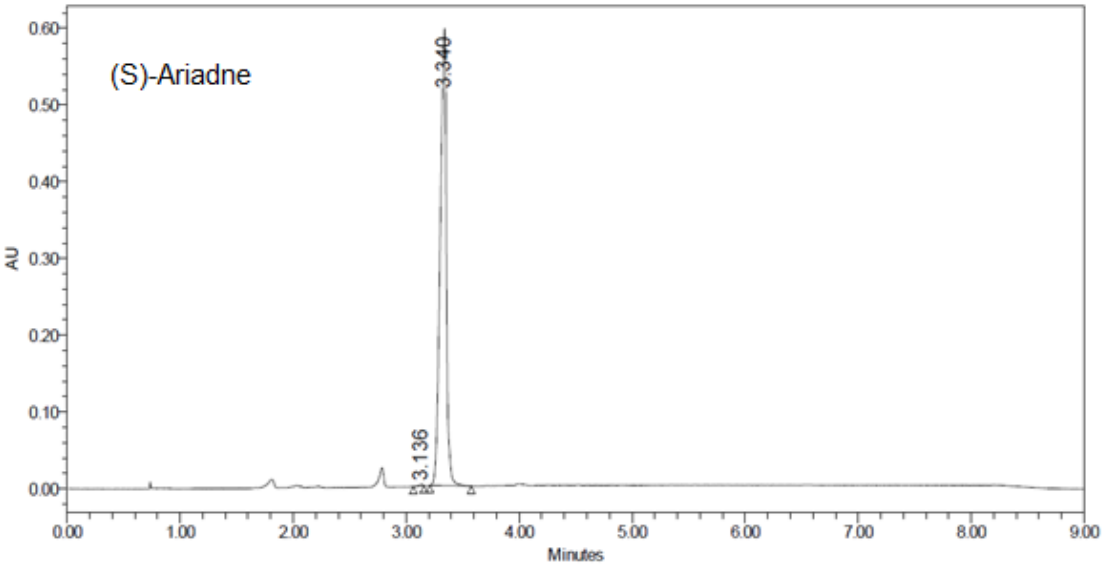
Analytical separation method:
Instrument: Waters UPC2 analytical SFC (SFC-H)
Column: ChiralPak AD, 150×4.6mm I.D., 3μm
Mobile phase: A for CO_2 and B for isopropanol (0.05% diethylamine)
Gradient: B 5-40%
Flow rate: 2.4 mL/min
Back pressure: 100 bar
Column temperature: 35 °C
Wavelength: 220nm

Preparative separation method:
Instrument: MG Ⅱ preparative SFC (SFC-14)
Column: ChiralPak AD, 250×30mm I.D., 10μm
Mobile phase: A for CO_2 and B for Isopropanol (0.1%NH_3H_2O)
Gradient: B 15%
Flow rate: 60 mL /min
Back pressure: 100 bar
Column temperature: 38 °C
Wavelength: 220nm
Cycle time: ~6 min

Figure S1: Chiral SFC profiles of Ariadne enantiomers



	RT	Area	% Area
1	3.120	5089312	100.00



	RT	Area	% Area
1	3.136	3255	0.14
2	3.340	2261157	99.86

Table S1. Eurofins SafetyScreen44 (Panlabs)

For species, "H" = human, "R" = rat. Test compound screened at all targets at concentration of 10μM. Eurofins Assay ID can be used to search catalog at *https://www.eurofinsdiscoveryservices.com* for complete details.

Table S1

Assay ID	**Target Name**	**% Response** @ 10μM	**Ligand or Substrate**	**Species**	**Tissue**
104010	Cholinesterase, Acetyl, ACES	12	Acetylthiocholine	H	recombinant
116030	Cyclooxygenase COX-1	-31	Arachidonic acid	H	recombinant
118030	Cyclooxygenase COX-2	-2	Arachidonic Acid	H	recombinant
140010	Monoamine Oxidase MAO-A	-3	Kynuramine	H	recombinant
152300	Phosphodiesterase PDE3A	-4	FAM-cAMP	H	recombinant
154420	Phosphodiesterase PDE4D2	-2	FAM-cAMP	H	recombinant
176020	Protein Tyrosine Kinase, LCK	13	Poly(Glu:Tyr)	H	recombinant
200610	Adenosine A2A	10	[3H]CGS-21680	H	recombinant
203110	Adrenergic alpha1A	24	[3H]Prazosin	H	recombinant
203630	Adrenergic alpha2A	34	[3H]Rauwolscine	H	recombinant
204010	Adrenergic beta1	17	[125I]Cyanopindolol	H	recombinant
204110	Adrenergic beta2	22	[3H]CGP-12177	H	recombinant
204410	Transporter, Norepinephrine (NET)	23	[125I]RTI-55	H	recombinant

206000	Androgen (Testosterone)	-8	[3H]Methyltrienolone	H	LNCaP clone FGC cells
214600	Calcium Channel L-Type, Dihydropyridine	-13	[3H]Nitrendipine	R	cerebral cortex
217050	Cannabinoid CB1	1	[3H]SR141716A	H	recombinant
217100	Cannabinoid CB2	-7	[3H]WIN-55,212-2	H	recombinant
218030	Cholecystokinin CCK1 (CCKA)	3	[125I]CCK-8	H	recombinant
219500	Dopamine D1	7	[3H]SCH-23390	H	recombinant
219700	Dopamine D2S	1	[3H]Spiperone	H	recombinant
220320	Transporter, Dopamine (DAT)	7	[125I]RTI-55	H	recombinant
224010	Endothelin ETA	-6	[125I]Endothelin-1	H	recombinant
226600	GABAA, Flunitrazepam, Central	-15	[3H]Flunitrazepam	R	brain (minus cerebellum)
232030	Glucocorticoid	-5	[3H]Dexamethasone	H	recombinant
232810	Glutamate, NMDA, Agonism	-5	[3H]CGP-39653	R	cerebral cortex
239610	Histamine H1	13	[3H]Pyrilamine	H	recombinant
239710	Histamine H2	21	[125I]Aminopotentidine	H	recombinant
252610	Muscarinic M1	-4	[3H]N-Methylscopolamine	H	recombinant
252710	Muscarinic M2	0	[3H]N-Methylscopolamine	H	recombinant

252810	Muscarinic M3	-11	[3H]N-Methylscopolamine	H	recombinant
260130	Opiate delta1 (OP1, DOP)	4	[3H]Naltrindole	H	recombinant
260210	Opiate kappa (OP2, KOP)	3	[3H]Diprenorphine	H	recombinant
260410	Opiate mu (OP3, MOP)	-11	[3H]Diprenorphine	H	recombinant
265510	Potassium Channel [KA]	-6	[125I]alpha-Dendrotoxin	R	cerebral cortex
265910	Potassium Channel hERG, [3H]Dofetilide	18	[3H]Dofetilide	H	recombinant
271110	Serotonin (5-Hydroxytryptamine) 5-HT1A	36	[3H]8-OH-DPAT	H	recombinant
271230	Serotonin (5-Hydroxytryptamine) 5-HT1B	12	[3H]GR125743	H	recombinant
271650	Serotonin (5-Hydroxytryptamine) 5-HT2A	72	[3H]Ketanserin	H	recombinant
271700	Serotonin (5-Hydroxytryptamine) 5-HT2B	72	[3H]Lysergic acid diethylamide (LSD)	H	recombinant
271910	Serotonin (5-Hydroxytryptamine) 5-HT3	-13	[3H]GR-65630	H	recombinant
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	11	[3H]Paroxetine	H	recombinant
279510	Sodium Channel, Site 2	47	[3H]Batrachotoxinin	R	brain (minus cerebellum)
287530	Vasopressin V1A	-5	[125I]PhenylacetylTyr(Me)PheGlnAsnArgProArgTyr	H	recombinant

299031	Nicotinic Acetylcholine alpha4beta2, Cytisine	-31	[3H]Cytisine	H	recombinant

Table S2.

