SUPPORTING INFORMATION

Active Metabolite of Aeruginascin (4-Hydroxy-*N*,*N*,*N*-trimethyltryptamine): Synthesis, Structure, and Serotonergic Binding Affinity

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Synthetic Procedure and Characterization of 4-AcO-TMT iodide.

250 mg of a commercial sample of 4-acetoxy-*N*,*N*-dimethyltryptammonium (4-AcO-DMT) fumarate (the Indole Shop) was dissolved in 10 mL of methanol in a 50 mL round bottom flask, and 10 mL of iodomethane was then added. The mixture was stirred for 24 hours under an atmosphere of nitrogen. The solvent was removed *in vacuo*. The resulting powder was washed with diethyl ether and filtered to yield 313 mg of yellow powder. This powder was dissolved in 75 mL of acetone. The solution was heated with stirring and reduced in volume to 40 mL. The mixture was cooled in an ice bath, producing a white precipitate. The powder was filtered to yield 142 mg of white powder (53.0 % yield). ^{1}H NMR (400 MHz, D_2O): δ 7.46 (d, *J* = 8.6 Hz, 1 H, Ar*H*), 7.34 (s, 1 H, Ar*H*), 7.26 (t, *J* = 7.8 Hz, 1 H, Ar*H*), 6.95 (d, *J* = 8.3 Hz, 1 H, Ar*H*) 3.63 (t, *J* = 7.9 Hz, 2 H, C*H*$_2$), 3.28 (t, *J* = 8.0 Hz, 2 H, C*H*$_2$), 3.21 (s, 9 H, C*H*$_3$), 2.47 (s, 3 H, C(O)*C*H$_3$); ^{13}C NMR (100 MHz, D_2O): δ 174.32 (*C*=O), 143.59 (Ar*C*), 139.28 (Ar*C*), 125.82 (Ar*C*), 123.01 (Ar*C*), 119.11 (Ar*C*), 112.92 (Ar*C*), 111.13 (Ar*C*), 107.32 (Ar*C*), 67.64 (N*C*H$_2$), 53.73 (N*C*H$_3$), 21.25 (*C*H$_2$), 20.48 (C(O)*C*H$_3$). Elemental analysis calcd. for $C_{15}H_{21}N_2O_2I$: C 46.40, H 5.45, N 7.22; Found: C 46.17, H 5.35, N 7.11.

