**Rudin, D, McCorvy, JD, Glatfelter, GC, Luethi, D, Szöllősi, D, Ljubišić, T, Kavanagh, PV, Dowling, G, Holy, M, Jaentsch, K, Walther, D, Brandt, SD, Stockner, T, Baumann, AL and Sitte, HH**

**(2-Aminopropyl)benzo[β]thiophenes (APBTs) are novel monoamine transporter ligands that lack stimulant effects but display psychedelic-like activity in mice**

# Supplementary data

# (2-Aminopropyl)benzo[β]thiophenes (APBTs) are novel monoamine transporter ligands that lack stimulant effects but display psychedelic-like activity in mice

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**Supplementary Material and Methods**

Material

Hydrochloride salts of all six racemic benzothiophene isomers used were synthesized as described previously [1]. For *in vivo* studies, racemic MDMA hydrochloride was provided by National Institute on Drug Abuse Drug Supply Program (Rockville, MD, US) and 5-APB hydrochloride as well as 6-APB hydrochloride were provided by Dr. Bruce Blough (Research Triangle International). [$^3$H]5-HT (43.1 µCI mmol$^{-1}$), [$^3$H]dopamine (51.4 µCI mmol$^{-1}$), [$^3$H]γ-aminobutyric acid (GABA) (12.3 µCI mmol$^{-1}$) and Ultima Gold$^{TM}$ XR liquid scintillation cocktail were purchased from Perkin Elmer (Boston, MA, USA); [$^3$H]MPP$^+$ (60 µCi mmol$^{-1}$) was obtained from American Radiolabled Chemicals (St. Louis, MO, USA). Cell culture dishes were purchased from Sarstedt (Nuembrecht, Germany). All other chemicals and cell culture supplies were from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture

Human embryonic kidney 293 (HEK293) cells stably expressing the human serotonin transporter (hSERT), dopamine transporter (hDAT), norepinephrine transporter (hNET) or GABA transporter 1 (hGAT1) were maintained in humidified atmosphere (37 °C, 5% $CO_2$) in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% heat-inactivated fetal calf serum (FCS), streptomycin (1 µg/mL) and penicillin (1 U/mL). Geneticin (50 µg/mL) was used as selection antibiotic.

Uptake inhibition experiments in HEK293 cells

For uptake experiments, cells expressing the respective transporter were seeded onto poly-D-lysine coated 96 well plates at a density of 30,000 cells per well in a final volume of 200 µL, 24 h prior to the experiment. Uptake inhibition assays were conducted as described previously [2] with some modifications. In brief, cell culture medium was replaced with 300 µL Krebs-HEPES buffer (KHB: 25 mM HEPES, 120 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 5 mM D-glucose, pH 7.3). Next, the cells were incubated with 50 µL of the respective benzothiophene isomer for 10 min at concentrations ranging from 1 nM to 1 mM. Subsequently, 0.1 µM [$^3$H]5-HT, 0.1 µM [$^3$H]dopamine, 0.05 µM [$^3$H]MPP+, or 0.03 µM [$^3$H]GABA were added. Uptake was terminated after 60 s (hSERT) or 180 s (hDAT, hNET, and hGAT) by washing the cells with 200 µL ice cold KHB. Next, the cells were lysed with 200 µL Ultima Gold$^{TM}$ XR liquid scintillation cocktail and the amount of tritium in the cells was measured with a Wallac 1450 MicroBeta® TriLux liquid scintillation counter. Non-specific uptake was determined in the presence of 100 µM paroxetine (hSERT), 100 µM cocaine (hDAT and hNET) or 100 µM tiagabine (hGAT).

Uptake inhibition experiments in rat brain synaptosomes

All experiments using animal tissue were performed according to the ARRIVE guidelines. Male Sprague-Dawley rats (Harlan, Frederick, MD, US) weighing 250–350 g were maintained under standard conditions (lights on at 07:00 hours, off at 19:00 hours) with food and water available *ad libitum*. The rats were housed three per cage for 2 weeks prior to being used in experiments. The animal facilities were fully accredited by the Association for Assessment and Accreditation of

Laboratory Animal Care (AALAC) and the Animal Care and Use Committee at the National Institute on Drug Abuse (NIDA), Intramural Research Program (IRP). On the day of the experiment, the rats were euthanized by $CO_2$ narcosis and the brains were harvested to prepare synaptosomes as described previously [3]. Rat caudate tissue was used for rDAT assays whereas rat forebrain minus caudate was used for rNET and rSERT assays. Brain tissue was homogenized in ice cold 10% sucrose followed by centrifugation at 1000 *g* for 10 min. The supernatant containing crude synaptosomes was kept on ice until use in uptake or release assays. A total of 42 rats was used for the *in vitro* synaptosome assays.

For uptake inhibition assays in synaptosomes, 5 nM [$^3$H]dopamine, [$^3$H]norepinephrine, or [$^3$H]5-HT was used to assess transport activity at rDAT, rNET, or rSERT, respectively. Assays were initiated by adding 100 µL of tissue to 900 µL Krebs-phosphate buffer (KPB: 126 mM NaCl, 2.4 mM KCl, 0.83 mM $CaCl_2$, 0.8 mM $MgCl_2$, 0.5 mM $KH_2PO_4$, 0.5 mM $Na_2SO_4$, 11.1 mM glucose, 0.05 µM pargyline, 1 mg/mL bovine serum albumin, and 1 mg/mL ascorbic acid, pH 7.4) containing test drug and [$^3$H]neurotransmitter. Test drug solutions were prepared in KPB at eight different concentrations in order to construct inhibition curves. Nonspecific uptake was measured by incubating synaptosomes in the presence of a large excess (1 µM) of the nonselective uptake inhibitor indatraline. Uptake inhibition assays were terminated by rapid vacuum filtration through Whatman GF/B filters, and retained radioactivity was quantified by liquid scintillation counting.