5-HT_{2A} and 5-HT_{2B} IP1 agonism assay summary (EC_{50} values, duplicate averages only), performed at Cerep, Eurofins France

#	"4C-X" code	5-HT2a	5-HT2b
32	4C-D	1.3E-06M	>3.2E-05M
35	4C-E	1.9E-06M	5.7E-07M
42	4C-PR	2.1E-07M	N.C.
37	4C-MOM	3.8E-06M	N.C.
33	4C-Tone	3.4E-06M	2.5E-05M
36	5-EtO-4C-TFM	2.8E-07M	9.5E-07M
29	4C-TFM	5.0E-08M	6.3E-07M
28	4C-T	1.6E-07M	6.6E-08M
27	4C-T-2	5.3E-08M	6.3E-07M
41	4C-C	1.9E-07M	1.4E-05M
31	4C-B	9.0E-08M	5.5E-06M
34	4C-I	9.7E-06M	1.8E-05M
39	5C-D	N.C.	2.3E-05M
38	4C(cycPr)-D	9.7E-06M	2.1E-05M
40	4C-psiD	1.0E-05M	N.C.
30	4C-CycPR	3.7E-06M	3.2E-05M
4	4C-CN	1.1E-05M	N.C.

Figure S2:

5-HT2A(h), agonist radioligand $^{[125]}$I(±)DOI) vs (*rac*)-, (*R*)-, and (*S*)-Ariadne

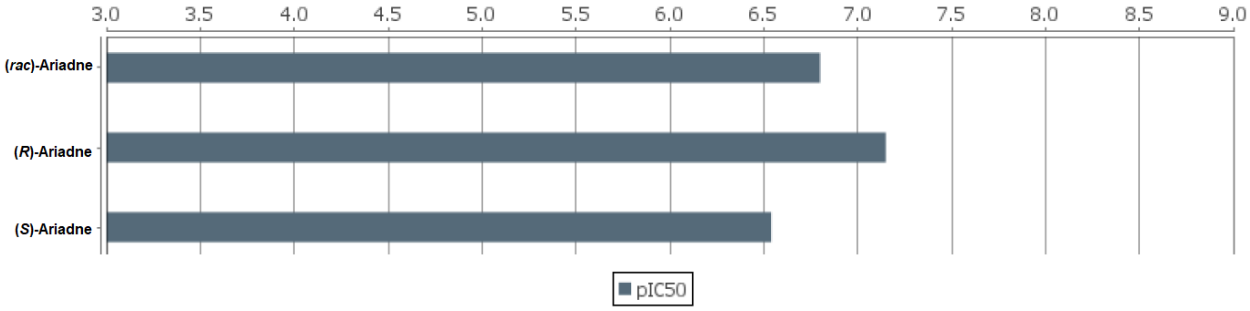
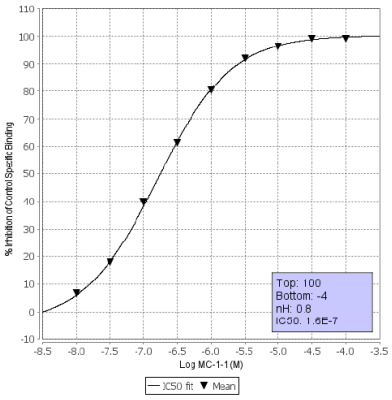


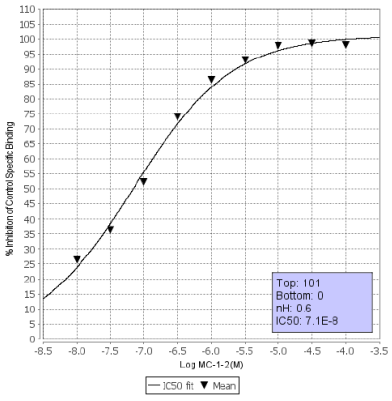
Figure S3: Radioligand displacement curves of (*rac*)-, (*R*)-, and (*S*)-Ariadne vs [125I](±)DOI)

IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	2nd	Mean
1.6E-07 M	1.2E-07 M	0.8	1.0E-08 M	2.6	10.8	6.7
			3.162E-08 M	9.7	26.2	18.0
			1.0E-07 M	37.9	42.0	39.9
			3.1623E-07 M	55.0	68.0	61.5
			1.0E-06 M	81.1	79.9	80.5
			3.1623E-06 M	93.5	90.6	92.0
			1.0E-05 M	95.7	97.2	96.4
			3.1623E-05 M	99.1	99.5	99.3
			1.0E-04 M	98.9	99.5	99.2



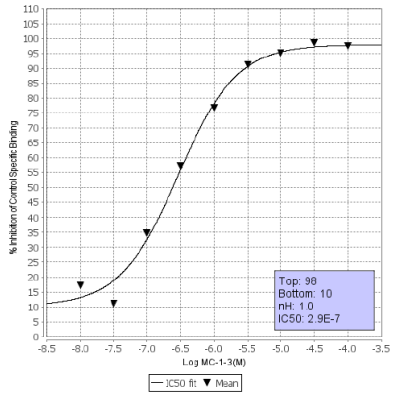
a/ (*rac*)-Ariadne

IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	2nd	Mean
7.1E-08 M	5.3E-08 M	0.6	1.0E-08 M	26.7	26.2	26.4
			3.162E-08 M	40.9	31.7	36.3
			1.0E-07 M	48.4	56.3	52.4
			3.1623E-07 M	72.6	76.1	74.3
			1.0E-06 M	86.9	85.9	86.4
			3.1623E-06 M	94.0	92.1	93.0
			1.0E-05 M	98.0	97.7	97.8
			3.1623E-05 M	98.3	98.8	98.6
			1.0E-04 M	98.0	98.1	98.0



b/ (*R*)-Ariadne

IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	2nd	Mean
2.9E-07 M	2.2E-07 M	1.0	1.0E-08 M	14.3	20.4	17.3
			3.162E-08 M	15.1	7.5	11.3
			1.0E-07 M	37.2	32.1	34.7
			3.1623E-07 M	60.9	53.8	57.3
			1.0E-06 M	77.7	75.6	76.7
			3.1623E-06 M	92.8	89.8	91.3
			1.0E-05 M	94.9	95.3	95.1
			3.1623E-05 M	98.3	98.6	98.5
			1.0E-04 M	97.0	98.2	97.6