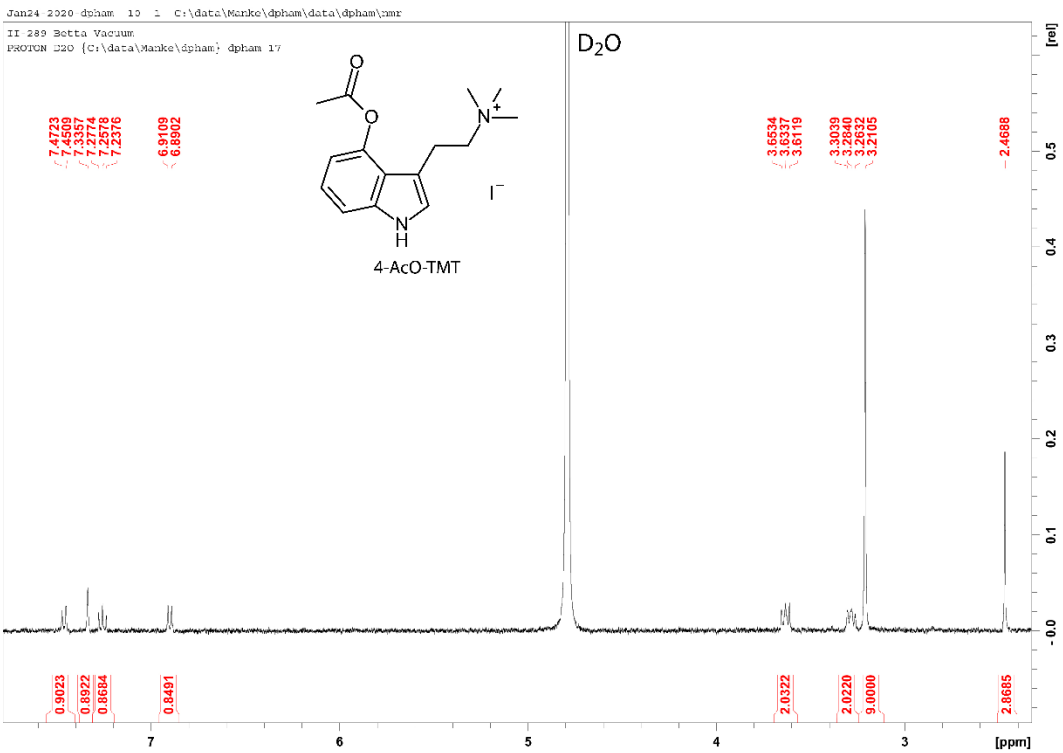


Figure S1. ^{1}H NMR of 4-AcO-TMT iodide in D_2O

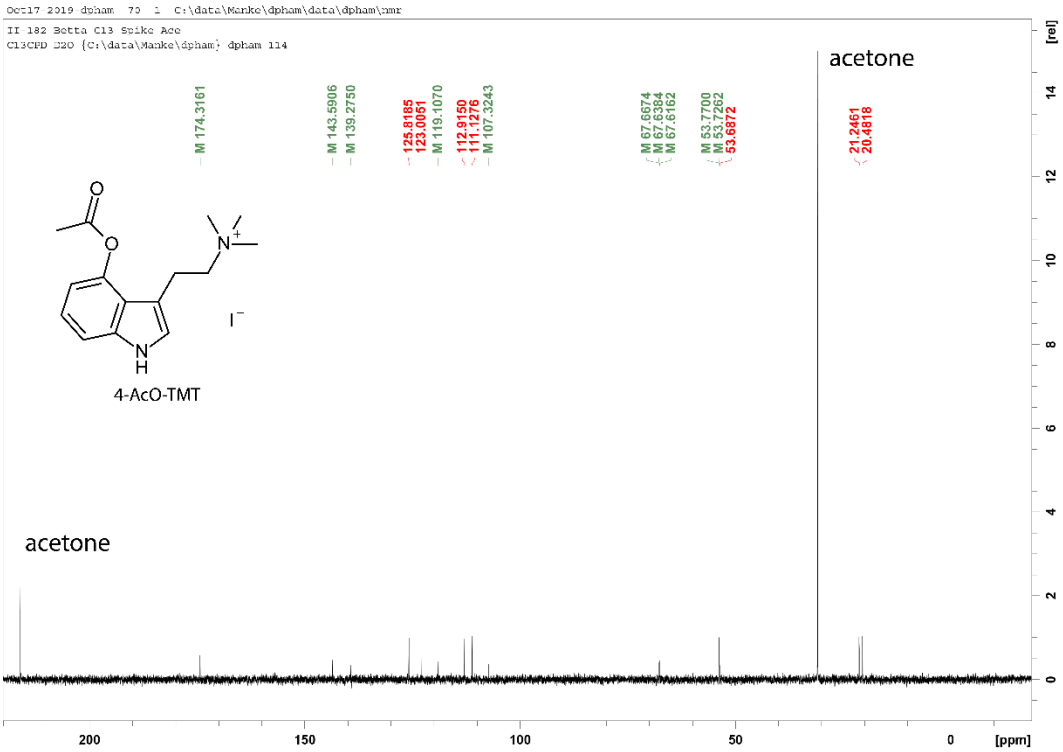
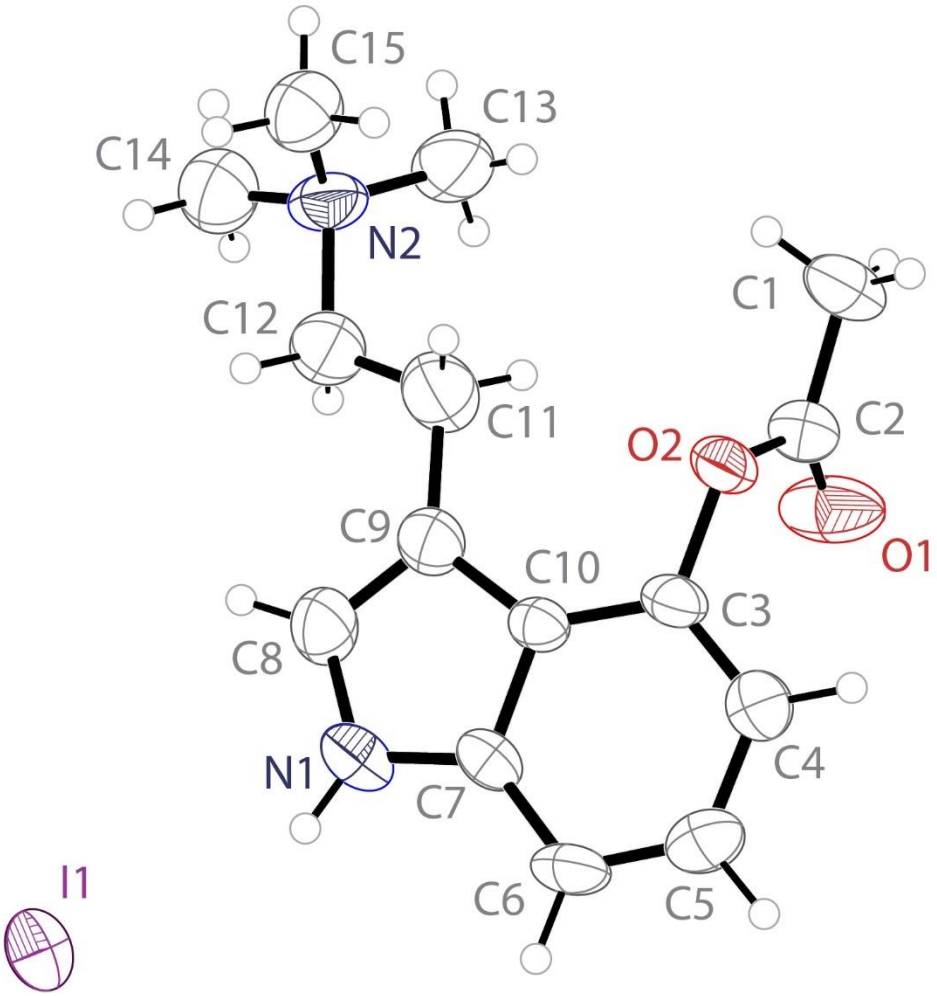


Figure S2. ^{13}C NMR of 4-AcO-TMT iodide in D_2O (referenced with an acetone internal standard)

Figure S3. Fully labelled displacement ellipsoid representation (50%) of the asymmetric unit of 4-AcO-TMT iodide.



X-Ray data collection and refinement details for 4-AcO-TMT iodide. Crystals suitable for X-ray diffraction studies were grown from the slow evaporation of an aqueous solution. All operations were performed on a Bruker D8 Venture CMOS diffractometer, using Mo Kα radiation with a TRIUMPH monochromator at a temperature of 298 K. Data collection was carried out using the Bruker *APEX3* software. Cell refinement and data reduction were performed with the *SAINT* program.[1] The structure solution was done with *SHELXS*[2] and structure refinement was performed with *SHELXL*.[3] Further refinement and molecular graphics were generated using the *OLEX2* and *Mercury CSD* software.[4,5]

All non-hydrogen atoms were refined anisotropically (*XL*) by full matrix least squares on F^2. Hydrogen atom H1 was found from a Fourier difference map, and refined with a fixed distance of 0.87 Å. Isotropic displacement parameters were set to 1.20 times U_{eq} of the parent N atoms. The remaining hydrogen atoms were placed in calculated positions and then refined with a riding model with C–H lengths of 0.93 Å (sp^2), 0.96 Å (CH_3) and 0.97 Å (CH_2) with isotropic displacement parameters set to 1.20 (sp^2 *and* CH_2) and 1.50 (CH_3) times U_{eq} of the parent C atom. Further details are in the tables below, and the final CIF is available as supporting material.

Table S1. Crystal data and structure refinement for 4-AcO-TMT iodide.

Identification code	UMD1786B_b
Empirical formula	$C_{15}H_{21}IN_2O_2$
Formula weight	388.24
Temperature/K	299.05
Crystal system	monoclinic
Space group	$P2_1$
a/Å	7.8459(9)
b/Å	9.8098(12)
c/Å	11.0823(12)
α/°	90
β/°	101.069(3)
γ/°	90
Volume/Å^3	837.10(17)
Z	2
ρ_{calc}g/cm^3	1.540
μ/mm^{-1}	1.916
F(000)	388.0
Crystal size/mm^3	0.2 × 0.1 × 0.05
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.866 to 50.852
Index ranges	$-9 \le h \le 9$, $-11 \le k \le 11$, $-13 \le l \le 13$
Reflections collected	18328
Independent reflections	3076 [R_{int} = 0.0424, R_{sigma} = 0.0298]
Data/restraints/parameters	3076/2/189
Goodness-of-fit on F^2	1.064
Final R indexes [$I \ge 2\sigma(I)$]	R_1 = 0.0300, wR_2 = 0.0548
Final R indexes [all data]	R_1 = 0.0396, wR_2 = 0.0583
Largest diff. peak/hole / e Å^{-3}	0.51/–0.40
Flack parameter	–0.001(11)

Table S2. Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for 4-AcO-TMT iodide. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{IJ} tensor.