Batch release experiments in HEK293 cells

For batch release experiments, cells expressing the respective transporter were seeded onto poly-D-lysine coated 96 well plates at a density of 30,000 cells per well in a final volume of 200 µL, 24 h prior to the experiment. HEK293 cells expressing the desired transporter were pre-loaded with [$^3$H]5-HT, [$^3$H]dopamine, or [$^3$H]MPP$^+$ by incubation with 0.05 µM of the respective tritiated neurotransmitter in KHB for 20 min at 37 °C. Subsequently, the cells were washed twice with KHB with or without 10 µM monensin and equilibrated for 20 min. Monensin is a selective $Na^+/H^+$ ionophore that increases intracellular sodium, whereby transporter mediated efflux is selectively augmented [2,4]. Next, the cells were incubated with 10 µM of the respective benzothiophene isomer or 10 µM 3,4-methylenedioxymethamphetamine (MDMA) for 10 min. Subsequently, the supernatant was transferred to another 96 well plate and 200 µL Ultima Gold™ XR liquid scintillation cocktail was added to the cells and supernatant. The amount of tritium in the cells and in the supernatant was assessed with a Wallac 1450 MicroBeta® TriLux liquid scintillation counter. The release of tritiated neurotransmitter was expressed as percentage of the total radioactivity of cells and supernatant together normalized to the basal efflux of untreated cells. Non-specific release was determined in the presence of 30 µM paroxetine, 10 µM mazindol, or 30 µM nisoxetine for hSERT, hDAT, or hNET, respectively.

Release experiments in rat synaptosomes

Rat brain synaptosomes were prepared as described above. For release assays, 9 nM [$^3$H]MPP$^+$ was used as substrate for rDAT and rNET and 5 nM [$^3$H]5-HT as substrate for rSERT. One micromolar reserpine was added to all buffers used for release assays to block vesicular uptake of substrates. To optimize the selectivity for a single transporter, the unlabeled compounds

nomifensine and 1-(2-diphenylmethoxyethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (GBR12935) were included for rSERT assays; citalopram and desipramine were included for rDAT assays; and GBR12935 and citalopram were included for rNET assays. The unlabeled blockers were used to prevent the uptake of the respective substrates by competing transporters. The synaptosomes were preloaded with radiolabeled substrate in KPB for 1 h to reach steady state. Efflux assays were started by addition of 850 µL preloaded synaptosomes to 150 µL of the respective benzothiophene isomer. Dose-response curves were generated from eight different concentrations of each isomer. The efflux assay was terminated by vacuum filtration through GF/B filters on a Brandel harvester (Gaithersburg, MD, USA), and the remaining radioactivity was quantified by liquid scintillation counting with a PerkinElmer TopCount.

Förster Resonance Energy Transfer imaging

Förster Resonance Energy Transfer (FRET) microscopy was employed to assess the potential of the ABPT isomers to induce conformational changes at SERT. These experiments were conducted using HEK293 cells stably expressing an hSERT construct with a fluorescence donor (CFP) and acceptor (YFP) attached to the N and C terminus, respectively [5,6]. This construct can be used to detect conformational changes of SERT exposed to substrates, as an accumulation of inward-facing SERT conformations results in decreased FRET [7]. The cells were seeded into 29 mm dishes with 20 mm bottom (# 1.5 glass; Cellvis, Mountain View, CA, USA) at a density of $10^5$ cells per dish one day prior to imaging. The following day, the cells were treated with the ABPT isomers for 10 min at ambient temperature at a concentration of 10 µM. The SERT substrates MDMA and *para*-chloroamphetamine (PCA) were included as controls. FRET was measured with an iMIC inverted

microscope (T.I.L.L. Photonics GmbH, Kaufbeuren, Germany) equipped with a 60X (N.A. 1.49) oil objective (Olympus). Fluorescence was excited with a 100 W Xenon Lamp (Polychrome, T.I.L.L Photonics GmbH, Kaufbeuren, Germany). The excitation light was filtered through 436/20 nm (CFP) or 514/10 nm (YFP) excitation filters (Semrock, Rochester, NY, USA) and directed to the sample by a 442/514 dual line dichroic mirror (Semrock, Rochester, NY, USA). The emitted fluorescence light was filtered through a 480/40 nm and 570/80 nm dual emission filter (Semrock, Rochester, NY, USA) and directed to a beamsplitter unit (Dichrotom, T.I.L.L Photonics, Kaufbeuren, Germany). The emission light was separated spatially according to the fluorescence wavelength using a 515 nm dichroic mirror (Semrock, Rochester, NY, USA) and the resultant channels (<515 nm & >515 nm) were projected side by side onto an EMCCD chip (iXon Ultra 897 Andor, Andor Technology, Belfast, UK). Live Acquisition software (version 2.5.0.21; T.I.L.L Photonics GmbH, Kaufbeuren, Germany) was used for recording. For optimal noise ratio and dynamic range, the camera was operated in 16-bit mode with a readout speed of 1 MHz. According to the manufacturer's recommendation, an EM-Gain of 16 was applied to overcome the noise floor. Two images were taken per set (donor and acceptor emission after donor excitation and acceptor emission after acceptor excitation). Per condition, ten sets were recorded on each experimental day and the images were then analyzed using Offline Analysis software (version 2.5.0.2; T.I.L.L Photonics GmbH, Kaufbeuren, Germany). Background fluorescence was subtracted from each image, and one region of interest (part of the plasma membrane) per cell was selected in the CFP channel. The average intensity of each region of interest was used for calculations. HEK293 cells expressing a CFP or YFP signal only were used to determine spectral bleed through (BT) for donor (0.57) and acceptor (0.04). Normalized FRET (NFRET) was calculated