c/ (*S*)-Ariadne

Table S3: 5-HTome data for 5-HT, (*rac*)-Ariadne and (*R*)-Ariadne

	5-HT					
Receptor, G Protein	**EC50, nM**	**pEC50**	**SEM**	**Emax**	**SEM**	**Log(Emax/EC50)**
5-HT1A, GoB	1.72	8.77	0.07	100	N.D.	8.76
5-HT1B, GoB	0.262	9.58	0.08	100	N.D.	9.58
5-HT1D, GoB	0.420	9.38	0.11	100	N.D.	9.38
5-HT1e, GoB	0.792	9.10	0.07	100	N.D.	9.10
5-HT1F, GoB	2.21	8.66	0.08	100	N.D.	8.66
5-HT2A, Gq	5.81	8.24	0.03	100	N.D.	8.24
5-HT2B, Gq	1.18	8.93	0.05	100	N.D.	8.93
5-HT2C, Gq	0.160	9.80	0.04	100	N.D.	9.80
5-HT4, Gs	7.13	8.15	0.03	100	N.D.	8.15
5-HT5a, GoB	60.3	7.22	0.11	100	N.D.	7.22
5-HT6, Gs	15.4	7.81	0.08	100	N.D.	7.81
5-HT7a, Gs	6.44	8.19	0.07	100	N.D.	8.19
	(*rac*)-Ariadne					
Receptor, G Protein	**EC50, nM**	**pEC50**	**SEM**	**Emax**	**SEM**	**Log(Emax/EC50)**
5-HT1A, GoB	656	6.18	0.18	67.6	5.97	6.01
5-HT1B, GoB	4023	5.40	0.19	89.5	12.1	5.35
5-HT1D, GoB	612	6.21	0.20	65.1	6.69	6.03
5-HT1e, GoB	727	6.14	0.09	79.0	3.56	6.04
5-HT1F, GoB	690	6.16	0.10	74.6	3.67	6.03
5-HT2A, Gq	171	6.77	0.03	81.3	1.04	6.68
5-HT2B, Gq	734	6.13	0.06	73.8	2.31	6.00
5-HT2C, Gq	216	6.67	0.07	85.1	2.62	6.60
5-HT4, Gs	N.D.	N.D.	N.D.	<20	N.D.	<5.00
5-HT5a, GoB	N.D.	N.D.	N.D.	<20	N.D.	<5.00
5-HT6, Gs	1038	5.98	0.27	41.9	6.15	5.61
5-HT7a, Gs	N.D.	N.D.	N.D.	<20	N.D.	<5.00
	(*R*)-Ariadne					
Receptor, G Protein	**EC50, nM**	**pEC50**	**SEM**	**Emax**	**SEM**	**Log(Emax/EC50)**
5-HT1A, GoB	306	6.52	0.35	40.4	5.83	6.12
5-HT1B, GoB	726	6.14	0.19	101.3	9.0	6.14
5-HT1D, GoB	636	6.20	0.24	67.0	7.5	6.02
5-HT1e, GoB	415	6.38	0.11	84.5	4.39	6.31
5-HT1F, GoB	391	6.41	0.20	66.1	6.16	6.23
5-HT2A, Gq	148	6.83	0.03	84.5	1.07	6.76
5-HT2B, Gq	658	6.18	0.08	71.7	2.71	6.04
5-HT2C, Gq	148	6.83	0.07	87.5	2.67	6.77
5-HT4, Gs	3553	5.45	0.23	36.1	5.83	5.01
5-HT5a, GoB	N.D.	N.D.	N.D.	<20	N.D.	<5.00
5-HT6, Gs	1215	5.92	0.26	53.6	8.09	5.64
5-HT7a, Gs	N.D.	N.D.	N.D.	<20	N.D.	<5.00
ND = not determined						

In Silico Receptor analysis

The receptor 5HT2A (PDBID: 6WHA) protein was obtained from the RCSB server representing 4-(2-((2-hydroxybenzyl)amino)ethyl)-2,5-dimethoxybenzonitrile (25CN-NBOH) agonist-bound active state. The protein was prepared by the addition and optimization of hydrogens and optimization of the side chain residues. The docking was performed with D155 being flexible.

Drug preparation and administration for animal studies

All samples are prepared the same day testing is performed. Solids are weighed into small vials and dissolved in USP grade 0.85% saline with addition of 2 molar equivalents of glacial acetic acid. Sonication and gentle heating are applied until complete dissolution. The compounds are subsequently filtered through 0.45 μm filters into a new glass vial. All compounds were administered at a 1 mg/kg subcutaneous dose at a volume of 10 mL/kg of body weight. Volinanserin hydrochloride salt (MDL 100907) was purchased from Toronto Research Chemicals.

General mouse use

All experimental procedures involving animals were approved by the Columbia University Institutional Animal Care and Use Committee (IACUC) and adhered to principles described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The studies were conducted at AAALAC accredited facilities. Animals received regular veterinary care (weekly by institutional veterinarians) including daily health monitoring (by experimenters) of the animals (observing home cage behaviors, nesting, and body weight). All procedures were designed to minimize any stress/distress. Healthy adult male mice C57BL/6J (9-14 weeks, 26-33 g) were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed 5 mice per cage with food and water available ad libitum. Mice were maintained on a 12-h light/dark cycle (lights on 7:00-19:00) and all testing was done in the light cycle. Temperature was kept constant at 22 ± 2 °C, and relative humidity was maintained at 50 ± 5%

Behavioral Assays in Mice

Open field (OF) locomotion protocol

Mice were allowed to habituate for 30 min. Immediately after receiving a subcutaneous injection of the compound solution, mice were then placed gently in a clear Plexiglass arena (27.31 × 27.31 × 20.32 cm, Med Associates ENV-510) lit with ambient light (~330 lux) and allowed to ambulate freely for 120 min. The locomotion of the animals was tracked by infrared beams embedded along the X, Y, Z axes of the area and automatically recorded. Data was collected on Activity Monitor by Med Associates.

Head twitch response (HTR) protocol

The head-twitch response evaluation was performed by trained observers who were blinded to both drug and dose. Mice were habituated to the testing room 30 min. The body weight of each mouse was recorded.

Dose-Response Curves: Mice were administered a single s.c. dose of compound solution. After injection, mice were allowed to rest for 5 min and then were observed for 15 min during which the HTR (defined as a rapid rotational movement of the head around the longitudinal axis of the animals' body) was scored. The total number of head-twitches over the 15-minute period was recorded. Five mice were used per dose.

Pretreatment of M100907: Mice were administered with vehicle or M100907 (0.01 mg/kg or 0.032 mg/kg ip) and placed into an observation cage. Fifteen minutes after the pretreatment injection mice were administered either (*R*)-Ariadne (10 mg/kg, sc) or 4C-TFM (10 mg/kg, sc). Observers scored HTRs in a 15-min period starting 5 minutes post second injection.

Time-Course of the Dose-Response Curves: After receiving a single dose of DOPR (0.1, 0.3, 1, and 3 mg/kg) or Ariadne/Ariadne analogs (1, 3, 10, and 30 mg/kg), mice were placed in an observation cage. An overhead camera (GoPro HERO9 at a 120 Hz) recorded their movements. Recordings were later scored for HTR in one-minute time bins for the subsequent 30-minute period. For vehicle, DOPR (1 mg/kg), and (*R*)-Ariadne (10 mg/kg), observers scored HTR from recorded footage for a 2 hr period.

Elevated Plus Maze (EPM)

Mice were weighed and transferred to the testing room 30 minutes prior to experimentation. The elevated plus-maze consists of two opposite open arms (L×W: 30cm x 5cm) and two closed arms (L×W×H: 30 x 5 x 15 cm) extending from a central area (L×W: 5 x 5 cm). Either 30 minutes or 4 hours post a drug dose of (R)-Ariadne, DOPR, or vehicle, mice were placed gently in the center of the maze and allowed to freely ambulate for 10 min. Mice were tracked by photo beams embedded at arm entrances. Data was collected on MEDPC V 64bit Software (Med Associates).

Light-Dark Box (LDB)

The apparatus of the light dark exploration tests consists of a clear, open-top compartment (20 x 40 x 35 cm, 350 lux) and a black, enclosed compartment (20 x 40 x 35 cm, 3 lux) separated by a divider with a small doorway. Forty-five minutes post-injection, and 5 min after the EPM, mice were placed in the light compartment and able to freely explore both compartments for a duration of 10 minutes. Time spent in each compartment and the number of transitions between the light (350 lux) and dark (3 lux) compartments were recorded with EthoVision (Noldus Information Technology Inc. Leesburg, VA).

Novelty Suppressed Feeding (NSF)

On day zero, mice were administered vehicle or (*R*)-Ariadne (6 and 10 mg/kg, sc) and underwent EPM and LDB. NSF was assessed on day 7. Mice were deprived of food for 24 hr prior to testing. On day 7, mice were transferred to the testing room and habituated for 30 minutes. Each mouse was gently placed in a corner of the novel arena (16.5 in * 16.5 in * 12 in, 150 lux), where a single food pellet affixed on top of a white filter paper (13 cm diameter) placed in the middle. The duration of the test was 10 minutes. Immediately after an eating event, the mouse was transferred to its home cage and allowed to free-feed for 5 min to assess for hunger drive. Sessions were recorded and latency to bite was manually scored. Mice that did not bite were assigned a latency of 600 s.

Forced Swim Test (FST)

Mice were weighed and transferred to the testing room 30 minutes prior to experimentation. After drug/vehicle injection (for timing see below) each mouse was gently placed into cylinder (plexiglass 40 cm tall, diameter 22 cm) filled with water (height 18 cm) kept at 24.0±1 °C. Mice were allowed to swim for a duration of 6 minutes. Video footage was analyzed with Noldus FST.