Atom	***x***	***y***	***z***	**U(eq)**
I1	–2180.9(5)	7190.0(6)	9177.6(3)	57.25(17)
O1	4938(6)	5097(6)	3837(6)	79.2(17)
O2	3333(6)	3405(4)	4358(4)	50.9(11)
N1	216(7)	5704(6)	6865(6)	52.8(14)
N2	6574(10)	2830(9)	8947(9)	59(2)
C1	6074(10)	2824(8)	4002(8)	73(2)
C2	4786(8)	3921(8)	4042(6)	50.7(17)
C3	1979(8)	4315(6)	4448(6)	40.9(14)
C4	886(9)	4737(7)	3399(6)	55.3(18)
C5	-528(9)	5571(8)	3499(7)	62(2)
C6	-881(8)	5947(7)	4607(7)	53.2(18)
C7	238(7)	5491(6)	5652(6)	39.1(14)
C8	1609(9)	5036(8)	7559(6)	58.8(19)
C9	2567(8)	4401(7)	6830(6)	49.0(16)
C10	1705(7)	4677(6)	5600(5)	35.2(13)
C11	4187(10)	3537(10)	7194(7)	72(2)
C12	4943(9)	3690(8)	8493(7)	58.8(19)
C13	7920(10)	3084(10)	8194(8)	82(3)
C14	7185(11)	3283(9)	10237(8)	74(2)
C15	6173(14)	1350(11)	8941(10)	64(3)

Table S3. Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for 4-AcO-TMT iodide. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
I1	53.7(2)	65.2(3)	57.1(2)	–7.3(4)	21.38(16)	–6.5(4)
O1	46(3)	56(3)	138(5)	26(3)	23(3)	4(2)
O2	56(3)	41(2)	64(3)	9(2)	30(2)	16(2)
N1	47(3)	54(4)	60(4)	–11(3)	18(3)	8(3)
N2	40(4)	64(5)	74(5)	25(4)	13(3)	14(3)
C1	66(5)	74(5)	88(6)	6(4)	34(4)	29(4)
C2	42(4)	57(5)	53(4)	3(3)	9(3)	10(3)
C3	40(3)	31(3)	55(4)	3(3)	18(3)	8(3)
C4	63(4)	57(5)	46(4)	–4(4)	12(3)	10(4)
C5	53(4)	73(5)	54(5)	8(4)	–3(4)	18(4)
C6	37(4)	54(4)	68(5)	4(4)	8(3)	19(3)
C7	31(3)	39(4)	50(4)	–8(3)	13(3)	–2(3)
C8	53(4)	81(5)	42(4)	–5(4)	6(3)	4(4)
C9	37(3)	61(4)	49(4)	3(3)	10(3)	2(3)
C10	31(3)	32(3)	44(4)	2(3)	11(3)	1(2)
C11	56(5)	102(7)	57(5)	–12(5)	6(4)	13(4)
C12	46(4)	69(5)	62(5)	4(4)	11(3)	2(4)
C13	46(4)	114(7)	89(6)	43(5)	22(4)	21(4)
C14	69(5)	87(6)	66(5)	2(5)	13(4)	4(5)
C15	69(7)	63(7)	60(6)	0(5)	9(5)	–2(5)

Table S4. Bond Lengths for 4-AcO-TMT iodide.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O1	C2	1.186(8)	C3	C4	1.370(9)
O2	C2	1.353(8)	C3	C10	1.381(8)
O2	C3	1.405(7)	C4	C5	1.400(9)
N1	C7	1.364(8)	C5	C6	1.360(10)
N1	C8	1.375(9)	C6	C7	1.386(9)
N2	C12	1.535(10)	C7	C10	1.411(8)
N2	C13	1.487(11)	C8	C9	1.355(9)
N2	C14	1.487(12)	C9	C10	1.426(8)
N2	C15	1.485(9)	C9	C11	1.516(10)
C1	C2	1.483(9)	C11	C12	1.456(10)

Table S5. Bond Angles for 4-AcO-TMT iodide.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C2	O2	C3	117.8(5)	C6	C5	C4	122.1(7)
C7	N1	C8	108.6(5)	C5	C6	C7	117.4(6)
C13	N2	C12	111.2(7)	N1	C7	C6	130.4(6)
C14	N2	C12	104.2(7)	N1	C7	C10	106.9(5)
C14	N2	C13	110.9(7)	C6	C7	C10	122.7(6)
C15	N2	C12	111.9(9)	C9	C8	N1	111.0(6)
C15	N2	C13	109.5(9)	C8	C9	C10	105.5(6)
C15	N2	C14	109.0(9)	C8	C9	C11	129.1(6)
O1	C2	O2	122.3(6)	C10	C9	C11	125.4(6)
O1	C2	C1	127.3(7)	C3	C10	C7	117.1(5)
O2	C2	C1	110.4(6)	C3	C10	C9	134.9(5)
C4	C3	O2	119.4(5)	C7	C10	C9	108.0(5)
C4	C3	C10	121.6(5)	C12	C11	C9	111.5(6)
C10	C3	O2	118.9(5)	C11	C12	N2	115.0(7)
C3	C4	C5	119.1(6)				

Table S6. Hydrogen Bonds for 4-AcO-TMT iodide.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
N1	H1	I	0.867(14)	2.98(4)	3.753(5)	150(6)
C4	H4	I[1]	0.93	3.23	4.068(7)	150.5
C6	H6	O2[2]	0.93	2.57	3.417(8)	152.4
C12	H12A	I[3]	0.97	3.02	3.959(8)	163.7
C12	H12B	I[4]	0.97	3.20	4.098(8)	154.7
C13	H13A	I[5]	0.96	3.18	4.085(8)	157.7
C14	H14B	I[4]	0.96	3.16	4.067(9)	157.6
C14	H14C	I[5]	0.96	3.13	3.995(8)	151.2
C15	H15B	O1[6]	0.96	2.33	3.274(13)	167.7
C15	H15C	I[3]	0.96	3.30	4.165(11)	151.2

[1]–x, –½ + y, 1 – z; [2]–x, ½ + y, 1 – z; [3]–x, –½ + y, 2 – z; [4]1 + x, y, z; [5]1 – x, –½ + y, 2 – z; [6]1 – x, –½ + y, 1 – z

Table S7. Torsion Angles for 4-AcO-TMT iodide.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
O2	C3	C4	C5	175.8(6)	C6	C7	C10	C3	–1.3(9)
O2	C3	C10	C7	–174.3(5)	C6	C7	C10	C9	179.6(6)
O2	C3	C10	C9	4.4(10)	C7	N1	C8	C9	0.9(8)
N1	C7	C10	C3	179.3(5)	C8	N1	C7	C6	180.0(7)
N1	C7	C10	C9	0.2(7)	C8	N1	C7	C10	–0.7(7)
N1	C8	C9	C10	–0.7(8)	C8	C9	C10	C3	–178.5(7)
N1	C8	C9	C11	–179.1(7)	C8	C9	C10	C7	0.3(7)
C2	O2	C3	C4	79.5(8)	C8	C9	C11	C12	–14.0(12)
C2	O2	C3	C10	–105.4(6)	C9	C11	C12	N2	179.0(7)
C3	O2	C2	O1	–0.5(10)	C10	C3	C4	C5	0.9(10)
C3	O2	C2	C1	179.5(6)	C10	C9	C11	C12	167.9(7)
C3	C4	C5	C6	–1.8(11)	C11	C9	C10	C3	0.0(12)
C4	C3	C10	C7	0.6(9)	C11	C9	C10	C7	178.8(7)
C4	C3	C10	C9	179.3(7)	C13	N2	C12	C11	54.4(10)
C4	C5	C6	C7	1.1(11)	C14	N2	C12	C11	173.9(7)
C5	C6	C7	N1	179.7(7)	C15	N2	C12	C11	–68.4(12)
C5	C6	C7	C10	0.5(10)					

Table S8. Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for 4-AcO-TMT iodide.