as follows:

$$\mathrm{NFRET} = \frac{I_{FRET} - BT_{Donor} \times I_{Donor} - BT_{Acceptor} \times I_{Acceptor}}{\sqrt{I_{Donor} \times I_{Acceptor}}}$$

Maximum FRET was determined using a fused CFP-YFP construct, and separate donor and acceptor fluorophores were included as a negative control.

*In silico* docking simulations at human SERT

For the docking simulations, the outward-open hSERT crystal structure was used (PDB ID: 5I71)[8]. Missing side chains and bound ions were positioned using MODELLER 9.20 [9,10] creating 100 structures from which the best was used based on the DOPE score [11]. The structure of hDAT and hNET was obtained by homology modeling using the above SERT structure. Again, 100 models were generated per transporter and the best were used. All proteins contained two $Na^+$ and one $Cl^-$ ion at their respective binding sites.

Initial ligand molecule conformations were prepared with Open Babel version 2.3.2 [12] using SMILES codes (Suppl. Table S2) except for the native substrates (5-HT, dopamine, and norepinephrine), for which conformations were downloaded from PubChem [13]. Transporters and ligand molecules were prepared for docking with AutoDockTools version 1.5.6 [14], adding missing hydrogens, determining Gasteiger charges and assigning ADT4 atom types. The bound ions are ensured to have correct unit charges before grid calculation. While in the ligands all

rotatable bonds were kept flexible, in the three transporters only the four homolog residues were kept flexible to directly influence the binding pose (hSERT: 98, 172, 176, 335; hDAT: 79, 152, 156, 320; hNET: 75, 148, 152, 317). Docking was performed with AutoDock4 [15] (docking parameters can be found in Suppl. Table S1) resulting in 1,000 docked poses for every compound.

Figures and statistical analyses were generated by R [16] and using the package ggplot2 [17]. For visualization, Pymol, v1.8.4 (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC) was used.

Receptor binding affinity and activation potency

For receptor binding affinity and activation potency assays as well as for subsequent *in vivo* behavioral investigations, only 3-APBT, 5-APBT, and 6-APBT were included; these isomers correspond to the most frequently encountered substitution profile of benzofurans and aminopropyl indoles. The benzothiophenes 3-APBT, 5-APBT, and 6-APBT were tested at the respective human 5-$HT_2$ receptors stably expressed in cells according to PDSP protocols [18], using 11 concentrations of each isomer to generate competition binding isotherms. $K_i$ values were obtained from best fit $IC_{50}$ values generated by nonlinear regression of the binding isotherms using the Cheng-Prusoff equation. [$^3$H]Ketanserin (5-$HT_{2A}$), [$^3$H]LSD (5-$HT_{2B}$), and [$^3$H]mesulergine (5-$HT_{2C}$) were used as radioligands.

5-$HT_2$ activation potency and efficacy was determined by Gq-mediated calcium flux assays performed in tetracycline-inducible HEK T-Rex cells stably expressing either the human 5-$HT_{2A}$, 5-$HT_{2B}$, or 5-$HT_{2C}$ receptor, as described previously [19]. One day before the assay, cells were

induced with 2 µg/mL tetracycline and seeded into poly-L-lysine-coated black 384 well clear-bottom tissue culture plates with DMEM (Invitrogen, Carlsbad, CA, USA) containing 1% dialyzed fetal bovine serum at a density of 10,000 cells in 40 µL per well. The following day, stock solutions of 3-APBT, 5-APBT, and 6-APBT were diluted in assay buffer (HBSS, 20 mM HEPES, 2.5 mM probenecid, 0.1% BSA, 0.01% ascorbic acid, pH 7.4) as 5x solutions. The media of each well were replaced with 20 µL of assay buffer containing Fluo-4 Direct dye (Invitrogen) and incubated for 1 h at 37 °C. After equilibration to room temperature, $Ca^{2+}$ flux was measured using a FLIPR$^{TETRA}$ system (Molecular Devices, Sunnyvale, CA, USA). To establish a baseline, plates were read for fluorescence initially for 10 s (one read per second) and then stimulated by addition of 5 µL per well of APBT solution or buffer and measured for additional 120 s. Peak fluorescence in each well was normalized to maximum-fold increase over baseline. Data were normalized to the maximum peak fold-over-basal fluorescence produced by 5-HT (100%) and baseline fluorescence (0%). Data were analyzed using the sigmoidal dose-response function of GraphPad Prism (Prism 9.0.2, GraphPad Software, San Diego, CA).

*In vivo* behavioral experiments

Locomotor activity and temperature assessment

Male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, USA) weighing 20–30 g were housed in the vivarium at the NIDA, IRP in Baltimore, MD, where facilities are fully accredited by AALAC. Mice were allowed at least 2 weeks of acclimation to the NIDA, IRP vivarium facilities prior to the start of experiments. All procedures described herein were approved by the Animal Care

and Use Committee of the NIDA, IRP.