Acute FST (4 hr post inj): Mice were administered vehicle (sc), (*R*)-Ariadne (30 mg/kg, sc), or DOPR (3 mg/kg, sc) and were immediately assessed in the open field for 30 minutes. Four hours post injection mice underwent the FST as described above.

Long-term FST (7 days post inj): On day zero, mice were administered vehicle (sc), (*R*)-Ariadne (6 mg/kg, sc), or Ariadne (10 mg/kg, sc) and underwent EPM and LDB tests. On day seven, mice underwent NSF and four hours afterwards underwent the FST as described above.

Pharmacokinetic assessment

PK assessments were performed at Sai Life (Telangana, India) according to the following general procedure. Total twenty-seven male C57BL/6 mice were used in this study with 3 mice per time point. Animals were administered subcutaneously at 10 mg/kg dose of test article. The formulation vehicle used was 0.9% normal saline. Blood samples (approximately 60 µL) were collected under light isoflurane anesthesia (Surgivet®) from retro orbital plexus from a set of three mice at 0.08, 0.25, 0.5, 1, 2, 4, 8, 24 and 48 hr. Immediately after blood collection, plasma was harvested by centrifugation at 4000 rpm, 10 min at 40°C and samples were stored at -70±10°C until bioanalysis. Following blood collection, immediately animals were sacrificed followed by cutting abdominal vena-cava and whole body was perfused from heart using 10 mL of normal saline. Brain samples were collected from set of three mice at 0.08, 0.25, 0.5, 1, 2, 4, 8, 24 and 48 hr. After isolation, brain samples were rinsed three times in ice cold normal saline (for 5-10 seconds/rinse using ~5-10 mL normal saline in disposable petri dish for each rinse) and dried on blotting paper. Brain samples were homogenized using ice-cold phosphate buffer

saline (pH-7.4). Total homogenate volume was three times the tissue weight. All homogenates were stored below -70±10 ºC until bioanalysis. All samples were processed for analysis by protein precipitation method and analyzed with fit-for-purpose LC-MS/MS method (LLOQ = 5.16 ng/mL for plasma and 2.06 ng/mL for brain).

Table S4

Pharmacokinetics data of rac-Ariadne in male C57BL/6 mice following a single subcutaneous administration (Dose: 10 mg/kg)

Matrix	**Route**	**Dose (mg/kg)**	**T_{max} (hr)**	**C_{max} (ng/mL)**	**AUC_{last} (hr*ng/mL)**	**$T_{1/2}$ (hr)**	**-**	**-**
Plasma	SC	10	0.25	801.64	1184.16	0.84	-	-
Matrix	**Route**	**Dose (mg/k**	**T_{max} (hr)**	**C_{max} (ng/g)**	**AUC_{last} (hr*ng/g)**	**$T_{1/2}$ (hr)**	**Brain-Kp (C_{max})**	**Brain-Kp**
Brain	SC	10	0.50	5606.44	10938.72	0.89	6.99	9.24

Table S5

Mean brain-to-plasma concentration ratio of rac-Ariadne in male C57BL/6 mice following a single subcutaneous administration (Dose: 10 mg/kg)

Route	Dose (mg/kg)	Time (hr)	Mean plasma Concentration (ng/mL)	Mean brain Concentration (ng/g)	Brain/Plasma ratio
SC	10	0.083	672.11	1631.82	2.43
		0.25	801.64	4755.16	5.93
		0.5	661.96	5606.44	8.47
		1	512.04	5239.35	10.23
		2	172.44	1711.49	9.93
		4	40.88	368.18	9.01
		8	BLQ	16.23	NC
		24	BLQ	BLQ	NC
		48	BLQ	BLQ	NC

Table S6

Pharmacokinetics data of 4C-TFM in male C57BL/6 mice following a single subcutaneous administration (Dose: 10 mg/kg)

Matrix	**Route**	**Dose (mg/kg)**	**T_{max} (hr)**	**C_{max} (ng/mL)**	**AUC_{last} (hr*ng/mL)**	**$T_{1/2}$ (hr)**	**-**	**-**
Plasma	SC	10	0.25	466.69	797.87	2.11	-	-
Matrix	**Route**	**Dose (mg/kg)**	**T_{max} (hr)**	**C_{max} (ng/g)**	**AUC_{last} (hr*ng/g)**	**$T_{1/2}$ (hr)**	**Brain-Kp (C_{max})**	**Brain-Kp (AUC_{last})**
Brain	SC	10	0.25	4156.28	10779.47	1.79	8.91	13.51

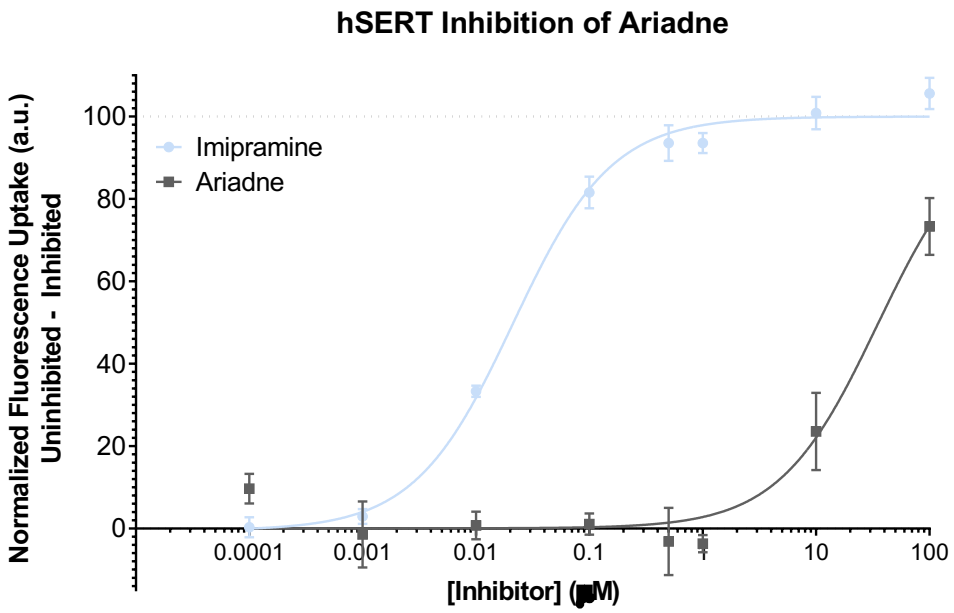
Table S7

Mean brain-to-plasma concentration ratio of 4C-TFM in male C57BL/6 mice following a single subcutaneous administration (Dose: 10 mg/kg)

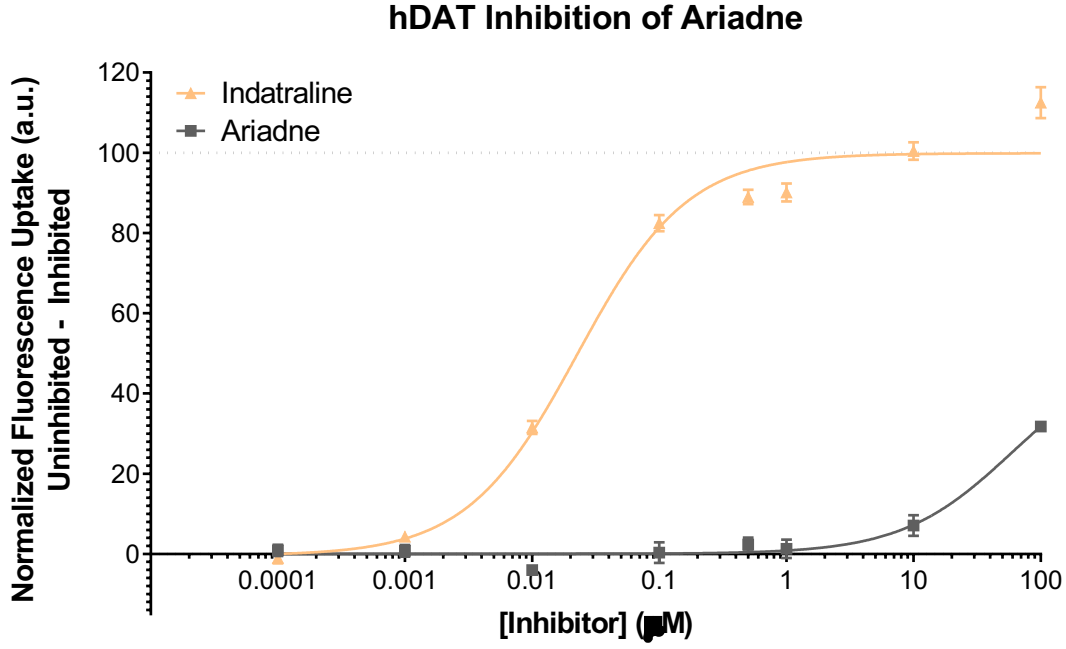
Route	Dose (mg/kg)	Time (hr)	Mean plasma Concentration (ng/mL)	Mean brain Concentration (ng/g)	Brain/Plasma ratio
SC	10	0.083	431.26	2011.16	4.66
		0.25	466.69	4156.28	8.91
		0.5	422.13	3770.22	8.93
		1	215.78	3900.97	18.08
		2	95.55	1742.53	18.24
		4	51.87	787.06	15.17
		8	13.45	170.36	12.67
		24	BLQ	BLQ	NC
		48	BLQ	BLQ	NC