Atom	***x***	***y***	***z***	**U(eq)**
H1	–590(70)	6170(60)	7120(60)	63
H1A	7168.02	3221.59	3929.11	110
H1B	5673.21	2245.51	3306.57	110
H1C	6215.02	2295.23	4743.07	110
H4	1081.3	4473.93	2630.97	66
H5	–1249.12	5877.73	2784.92	74
H6	–1836.61	6488.32	4660.69	64
H8	1859.68	5021.26	8413.44	71
H11A	3895.74	2587.12	7020.57	87
H11B	5034.39	3803.19	6705.99	87
H12A	4071.83	3449.01	8969.96	71
H12B	5233.97	4642.72	8653.65	71
H13A	9013.03	2723.65	8611.09	123
H13B	8029.2	4047.21	8075.92	123
H13C	7586.87	2645.41	7410.08	123
H14A	6296.95	3110.4	10705.3	111
H14B	7434.3	4241.97	10248.94	111
H14C	8217.46	2791.15	10591.33	111
H15A	7219.35	848.52	9240.09	97
H15B	5691.26	1065.29	8117.53	97
H15C	5349.34	1180.65	9461.79	97

Synthetic Procedure and Characterization of 4-HO-TMT iodide

95 mg of 4-AcO-TMT iodide was dissolved in 4 mL of deionized water. 20 mL of acetic acid was added to the mixture, and it was refluxed under an atmosphere of nitrogen for 2 days. Solvent was removed *in vacuo* to obtain a green/blue oil. 3 mL of methanol and 20 mL of ethyl acetate were added to the green/blue oil, leaving a green/blue powder that was removed via filtration. Solvent was removed from the filtrate *in vacuo*. The resulting oil was dissolved in 5 mL of ethanol, and 30 mL of pentane was added to generate a precipitate. The resulting powder was isolated via filtration to give 53.1 mg (60 % yield) of an off-white powder. ^{1}H NMR (400 MHz, D_2O): δ 7.19 (s, 1 H, Ar*H*), 7.12-7.07 (m, 2 H, Ar*H*), 6.56 (dd, *J* = 5.9, 2.5 Hz, 1 H, Ar*H*), 3.66-3.62 (m, 2 H, CH_2), 3.40-3.36 (m, 2 H, CH_2), 3.20 (s, 9 H, CH_3); ^{13}C NMR (100 MHz, D_2O): δ 150.62 (Ar*C*), 139.37 (Ar*C*), 123.89 (Ar*C*), 123.86 (Ar*C*), 116.56 (Ar*C*), 108.72 (Ar*C*), 105.23 (Ar*C*), 104.43 (Ar*C*), 68.20 (N*C*H_2), 53.59 (N*C*H_3), 21.20 (*C*H_2). Elemental analysis calcd. For $C_{13}H_{19}N_2OI$: C 45.10, H 5.53, N 8.09; Found: C 44.84, H 5.23, N 7.98.