At 6–7 weeks of age, mice were briefly anesthetized with isoflurane, and subcutaneous temperature transponders (Bio Medic Data Systems, Inc. Seaford, Delaware, US) were implanted. Mice were single housed post-operatively and allowed 1 week for recovery prior to the start of drug treatments and testing.

At 7–8 weeks of age, mice were randomized to receive treatment with 0.01–30 mg/kg s.c. 3-APBT, 5-APBT, 6-APBT, or vehicle (saline 0.9%) and tested once per week for 5 weeks. Two weeks after the last APBT administration, one group of mice was used to test the effects of 10 mg/kg s.c. 5-APB on locomotor activity. A separate cohort of mice was used to assess the effects of 10 mg/kg s.c. 6-APB and 30 mg/kg s.c. MDMA on locomotor activity. Locomotor activity was assessed using plexiglass open field arenas equipped with photobeam arrays to measure distance traveled (cm) in the horizontal plane (TruScan, Harvard Apparatus, Holliston, MA, USA). This system was also used to assess the total number of movements, rest time (seconds), and the number of stereotypy-like repetitive episodes (comprised of at least 3 movements less than 0.999 beam spaces occurring 2 seconds or less apart). Mice were moved from the vivarium to the testing room in their home cages. After at least a 1 h of acclimation to the testing room, mice were placed into open field arenas and allowed 30 min for habituation to the testing chamber. After habituation, mice received s.c. injections of test drug or saline vehicle and were immediately returned to the chamber for 60 min. Temperature (°C) was measured non-invasively with a handheld receiver 30 min prior to injections, at the time of injection, as well as 30 and 60 min after injections. The effects of each drug on cumulative locomotor activity, number of movements, rest time, stereotypy episodes, and temperature change across the session were evaluated using a one-way

ANOVA (dose) followed by Dunnett's post-hoc test to compare drug effects to saline control. *$P$ < 0.05 was used as the minimum criterion for statistical significance for all experiments.

Head-twitch response

Male C57BL/6J mice were housed in a vivarium at the University of California San Diego (UCSD), which is an AAALAC-approved animal facility that complies with Federal and State requirements for care and treatment of laboratory animals. The mice (6–8 weeks old) were housed in a climate-controlled room with a reversed light-cycle (lights on at 19:00 hours, off at 07:00 hours) up to 4 animals per cage. Food and water were provided ad libitum, except during behavioral testing which occurred between 10:00 and 18:00 hours. All experiments were conducted according to NIH guidelines and were approved by the UCSD animal care committee.

Head movement was recorded using a head-mounted magnet and a magnetometer coil [20]. Mice were anesthetized, a small incision was made in the scalp, and a neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a 1–2-week recovery period, behavioral experiments were conducted in a well-lit room with at least 7 days between sessions to avoid any carryover effects. Mice were treated IP with vehicle (saline) or APBT isomer and then placed in a glass cylinder surrounded by a magnetometer coil and tested for 30 min. Coil voltage was filtered (5–10 kHz lowpass), digitized (20 kHz sampling rate) and saved to disk using a Powerlab/8SP with LabChart v 7.3.2 (ADInstruments, Colorado Springs, CO). Head twitches were identified in the recordings using established procedures based on artificial intelligence [21]. HTR counts were analyzed using one-way analyses of variance (ANOVA). Post hoc pairwise comparisons between selected groups were performed using Tukey's studentized range method.

Significance was demonstrated when an α-level of 0.05 was surpassed. Median effective doses ($ED_{50}$ values) and 95% confidence intervals (95% CI) were calculated by nonlinear regression (Prism 9.0.2, GraphPad Software, San Diego, CA).

**Supplementary Figure Legends**

**Figure S1**

**Transporter-mediated uptake in HEK293 cells and rat brain synaptosomes. A)** Transporter-mediated uptake in HEK293 cells expressing human SERT, DAT, or NET. Cells were incubated with 0.2 µM [$^3$H]5HT for hSERT assays, 0.1 µM [$^3$H]dopamine for hDAT assays, or 0.05 µM [$^3$H]MPP+ for hNET assays, along with various concentrations of the APBT isomers. Non-specific uptake was determined in the presence of 100 µM paroxetine for hSERT, 10 µM mazindol for hDAT, or 1 mM cocaine for hNET. All data are presented as mean ± SD from at least 3 experiments performed in triplicate. **B)** Effects of APBT isomers on transporter-mediated uptake at SERT, DAT, or NET in rat brain synaptosomes. Rat brain synaptosomes were incubated with 5 nM [$^3$H]5HT for SERT assays, 5 nM [$^3$H]dopamine for DAT assays, or 5 nM [$^3$H]norepinephrine for NET assays, along with various concentrations of the APBT isomers. Non-specific uptake was determined in the presence of 1 µM indatraline for DAT, NET and SERT. All data are presented as mean ± SD from at least 3 experiments performed in triplicate

**Figure S2**

**Transporter-mediated uptake in HEK293 cells expressing human GAT1.** Cells were incubated with 0.03 µM [$^3$H]-GABA along with APBT isomers. Non-specific uptake was determined in the presence of 100 µM tiagabine. All data are presented as mean ± SD from 3 experiments performed in triplicate.