Figure S4: (*rac*)-Ariadne hSERT and hDAT inhibiton curves
(a) Ariadne is not a potent hSERT inhibitor (IC_{50} > 50 μM). Imipramine, a potent hSERT inhibitor, was utilized as the comparative positive control (IC_{50} = 21 $\pm$ 3 nM). **(b)** Ariadne is additionally not a potent hDAT inhibitor (IC_{50} > 50 μM) and was compared to a potent hDAT inhibitor, indatraline (23 $\pm$ 3 nM). Inhibition by (*rac*)-Ariadne, imipramine, and indatraline is presented as normalized fluorescence uptake (uninhibited – inhibited) ± SEM and compiled from four separate experiments. The x-axis, concentration of inhibitor (micromolar).

a/



b/



Scheme S5: Timeline of Aux-KO experiments

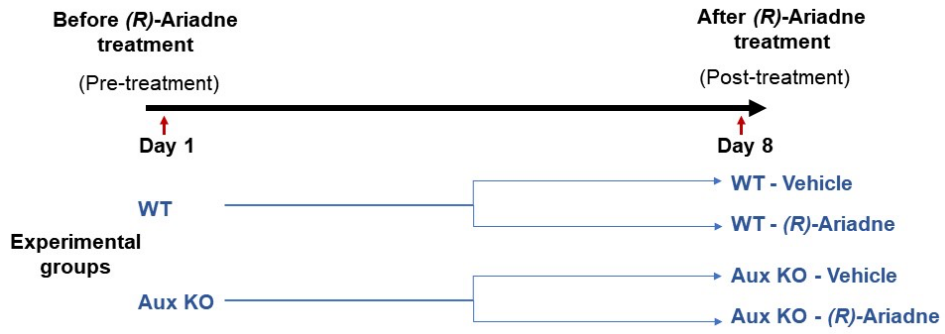


Figure S5: Vehicle treatment comparison for auxilin KO mouse model

(a) Number of balance beam runs performed over 60 seconds by WT and Aux KO mice before (Pre) and after (Post) vehicle treatment. **(b)** Time taken per run on balance beam of WT and Aux KO mice before (Pre) and after (Post) vehicle treatment. **(c)** Hind limb clasping duration in WT and Aux KOs, and its response to vehicle treatment **(d)** Hindlimb clasping score in WT and Aux KOs, and its response to vehicle treatment. Note that Aux KO exhibit severe motor phenotypes and these are not changed by vehicle treatment.

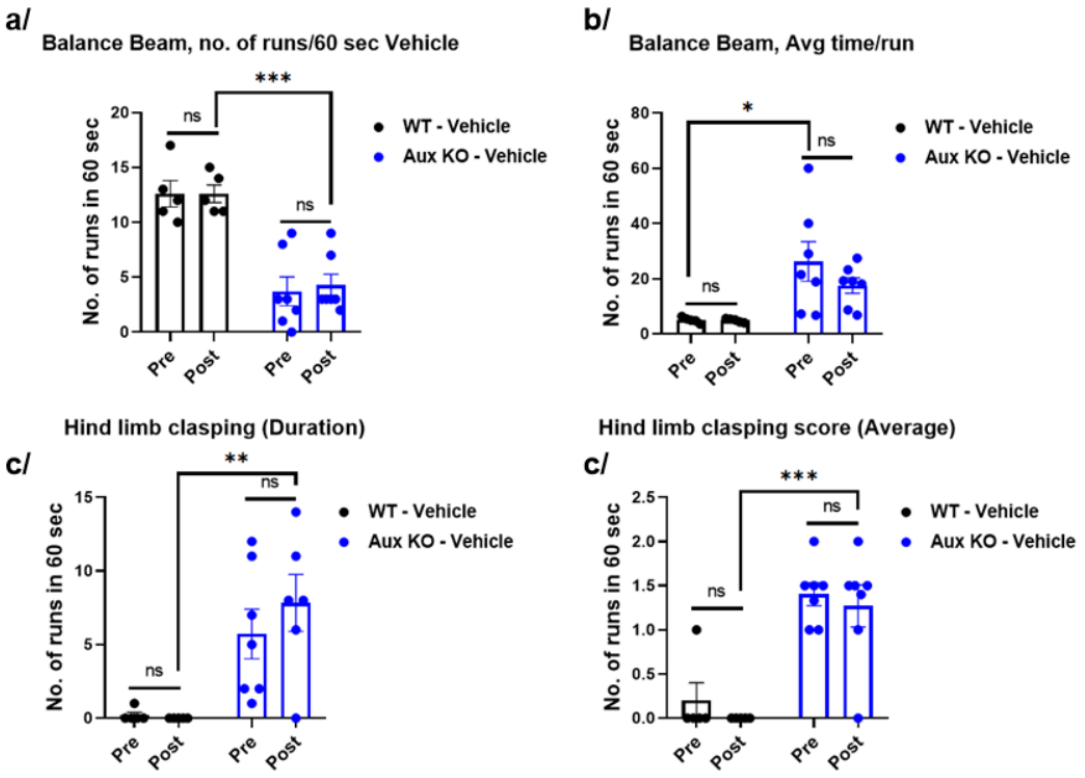


Figure S6: Predicted binding poses of 25CN-NBOH (blue) in the active state of 5-HT2A receptor with the following compounds **(a)** (*R*)-DOM (green) **(b)** (*R*)-Adriane (pink). **(c)** (*S*)-Adriane (yellow) **(d)** (*R*)-5-CD, (Ariadne's propyl homolog, coral)