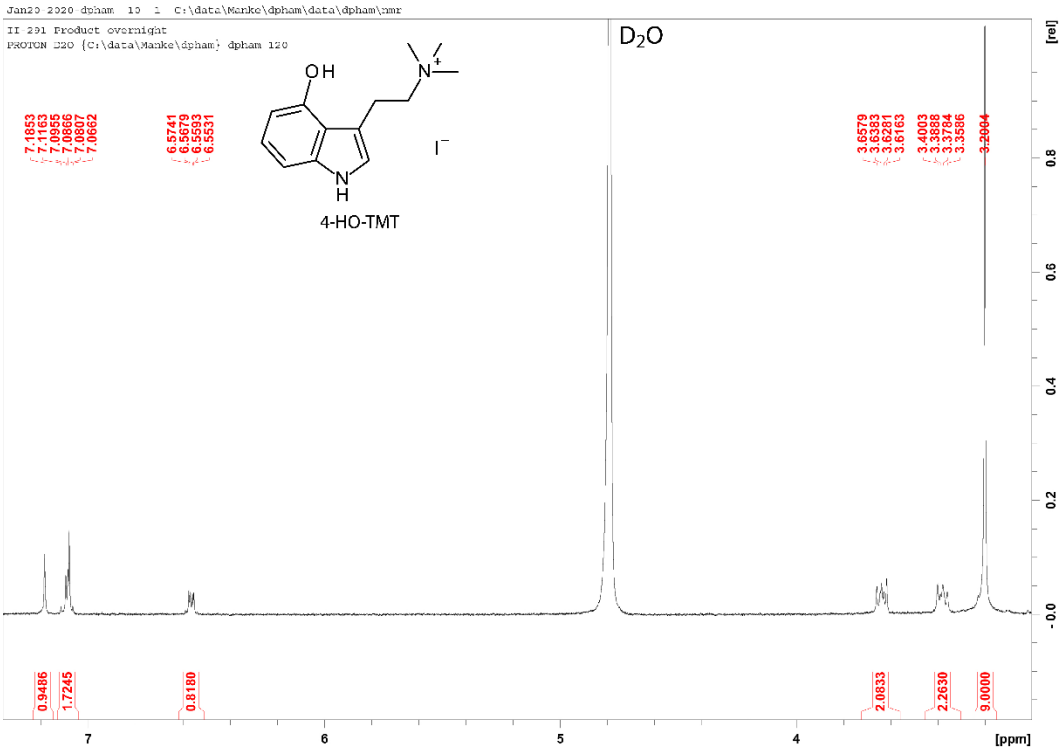


Figure S4. ^{1}H NMR of 4-HO-TMT iodide in D_2O

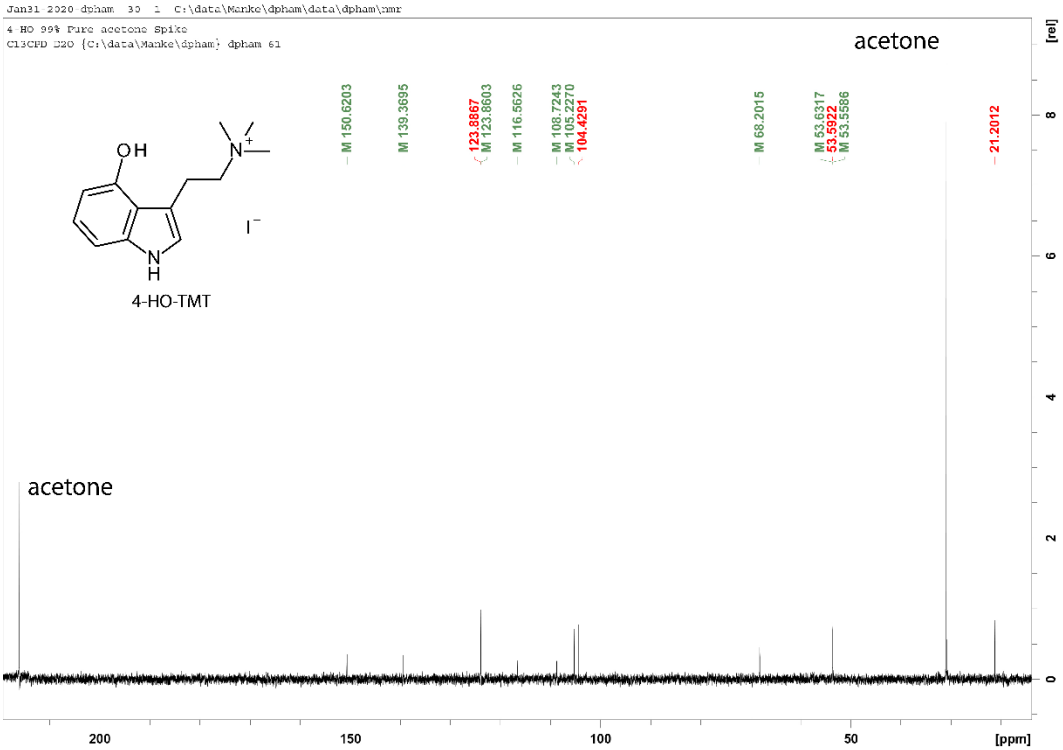
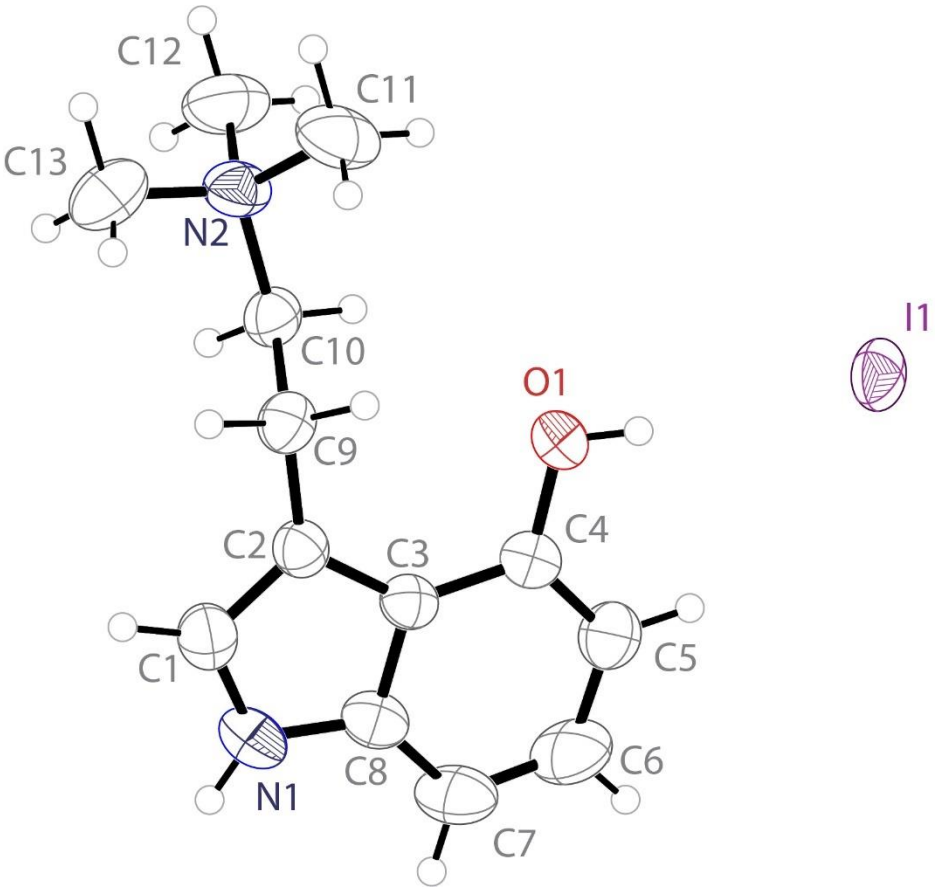


Figure S5. ^{13}C NMR of 4-HO-TMT iodide in D_2O (referenced to an acetone internal standard)

Figure S6. Fully labelled displacement ellipsoid representation (50%) of the asymmetric unit of 4-HO-TMT iodide.



X-Ray data collection and refinement details for 4-HO-TMT iodide. Crystals suitable for X-ray diffraction studies were grown from the slow evaporation of an aqueous solution. All operations were performed on a Bruker D8 Venture CMOS diffractometer, using Mo Kα radiation with a TRIUMPH monochromator at a temperature of 200 K. Data collection was carried out using the Bruker *APEX3* software. Cell refinement and data reduction were performed with the *SAINT* program. The structure solution was done with *SHELXS* and structure refinement was performed with *SHEXL*. Further refinement and molecular graphics were generated using the *OLEX2* and *Mercury CSD* software.

All non-hydrogen atoms were refined anisotropically (*XL*) by full matrix least squares on F^2. Hydrogen atom H1 and H1A were found from a Fourier difference map, and refined with a fixed distance of 0.86 Å and 0.85 Å respectively. Isotropic displacement parameters were set to 1.20 times U_{eq} of the parent N atom, and 1.50 times U_{eq} of the parent O atom. The remaining hydrogen atoms were placed in calculated positions and then refined with a riding model with C–H lengths of 0.95 Å (sp^2), 0.98 Å (CH_2) and 0.99 Å (CH_3) with isotropic displacement parameters set to 1.20 (sp^2 *and* CH_2) and 1.50 (CH_3) times U_{eq} of the parent C atom. Further details are in the tables below, and the final CIF is available as supporting material.

Table S9. Crystal data and structure refinement for 4-HO-TMT iodide.

Identification code	UMD1827_0m_a
Empirical formula	$C_{13}H_{19}IN_2O$
Formula weight	346.20
Temperature/K	200.0
Crystal system	monoclinic
Space group	$P2_1/n$
a/Å	11.3057(9)
b/Å	11.2370(10)
c/Å	12.7785(10)
α/°	90
β/°	113.087(2)
γ/°	90
Volume/Å^3	1493.4(2)
Z	4
ρ_{calc}g/cm^3	1.540
μ/mm^{-1}	2.133
F(000)	688.0
Crystal size/mm^3	0.4 × 0.2 × 0.1
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	6.164 to 52.884
Index ranges	$-13 \leq h \leq 14$, $-14 \leq k \leq 14$, $-15 \leq l \leq 15$
Reflections collected	29269
Independent reflections	3063 [R_{int} = 0.0564, R_{sigma} = 0.0277]
Data/restraints/parameters	3063/2/161
Goodness-of-fit on F^2	1.073
Final R indexes [I ≥ 2σ (I)]	R_1 = 0.0368, wR_2 = 0.0797
Final R indexes [all data]	R_1 = 0.0574, wR_2 = 0.0885
Largest diff. peak/hole / e Å^{-3}	1.05/–0.93

Table S10. Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for 4-HO-TMT iodide. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{IJ} tensor.

