Supporting Information

(Supplemental materials may be accessed free of charge online at http://pubs.acs.org)

Noncompetitive Inhibition of Indolethylamine *N*-Methyltransferase by *N,N*-Dimethyltryptamine (DMT) and *N,N*-Dimethylaminopropyltryptamine

Uyen B. Chu

Sevahn K. Vorperian

Kenneth Satyshur

Kelsey Eickstaedt

Nicholas Cozzi

Timur Mavlyutov

Abdol R. Hajipour

Arnold E. Ruoho

U.B.C., S.K.V., K.E., T.M., and A.E.R.

Department of Neuroscience, University of Wisconsin School of Medicine and Public Health, 1300 University Avenue, Madison, Wisconsin 53706

S.K.V.

West High School, 30 Ash St., Madison, WI 53726

N.V.C

Department of Cell and Regenerative Biology

University of Wisconsin School of Medicine and Public Health, 1300 University Avenue, Madison, Wisconsin 53706

K.S.

University of Wisconsin Carbone Cancer Center, Small Molecule Screening and Synthesis Facility, Wisconsin Institutes for Medical Research, 1111 Highland Avenue, Madison, WI 53705

A.R.H.

Pharmaceutical Research Laboratory, College of Chemistry, Isfahan University of Technology, Isfahan 84156, Iran

Figure Legend

Figure S1: A representative thin layer chromatography showing the end products of rabINMT methylation reactions. The INMT methylation of tryptamine shows two products corresponding to N-methyl (MMT) and N,N-dimethyltryptamine (DMT) as detected by autoradiography. Cold tryptamine, MMT, and DMT were added to each sample after the reaction was quenched with potassium borate and prior to extraction. Samples were spotted on silica plate and separated using 12 n-Butanol: 5 H_2O: 3 acetic acid solvent system. A control condition was performed in the absence of rabbit lung homogenate. The relative location of each compound on TLC plates was determined separately. Co-migration of cold MMT and DMT with the radioactive products was confirmed and shown by the dotted lines around UV-active spots. MMT, N-methyltryptamine; DMT, N,N-dimethyltryptamine; RLH, Rabbit lung homogenates. Rf values for DMT is 0.39; MMT, 0.48; and Tryptamine, 0.57.

Figure S2: Sequence alignment of human and rabbit INMT. Amino acids highlighted in red are identical between two species and those highlighted in yellow show sequence similarity. The blue box highlights the loop region identified by *in silico* docking of DMT and PDAT. The proposed allosteric N-terminal Helix-loop-Helix region is not within the active site of the enzyme as assessed by the position of SAH in the co crystal structure of hINMT (PDB code 2A14).

Figure S3: Binding mechanism of DMT for rabINMT. **A.** A repeat experiment of the Michaelis-Menten plot for DMT in the absence or presence of cold DMT (100 µM). Concentrations of tryptamine varied from 100 µM to 1000 µM. **B.** Lineweaver-Burk

transformation of the data presented in A. Velocity (V) was calculated by determining the [^{14}C]DMT formed over the time course of 60 min) and [S] represents the tryptamine concentrations. Inset shows a close up image of part B showing the intersections of the two lines. In the absence of inhibitor, the mean Km (from two separate experiments) for DMT is 852.2 ± 61.35 µM and 1618 ± 275.5 µM in the presence of cold DMT. The Km values were determined using the Michaelis-Menten analyses from Graphpad Prism and standard error of the mean was calculated from two separate experiments performed in duplicates.

Figure S4: Binding mechanism of PDAT for rabINMT. **A.** A repeat experiment for the Michaelis-Menten plot for DMT in the absence or presence of 100 µM of PDAT. Concentrations of tryptamine varied from 100 µM to 1000 µM. **B.** Lineweaver-Burk transformations of the data presented in A. Velocity (V) was calculated by determining the ^{14}C-DMT formed over the time course of 60 min) and [S] represents the tryptamine concentrations. Inset shows a close up image of part B showing the intersections of the two lines. In the absence of inhibitor, the mean Km (from two separate experiments) for DMT is 499.6 ± 68.2 µM and 523.4 ± 85.4 µM in the presence of 100 µM PDAT. The Km values were determined using the Michaelis-Menten analyses from Graphpad Prism and standard error of the mean was calculated from two separate experiments performed in duplicates.

Figure S5: Schematic representation of the kinetic mechanisms of binding of DMT (A) and PDAT (B). **A.** The inhibition of INMT by DMT fits a mixed-noncompetitive mechanism model. **B.** The inhibition of INMT by PDAT follows a pure non-competitive model.