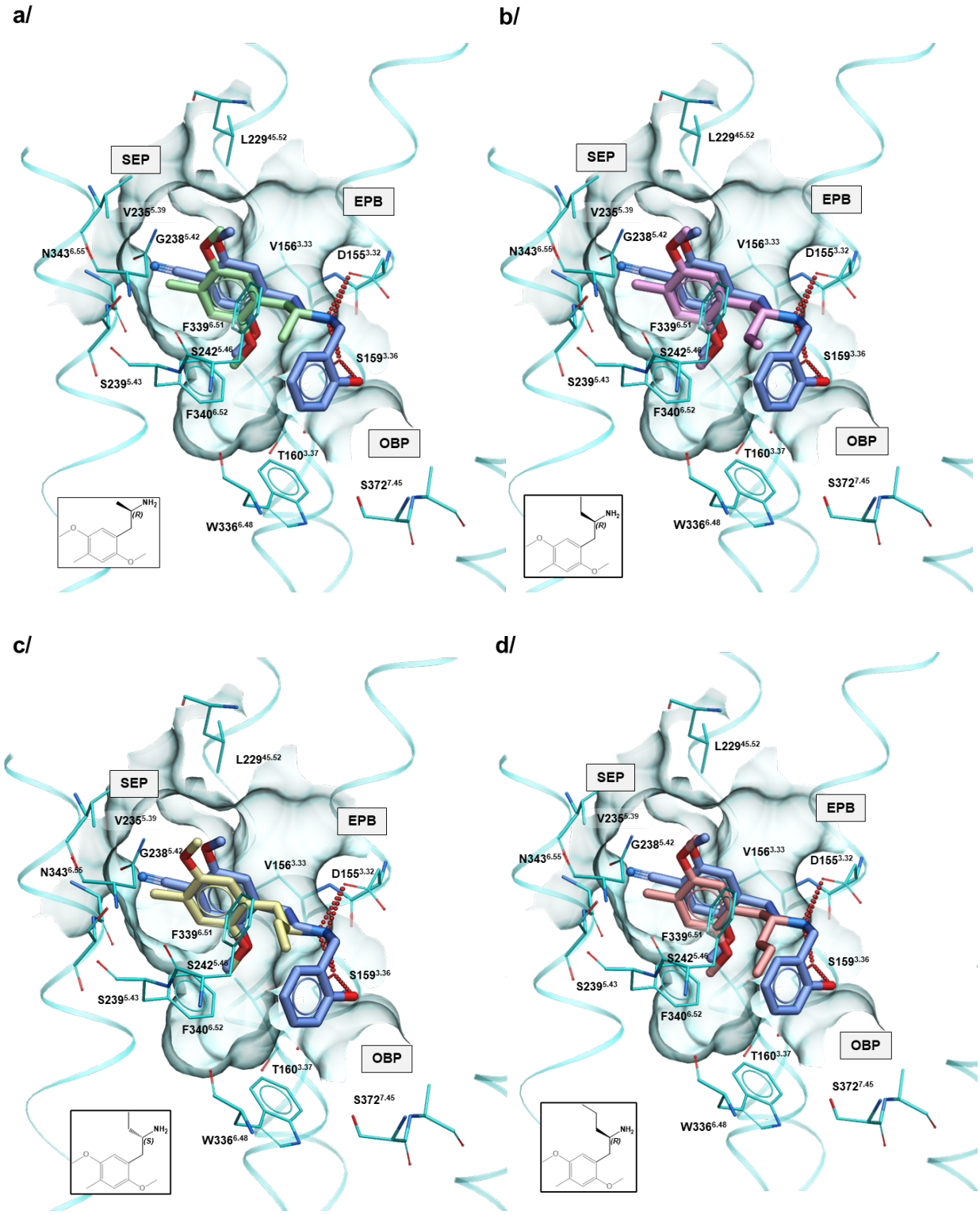


Table S8: Mouse 5-HT_{2A} Receptor Gq dissociation

	m5-HT_{2A}-Gq Dissociation				
Ligand	EC50, nM	pEC50	SEM	Emax	SEM
5-HT	32.5	7.49	0.04	100.0	ND
rac-Ariadne	455	6.34	0.04	75.8	1.3
(R)-Ariadne	383	6.42	0.03	81.7	1.3
(S)-Ariadne	917	6.04	0.05	72.6	1.8
DOPr	3.23	8.49	0.02	97.6	0.7
4C-TFM	82.5	7.08	0.04	79.8	1.3

Figure S7: Timeline of HTR events induced by DOPR and (R)-Ariadne

N = 4/group, points represented as Mean ± SEM.

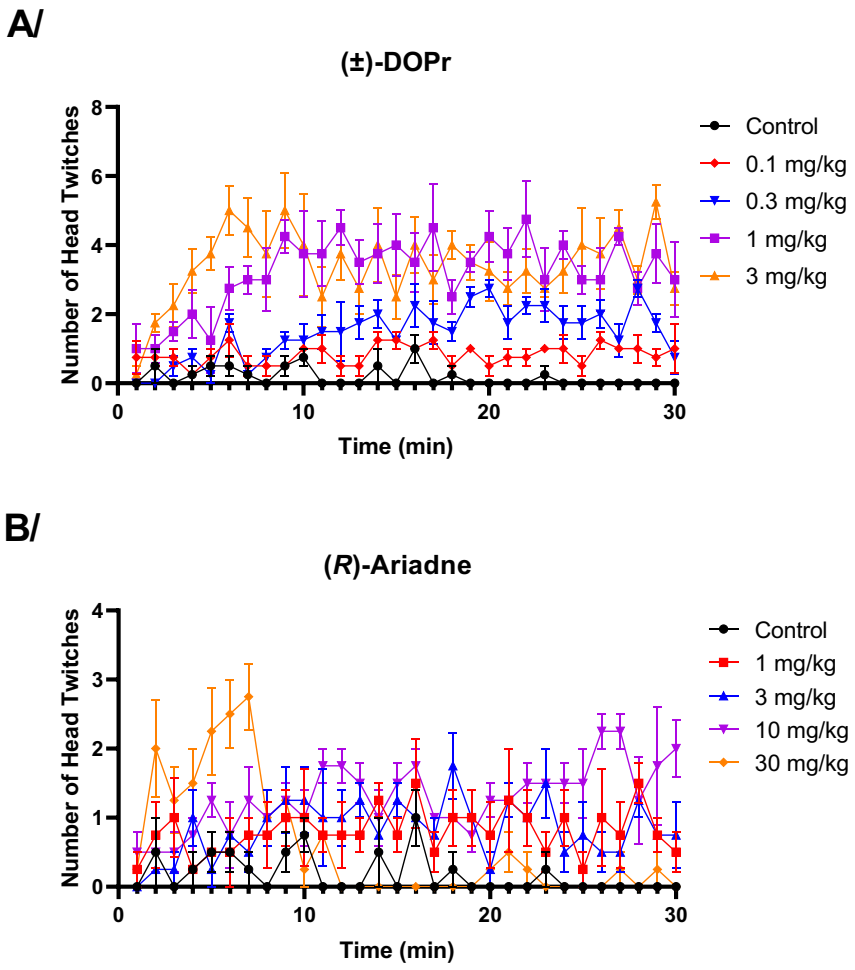


Figure S8: (a) The total counts of HTRs over various time intervals. Kruskal-Wallis with post-hoc Dunn's test, **p < 0.01. (b) To compare magnitude of DOPR and (*R*)-Ariadne at the various time bins, a t-test was performed, **p < 0.01. (c) The evaluation of (*R*)-Ariadne in open field for two hours (n = 4/group). All values represented as mean ± S.E.M.