Atom	***x***	***y***	***z***	**U(eq)**
I1	2145.7(3)	2150.1(3)	6167.0(2)	52.35(15)
N1	3443(4)	5712(4)	1526(3)	50.9(9)
N2	7618(4)	2762(3)	4737(3)	48.4(9)
O1	3640(3)	3212(3)	4425(2)	45.5(7)
C1	4692(4)	5503(4)	2233(4)	49.9(11)
C2	4734(4)	4775(4)	3098(3)	38.5(9)
C3	3431(4)	4524(4)	2918(3)	36.4(9)
C4	2839(4)	3835(4)	3494(3)	41.0(9)
C5	1520(4)	3802(4)	3097(4)	55.7(12)
C6	763(5)	4448(5)	2110(5)	67.5(15)
C7	1311(5)	5116(5)	1521(4)	58.8(13)
C8	2640(4)	5138(4)	1929(3)	42.5(10)
C9	5948(4)	4404(4)	4058(4)	42.9(10)
C10	6302(4)	3131(4)	3906(3)	42.4(10)
C11	7716(6)	2825(5)	5931(4)	72.1(16)
C12	7832(6)	1499(5)	4459(5)	72.0(16)
C13	8639(5)	3539(6)	4607(5)	72.0(16)

Table S11. Anisotropic Displacement Parameters ($Å^2×10^3$) for 4-HO-TMT iodide. The Anisotropic displacement factor exponent takes the form: $–2π^2[h^2a^{*2}U_{11}+2hka*b*U_{12}+…]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
I1	48.1(2)	77.5(3)	37.08(18)	–2.51(15)	22.74(13)	0.33(16)
N1	59(2)	50(2)	39(2)	10.4(18)	14.9(18)	1.0(19)
N2	55(2)	51(2)	35.2(18)	4.8(17)	14.1(16)	14.3(19)
O1	47.5(17)	51.7(18)	40.0(16)	10.9(14)	20.2(13)	5.3(14)
C1	48(3)	54(3)	50(3)	4(2)	22(2)	–2(2)
C2	42(2)	37(2)	39(2)	–0.3(18)	18.0(18)	0.4(18)
C3	40(2)	37(2)	29.8(19)	–1.8(17)	11.4(16)	1.7(17)
C4	43(2)	39(2)	40(2)	1.4(18)	14.4(19)	1.0(18)
C5	40(2)	58(3)	69(3)	9(3)	21(2)	–6(2)
C6	37(3)	68(4)	81(4)	7(3)	5(2)	–1(2)
C7	49(3)	57(3)	50(3)	6(2)	–2(2)	2(2)
C8	47(2)	40(2)	34(2)	–1.3(18)	8.1(18)	–0.4(19)
C9	38(2)	46(3)	45(2)	–1(2)	16.6(19)	–1.4(19)
C10	39(2)	52(3)	37(2)	0.6(18)	15.8(18)	4.2(19)
C11	101(4)	72(4)	40(3)	10(3)	24(3)	30(3)
C12	82(4)	65(4)	64(3)	6(3)	24(3)	34(3)
C13	36(3)	90(4)	81(4)	3(3)	13(2)	6(3)

Table S12. Bond Lengths for 4-HO-TMT iodide.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
N1	C1	1.365(6)	C2	C9	1.497(6)
N1	C8	1.368(6)	C3	C4	1.406(6)
N2	C10	1.508(5)	C3	C8	1.410(5)
N2	C11	1.488(6)	C4	C5	1.374(6)
N2	C12	1.505(6)	C5	C6	1.416(7)
N2	C13	1.506(7)	C6	C7	1.371(7)
O1	C4	1.371(5)	C7	C8	1.384(6)
C1	C2	1.360(6)	C9	C10	1.519(6)
C2	C3	1.428(6)			

Table S13. Bond Angles for 4-HO-TMT iodide.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C1	N1	C8	109.7(4)	C8	C3	C2	107.4(4)
C11	N2	C10	111.4(4)	O1	C4	C3	116.6(4)
C11	N2	C12	109.4(4)	O1	C4	C5	124.0(4)
C11	N2	C13	109.5(4)	C5	C4	C3	119.4(4)
C12	N2	C10	107.4(4)	C4	C5	C6	120.4(4)
C12	N2	C13	108.7(4)	C7	C6	C5	121.6(5)
C13	N2	C10	110.5(3)	C6	C7	C8	117.4(4)
C2	C1	N1	109.8(4)	N1	C8	C3	106.7(4)
C1	C2	C3	106.4(4)	N1	C8	C7	130.5(4)
C1	C2	C9	124.2(4)	C7	C8	C3	122.8(4)
C3	C2	C9	129.3(4)	C2	C9	C10	111.1(3)
C4	C3	C2	134.3(4)	N2	C10	C9	114.0(3)
C4	C3	C8	118.3(4)				

Table S14. Torsion Angles for 4-HO-TMT iodide.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
N1	C1	C2	C3	–0.4(5)	C4	C3	C8	N1	–178.8(4)
N1	C1	C2	C9	–177.3(4)	C4	C3	C8	C7	1.9(6)
O1	C4	C5	C6	–178.2(4)	C4	C5	C6	C7	0.2(8)
C1	N1	C8	C3	–1.3(5)	C5	C6	C7	C8	0.0(8)
C1	N1	C8	C7	177.9(5)	C6	C7	C8	N1	179.8(5)
C1	C2	C3	C4	179.4(5)	C6	C7	C8	C3	–1.1(7)
C1	C2	C3	C8	–0.4(5)	C8	N1	C1	C2	1.1(5)
C1	C2	C9	C10	–104.1(5)	C8	C3	C4	O1	177.3(4)
C2	C3	C4	O1	–2.5(7)	C8	C3	C4	C5	–1.6(6)
C2	C3	C4	C5	178.6(5)	C9	C2	C3	C4	–3.9(8)
C2	C3	C8	N1	1.1(5)	C9	C2	C3	C8	176.2(4)
C2	C3	C8	C7	–178.3(4)	C11	N2	C10	C9	61.5(5)
C2	C9	C10	N2	170.7(3)	C12	N2	C10	C9	–178.8(4)
C3	C2	C9	C10	79.8(5)	C13	N2	C10	C9	–60.4(5)
C3	C4	C5	C6	0.6(7)					

Table S15. Hydrogen Atom Coordinates (Å×10⁴) and Isotropic Displacement Parameters (Å²×10³) for 4-HO-TMT iodide.

Atom	***x***	***y***	***z***	**U(eq)**
H1	3200(40)	6110(40)	900(20)	61
H1A	3240(50)	3020(40)	4830(40)	68
H1B	5421.37	5819.48	2136.18	60
H5	1114.54	3343.98	3486.51	67
H6	–147.54	4417.06	1848.53	81
H7	798.29	5547.87	858.59	71
H9A	5833.64	4474.42	4784.75	51
H9B	6655.81	4942.21	4092.89	51
H10A	5654.76	2589.56	3992.38	51
H10B	6262.69	3034.95	3123.35	51
H11A	7577.64	3647.19	6112.79	108
H11B	7062.84	2308.57	6019.11	108
H11C	8572.74	2560.96	6448.04	108
H12A	7767.38	1456.46	3672.79	108
H12B	8687.99	1235.46	4976.68	108
H12C	7178.1	983.07	4547.74	108
H13A	8574.28	3497.44	3820.28	108
H13B	8515.45	4363.34	4793.01	108
H13C	9489.53	3260.9	5122.83	108

Description of Cellular Assays

Cellular assays were performed by Eurofins CEREP SA, Celle-Lévescault, France. All receptors were separately expressed in HEK-293 cells. Cell membrane homogenates (30 µg protein) are incubated for 60 min (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}) or 120 min (5-HT_3) at 22°C with radiolabeled ligand in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 10 µM pargyline and 0.1% ascorbic acid. For 5-HT3, the buffer contained 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, and 1mM EDTA.

Ligands used for each receptor were:

5-HT_{1A}: [^{3}H] 8-OH-DPAT

5-HT_{2A}: [^{125}I] (±)DOI

5-HT_{2B}: [^{125}I] (±)DOI

5-HT_3: [^{3}H] BRL 43694

Nonspecific binding was determined in the presence of 1 µM unlabeled ligand listed above. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters were dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The results are expressed as a percent inhibition of the control radioligand specific binding.