**Figure S3**

**FRET imaging studies and docking simulations. A)** HEK293 cells stably expressing a human SERT construct with a fluorescence donor (CFP) and acceptor (YFP) attached to the N- and C-terminus were used, and surface PCA (10 µM) and MDMA (10 µM) were used as positive controls. Average NFRET values (×100) are presented as mean and SD. *n* values are provided for each condition. **B)** Docking results are shown by representative docking poses in a substrate binding site S1 for SERT, DAT, and NET. The docked compounds are colored as in the right panel. The four sidechains of the transporter that were defined as flexible in the docking runs are highlighted. Distributions of the estimated binding energies are shown as a violin plot in the left panel.

**Figure S4**

**Serotonin receptor binding affinities and activation potencies. A-C)** Binding data were generated according to PDSP protocols and represent means ± SEM from three independent experiments performed in triplicate. **D-G)** Receptor activation was assessed by Gq-mediated calcium flux. Data were collected in HEK T-Rex-293 inducible cell lines stably expressing either human 5-$HT_{2A}$, human 5-$HT_{2B}$, or human 5-$HT_{2C}$ receptors. Data represent means ± SEM from three independent experiments performed in triplicate.

**Figure S5**

**3-APBT, 5-APBT, and 6-APBT do not alter the number of movements, rest time, or stereotypy episodes.** Plots of number of movements, rest time, and stereotypy episodes for mice treated with 3-APBT **(A-C)**, 5-APBT **(D-F)**, and 6-APBT **(G-I)** in locomotor activity experiments. All values are compared to vehicle controls (*$P < 0.05$).

**Figure S6**

**Effects of 3-APBT, 5-APBT, and 6-APBT on core body temperature in mice.** APBT-treated mice received s.c. injections of 0.01 – 30 mg/kg s.c. 3-APBT, 5-APBT, 6-APBT, or vehicle (saline 0.9%). Core body temperature was measured at 30 and 60 min after injections and is expressed as an average change at both timepoints from baseline temperature measured prior to injections. MDMA (30 mg/kg), 5-APB (10 mg/kg), and 6-APB (10 mg/kg) were administered s.c. for comparison to temperature effects of APBT compounds tested. Data are mean ± SEM for $n = 5$ mice per group and were compared via one-way ANOVA with Dunnett's post hoc test comparing all groups to 0 mg/kg vehicle controls (****$P < 0.0001$, ***$P < 0.001$).