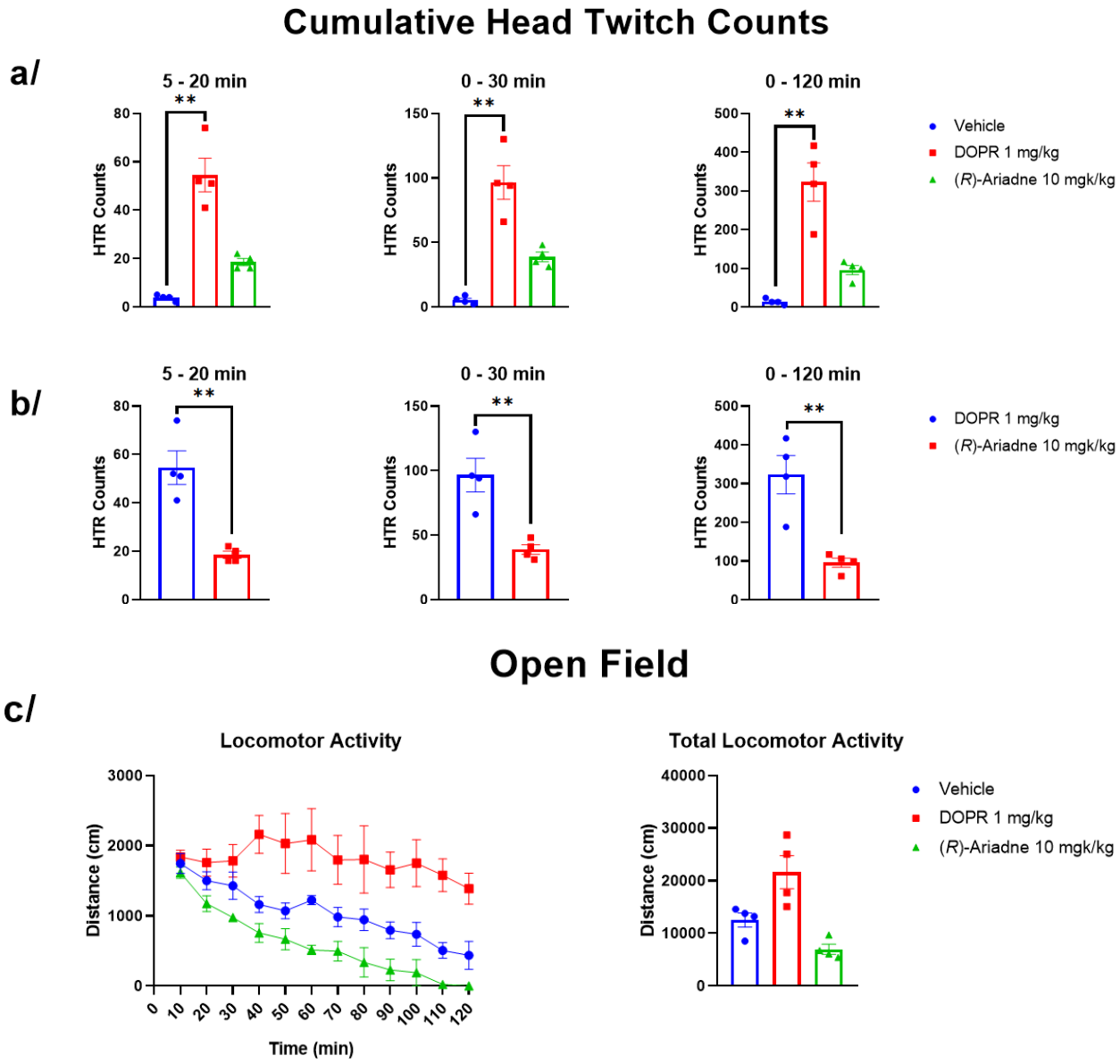


Figure S9: The effect of (*R*)-Ariadne 15 minutes post drug injection in the FST. **(a)** Evaluation of immobility time **(b)** The last 4 minutes of the 6 minutes test were analyzed for time spent immobile (n = 10/group). One-way ANOVA (b) with post-hoc Dunnett's test. No statistical significance. All values represented as mean ± S.E.M.

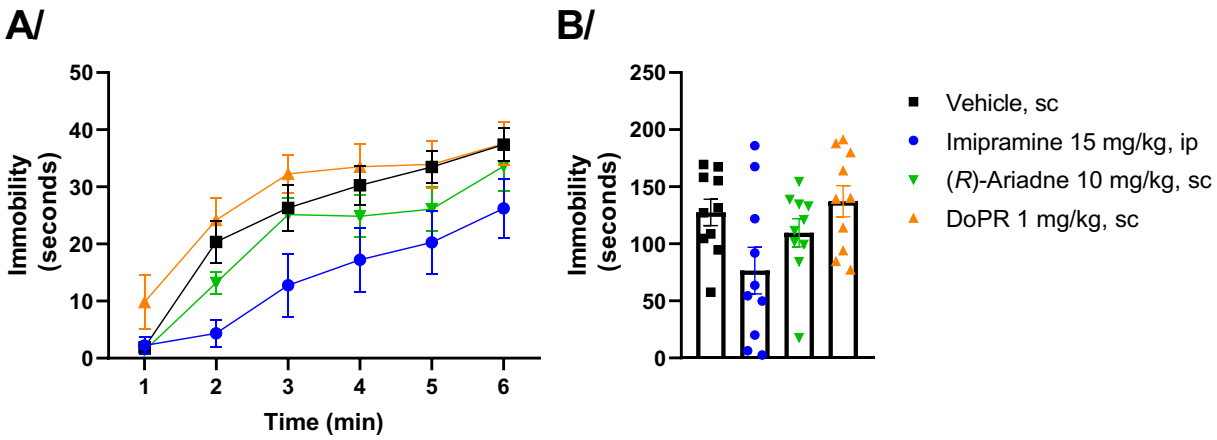


Figure S10: Linked OF and FST experiments
(a) Schematic of experimental design. Evaluation of (R)-Ariadne's and DOPR's effect on total distance **(b & c)** and time in center **(d & e)** in open field for 30 minutes (n = 8/group). **(f)** The effect of (R)-Ariadne four hours post injection (n = 8/group) on the forced swim test. The last 4 minutes of the 6 minutes test were analyzed for time spent immobile. One-way ANOVA with post-hoc Dunnett's test. All values represented as mean ± S.E.M, ****p < 0.0001, ***p < 0.001, *p < 0.05

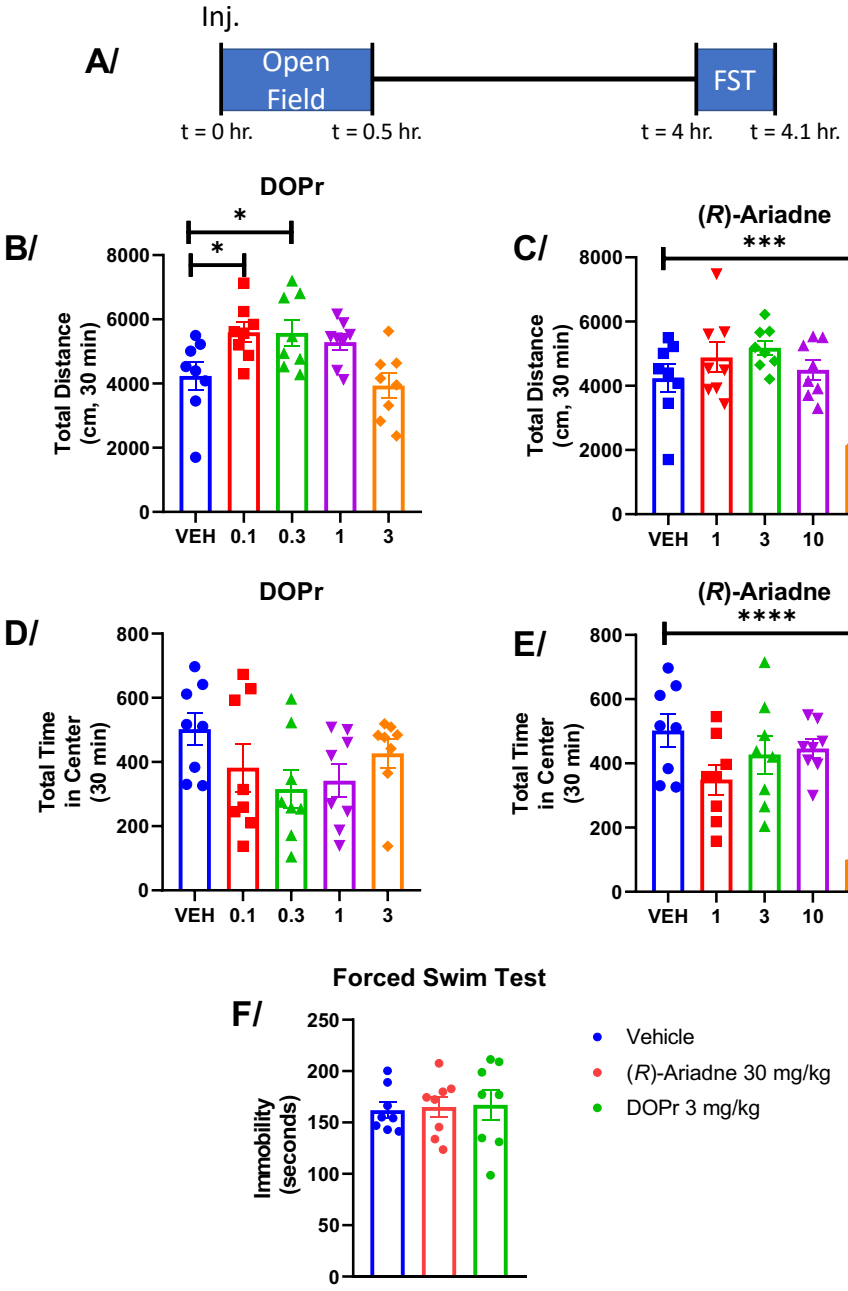


Figure S11: Linked behavioral experiments continued
(A) Schematic of experimental design. **(B)** The effect of (R)-Ariadne was evaluated in the light-dark box. **(C)** Mice underwent FST 7 days post injection. The last 4 minutes of the 6 minutes test were analyzed for time spent immobile. One-way ANOVA with post-hoc Dunnett's test. No statistical significance. All values represented as mean ± S.E.M.