The IC_{50} values and Hill coefficients (nH) were determined by non-linear regression analysis of the competition curves generated with mean values of duplicate data points, using Hill equation curve fitting $Y=D+[(A-D) / (1+(C/C_{50})^{nH})]$ where Y = specific binding, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, $C_{50} = IC_{50}$, and nH = slope factor.

Analysis was performed using software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot® 4.0 for Windows® (© 1997 by SPSS Inc.). The inhibition constants (K_i) were calculated using the Cheng Prusoff equation: $K_i = IC_{50} (1+L/K_D)$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor. A scatchard plot is used to determine the K_D.

In Vitro Pharmacology: Binding Assays

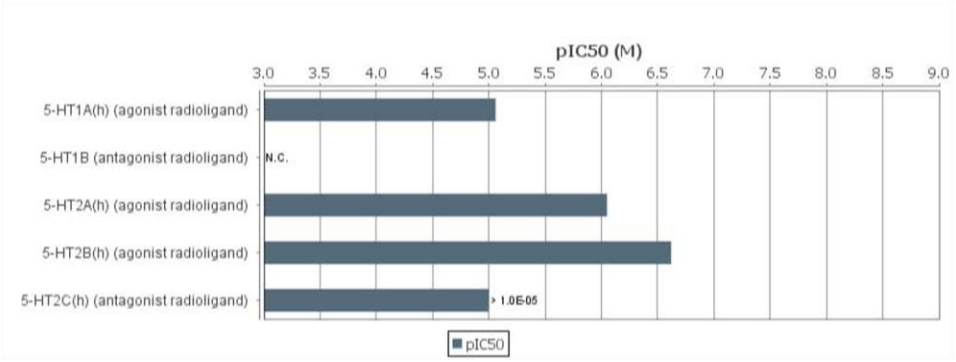


Figure S7. Histogram for 4-HO-TMT iodide.

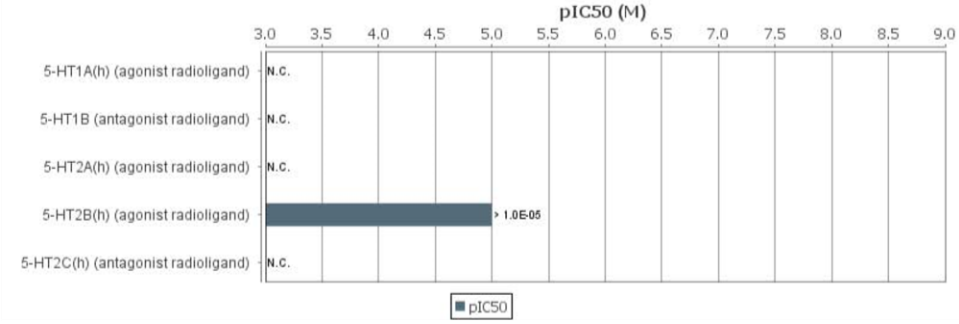


Figure S8. Histogram for 4-AcO-TMT iodide.

Compound I.D.	Client Compound I.D.	IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	2nd	Mean	Flags 1st	2nd
5-HT_{1A}*(h)* (agonist radioligand)										
100051748-1	CT2001PR01	N.C.	n/a	n/a	3.0E-09 M	-11.2	15.9	2.4		
					1.0E-08 M	-19.8	33.4	6.8		
					3.0E-08 M	-27.6	22.7	-2.5		
					1.0E-07 M	-13.3	-10.9	-12.1		
					3.0E-07 M	1.1	20.3	10.7		
					1.0E-06 M	-21.2	15.1	-3.0		
					3.0E-06 M	15.6	-18.2	-1.3		
					1.0E-05 M	-0.1	20.1	10.0		

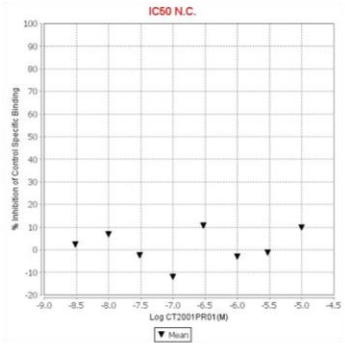


Figure S9. 4-AcO-TMT iodide on 5-HT_{1A} (agonist radioligand)

100051748-2	CT2001AM01	8.8E-06 M	4.4E-06 M	1.8	3.0E-09 M	14.8	-10.5	2.2		
					1.0E-08 M	-6.6	-11.1	-8.9		
					3.0E-08 M	19.5	-13.6	2.9		
					1.0E-07 M	-5.2	35.8	-5.2		{}
					3.0E-07 M	-11.0	-0.7	-5.9		
					1.0E-06 M	-30.1	-4.2	-17.2		
					3.0E-06 M	22.5	3.9	13.2		
					1.0E-05 M	59.3	45.7	52.5		

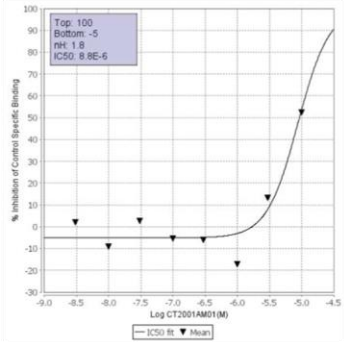


Figure S10. 4-HO-TMT iodide on 5-HT_{1A} (agonist radioligand)

Compound I.D.	Client Compound I.D.	IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	2nd	Mean	Flags 1st	2nd
5-HT_{1B} (antagonist radioligand)										
100051748-1	CT2001PR01	N.C.	n/a	n/a	3.0E-09 M	-7.3	-11.1	-9.2		
					1.0E-08 M	-9.2	1.5	-3.9		
					3.0E-08 M	6.7	-11.3	-2.3		
					1.0E-07 M	0.4	5.3	2.8		
					3.0E-07 M	9.5	10.5	10.0		
					1.0E-06 M	-4.2	6.9	1.3		
					3.0E-06 M	9.1	3.0	6.0		
					1.0E-05 M	-1.8	9.3	3.8		

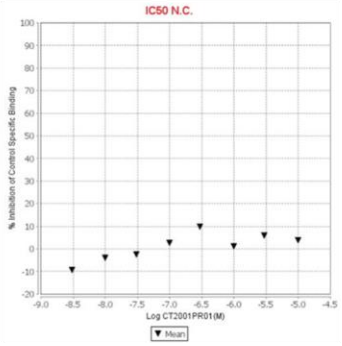


Figure S11. 4-AcO-TMT iodide on 5-HT_{1B} (antagonist radioligand)

100051748-2	CT2001AM01	N.C.	n/a	n/a	3.0E-09 M	0.6	-3.7	-1.5		
					1.0E-08 M	11.8	-0.6	5.6		
					3.0E-08 M	6.1	-11.0	-2.4		
					1.0E-07 M	2.0	12.8	7.4		
					3.0E-07 M	0.4	-4.5	-2.0		
					1.0E-06 M	12.3	-6.1	3.1		
					3.0E-06 M	-3.8	-3.7	-3.7		
					1.0E-05 M	22.4	19.6	21.0		