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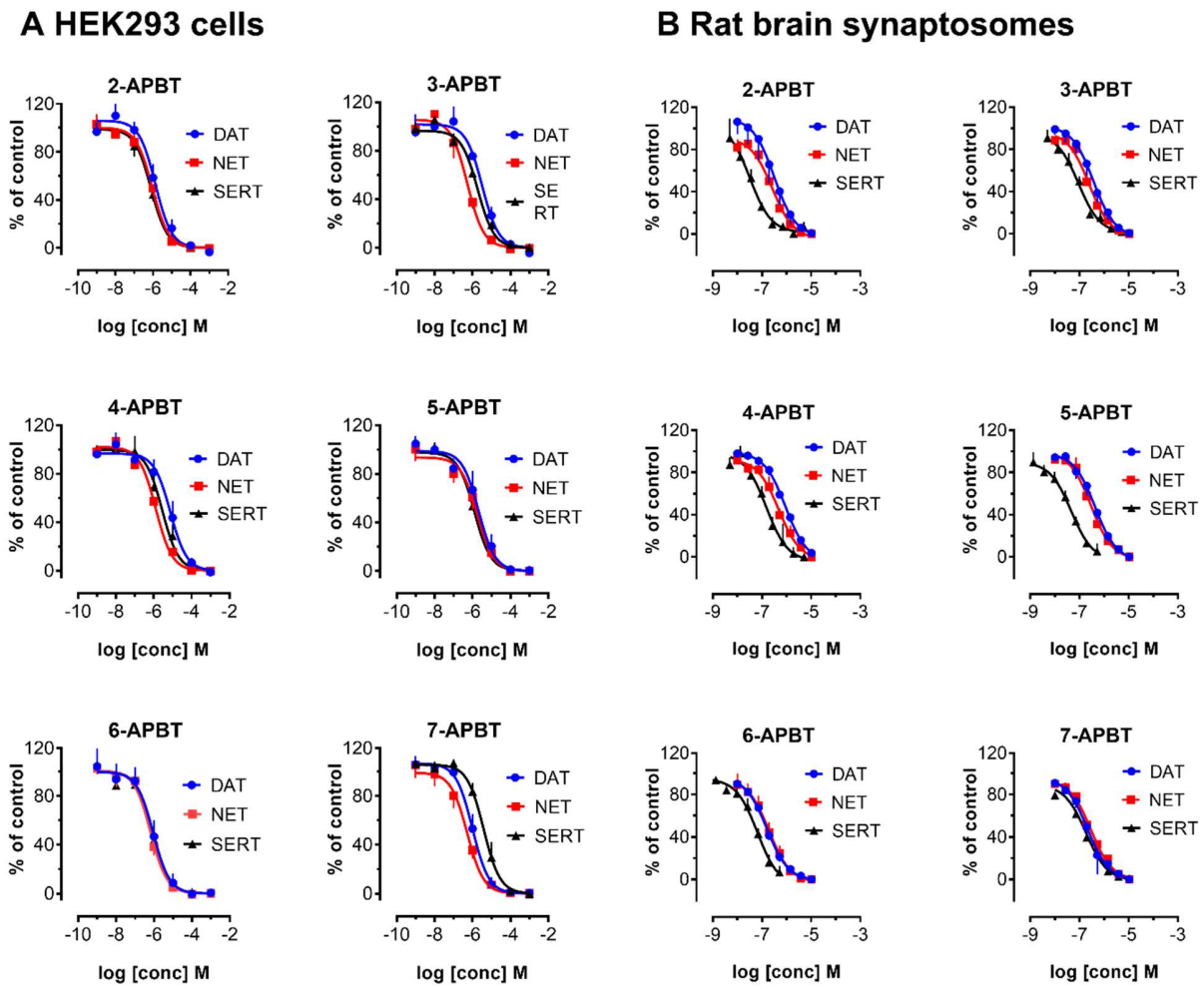
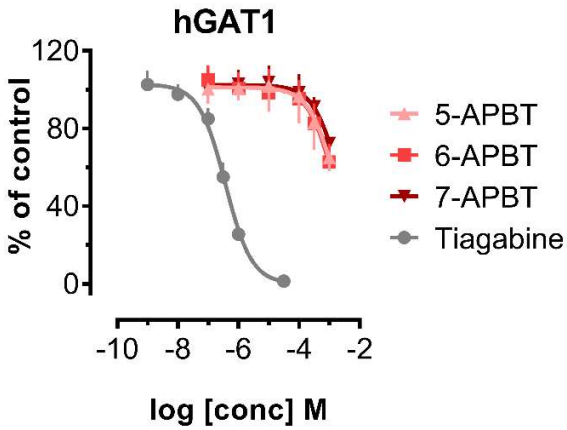
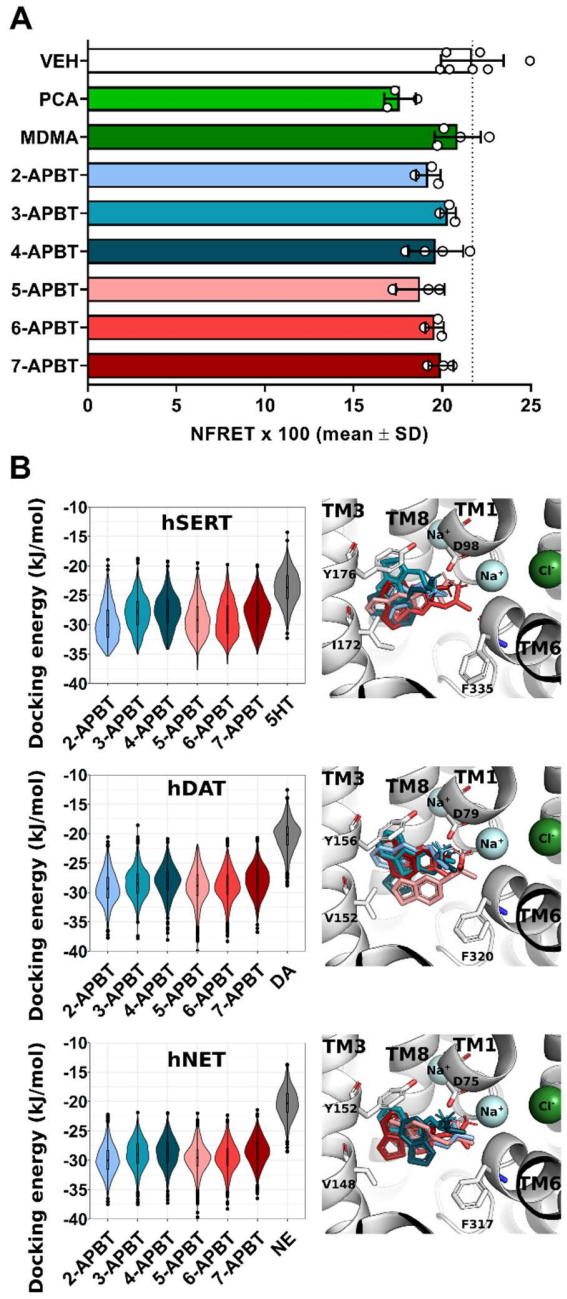
**Supplementary Figure S1**