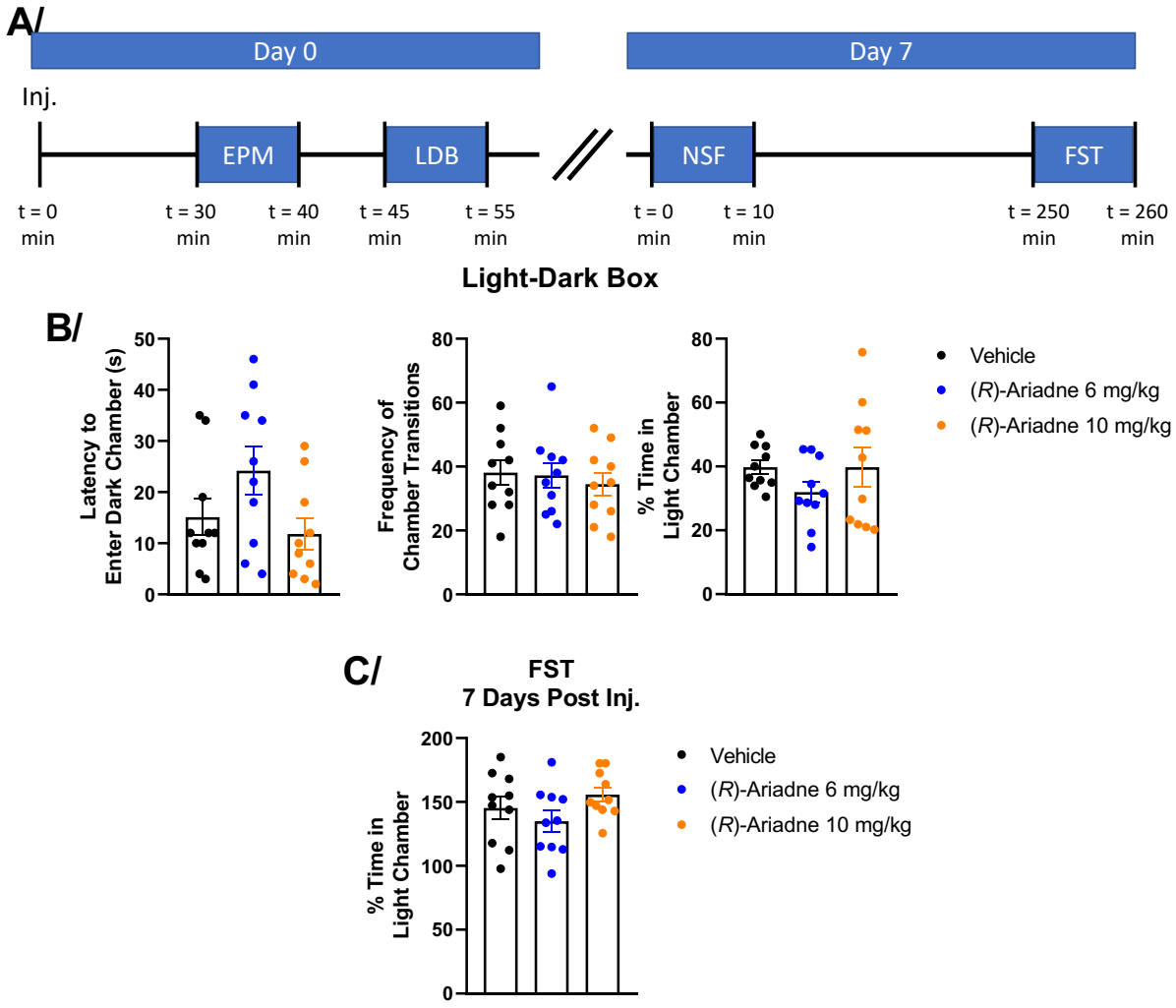


Figure S12: (A) Effect of MDL100907, 5-HT2A antagonist, on 4C-TFM-induced HTR (n = 5/group). (B) MDL100907 effect on locomotor activity. Beige band shows the time-period used for HTR scoring in these studies. One-way ANOVA with post-hoc Tukey's test (A). All values represented as mean ± S.E.M, ****p < 0.0001

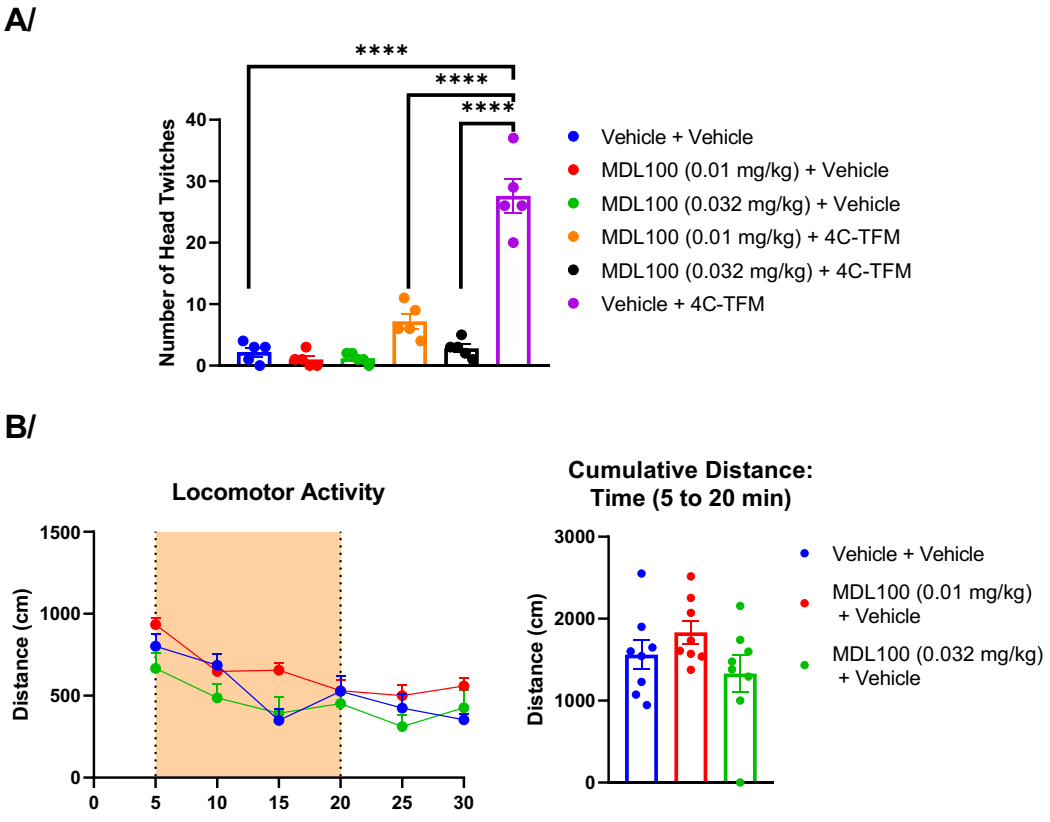


Figure S13: Contribution of alpha substitution relative to amine on head twitch response. N = 5/group. All values represented as mean ± S.E.M. over a 15 min period.