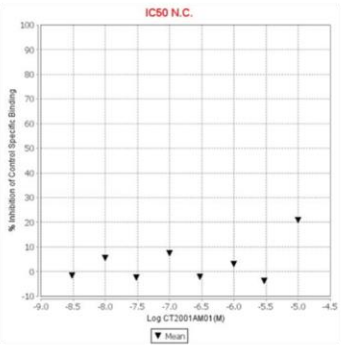


Figure S12. 4-HO-TMT iodide on 5-HT_{1B} (antagonist radioligand)

Compound I.D.	Client Compound I.D.	IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	2nd	Mean	Flags 1st	2nd
5-HT_{2A}*(h)* (agonist radioligand)										
100051748-1	CT2001PR01	N.C.	n/a	n/a	3.0E-09 M	-15.3	-7.7	-11.5		
					1.0E-08 M	-0.8	-11.3	-6.1		
					3.0E-08 M	-7.0	-3.6	-5.3		
					1.0E-07 M	0.2	-26.6	-13.2		
					3.0E-07 M	7.0	2.3	4.7		
					1.0E-06 M	-7.4	1.8	-2.8		
					3.0E-06 M	3.5	-3.5	0.0		
					1.0E-05 M	16.3	3.7	10.0		

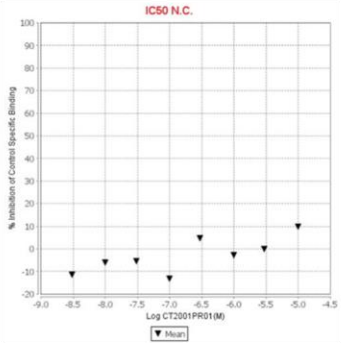


Figure S13. 4-AcO-TMT iodide on 5-HT_{2A} (agonist radioligand)

100051748-2	CT2001AM01	9.0E-07 M	6.7E-07 M	0.6	3.0E-09 M	-10.3	-12.0	-11.2		
					1.0E-08 M	-2.7	-10.3	-6.5		
					3.0E-08 M	13.7	-5.9	3.9		
					1.0E-07 M	17.4	19.4	18.4		
					3.0E-07 M	18.4	15.4	16.9		
					1.0E-06 M	45.8	49.4	47.6		
					3.0E-06 M	65.1	59.1	62.1		
					1.0E-05 M	79.0	79.1	79.0		

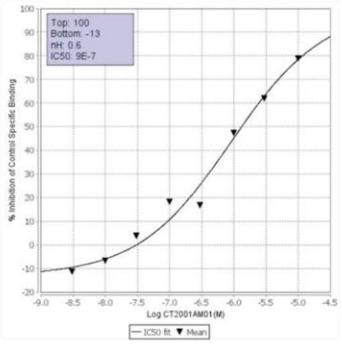


Figure S14. 4-HO-TMT iodide on 5-HT_{2A} (agonist radioligand)

Compound I.D.	Client Compound I.D.	IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	2nd	Mean	Flags 1st	2nd
5-HT_{2B}*(h)* (agonist radioligand)										
100051748-1	CT2001PR01	> 1.0E-05	n/a	n/a	3.0E-09 M	8.6	-1.5	3.6		
					1.0E-08 M	1.6	-13.0	-5.7		
					3.0E-08 M	8.0	1.0	4.5		
					1.0E-07 M	11.0	-5.2	2.9		
					3.0E-07 M	12.3	-1.0	5.6		
					1.0E-06 M	13.7	6.7	10.2		
					3.0E-06 M	28.7	20.0	24.3		
					1.0E-05 M	45.5	47.4	46.5		

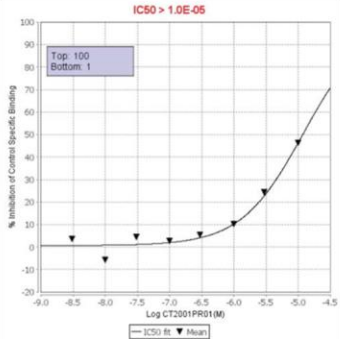


Figure S15. 4-AcO-TMT iodide on 5-HT_{2B} (agonist radioligand)

100051748-2	CT2001AM01	2.4E-07 M	1.2E-07 M	1.1	3.0E-09 M	-3.5	-7.9	-5.7		
					1.0E-08 M	3.6	1.0	2.3		
					3.0E-08 M	17.4	-1.5	7.9		
					1.0E-07 M	26.9	14.2	20.6		
					3.0E-07 M	53.9	50.7	52.3		
					1.0E-06 M	77.9	74.2	76.0		
					3.0E-06 M	89.2	89.1	89.1		
					1.0E-05 M	88.2	91.9	90.1		

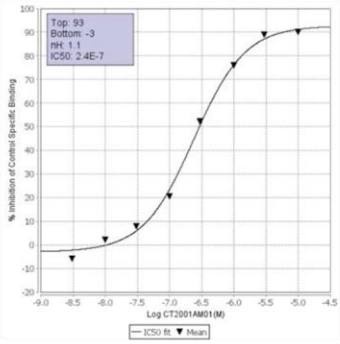


Figure S16. 4-HO-TMT iodide on 5-HT_{2B} (agonist radioligand)

Compound I.D.	Client Compound I.D.	IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	2nd	Mean	Flags 1st	2nd
5-HT_{2C}*(h)* (antagonist radioligand)										
100051748-1	CT2001PR01	N.C.	n/a	n/a	3.0E-09 M	-6.0	19.4	6.7		
					1.0E-08 M	4.3	12.5	8.4		
					3.0E-08 M	7.3	4.0	5.6		
					1.0E-07 M	1.7	-0.5	0.6		
					3.0E-07 M	-4.5	-2.9	-3.7		
					1.0E-06 M	1.2	6.9	4.1		
					3.0E-06 M	1.7	7.8	4.8		
					1.0E-05 M	13.7	20.7	17.2		

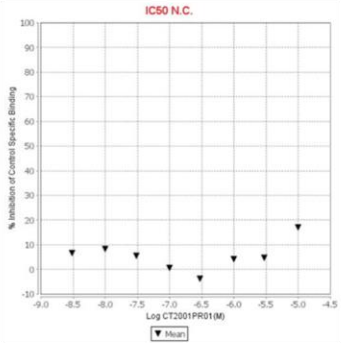


Figure S17. 4-AcO-TMT iodide on 5-HT_3 (antagonist radioligand)

100051748-2	CT2001AM01	> 1.0E-05	n/a	n/a	3.0E-09 M	2.6	26.1	14.4		
					1.0E-08 M	5.1	5.8	5.5		
					3.0E-08 M	9.4	9.4	9.4		
					1.0E-07 M	1.6	5.1	3.3		
					3.0E-07 M	3.5	12.6	8.1		
					1.0E-06 M	19.2	23.0	21.1		
					3.0E-06 M	25.3	25.4	25.3		
					1.0E-05 M	50.5	53.1	51.8		

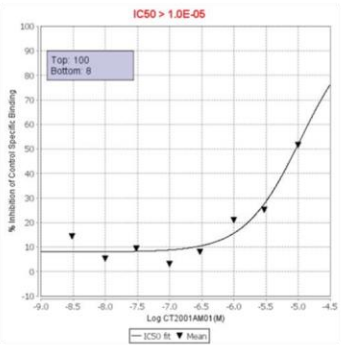


Figure S18. 4-HO-TMT iodide on 5-HT_3 (antagonist radioligand)

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