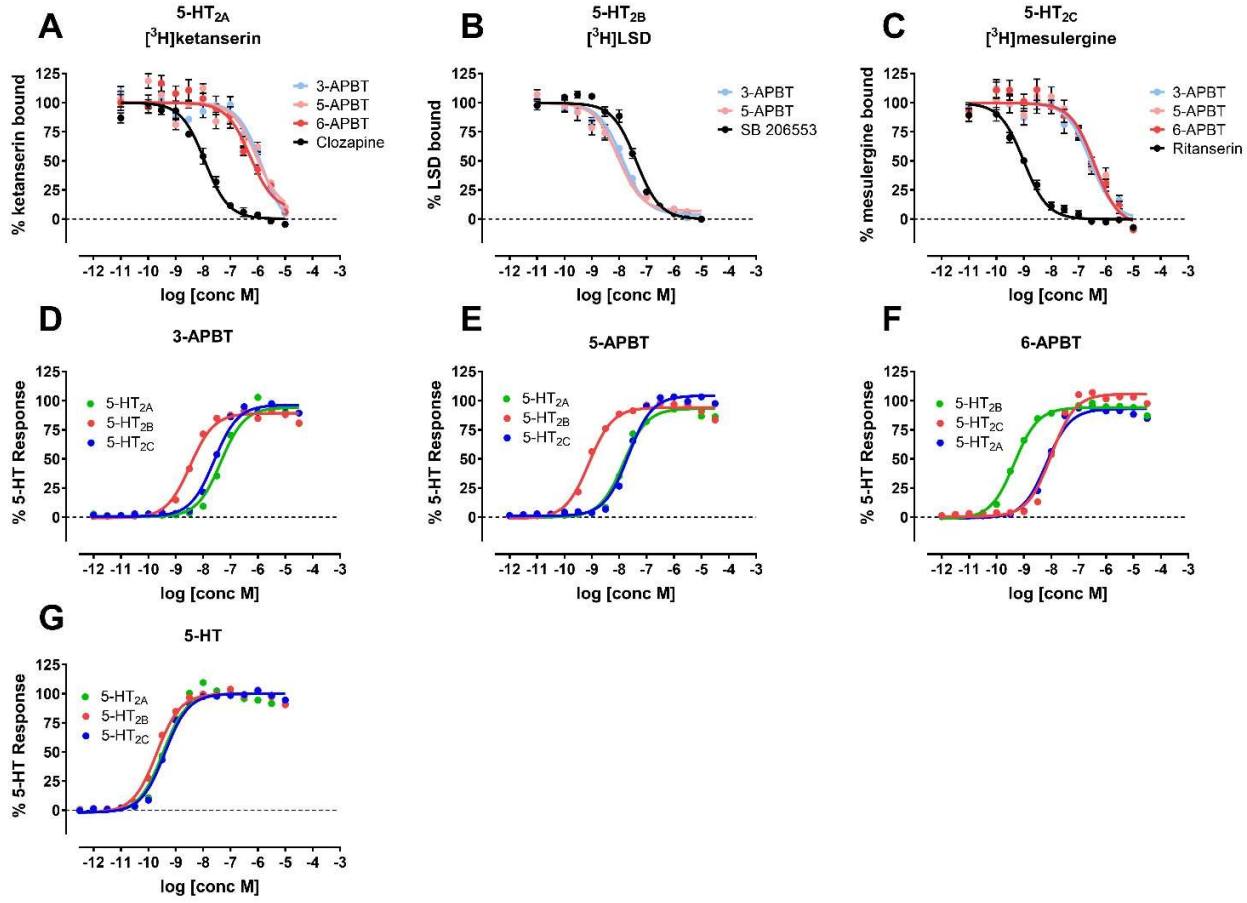
**Supplementary Figure S2**



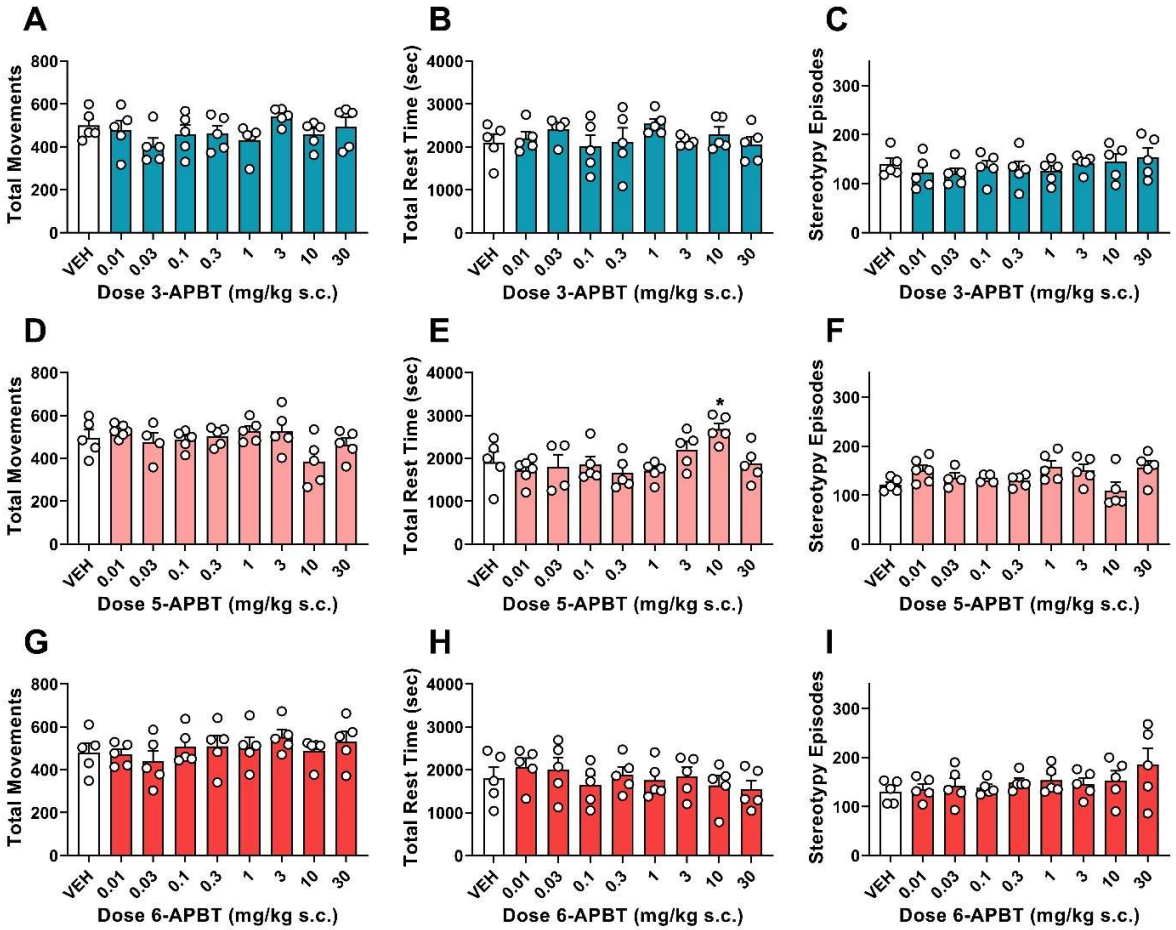
## Supplementary Figure S3



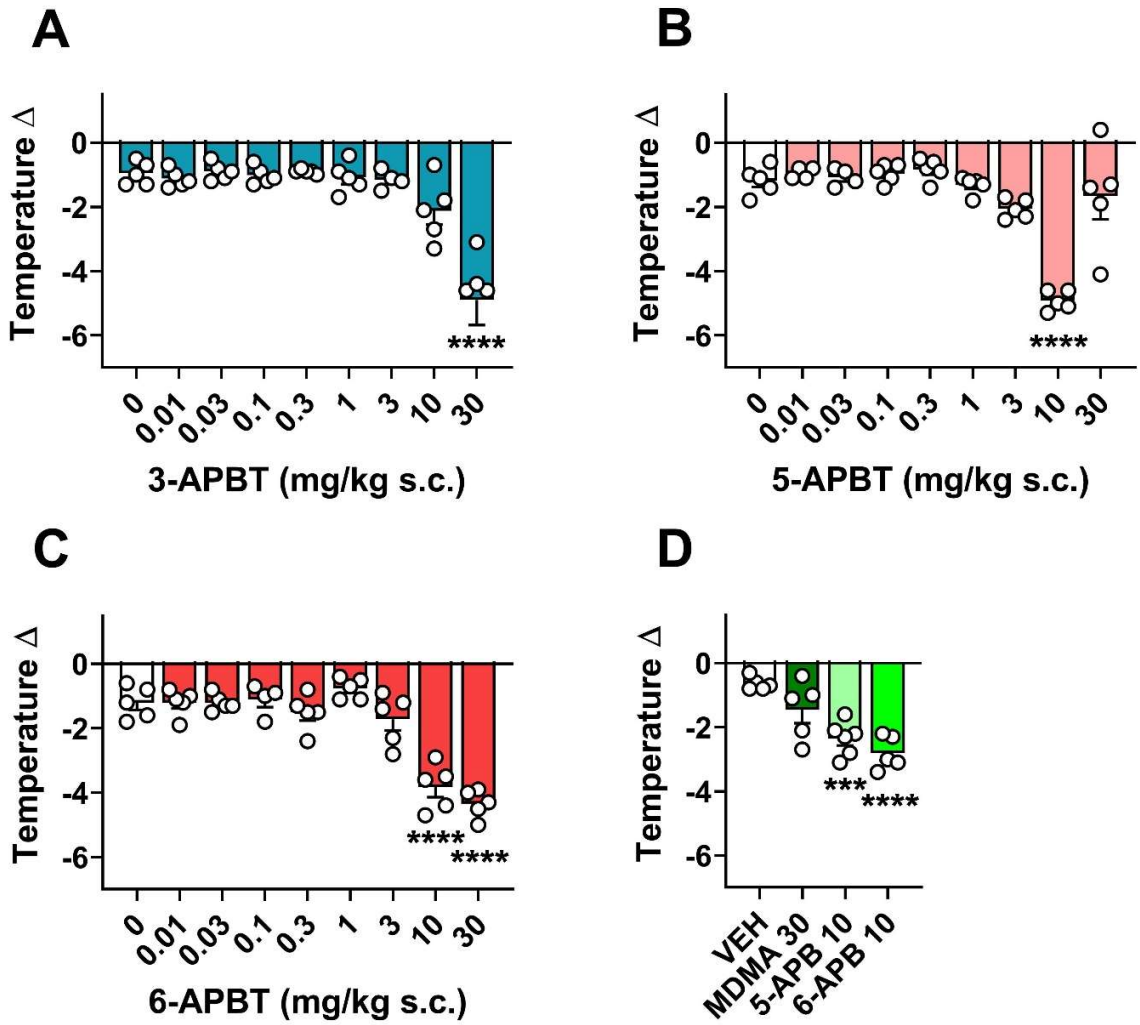
## Supplementary Figure S4



**Supplementary Figure S5**



**Supplementary Figure S6**



**Supplementary Table S1**

## HEK293 cells

| | GAT1 $IC_{50}$ [µM] (95% CI) |
|---|---|
| **5-APBT** | >100000 |
| **6-APBT** | >100000 |
| **7-APBT** | >100000 |
| **Tiagabine** | 372.6 (328.5–422.5) |

**Table S1: Inhibition of GAT in HEK293 cells**. $IC_{50}$ values for inhibition of transporter-mediated uptake by selected APBT isomers compared to the selective GAT inhibitor tiagabine in HEK293 cells expressing human GAT. Values are given as mean and 95% confidence intervals obtained from nonlinear curve fits obtained from 3 independent experiments, performed in triplicate (data shown in Fig. S1).

**Supplementary Table S2**

| Name | SMILES code |
|---|---|
| 2-APBT | CC([NH3+])Cc2cc1ccccc1s2 |
| 3-APBT | CC([NH3+])Cc1csc2ccccc12 |
| 4-APBT | CC([NH3+])Cc1cccc2sccc12 |
| 5-APBT | C[C@H]([NH3+])Cc1ccc2sccc2c1 |
| 6-APBT | C[C@H]([NH3+])Cc2ccc1ccsc1c2 |
| 7-APBT | CC([NH3+])Cc1cccc2ccsc12 |

**Table S2: Names and SMILES codes of docked compounds**