Supporting Information

Identification of Potent, Selective and Peripherally Restricted Serotonin Receptor 2B Antagonists from a High-Throughput Screen

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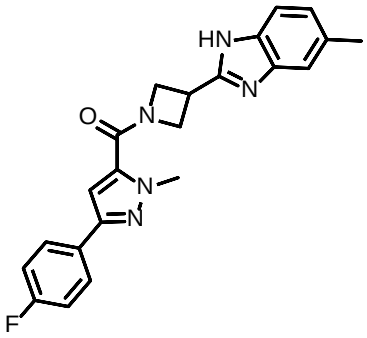
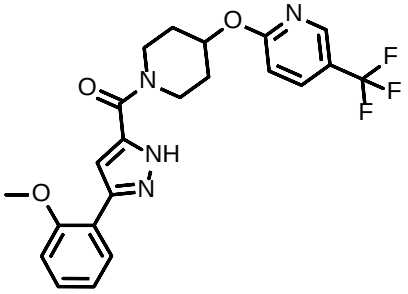
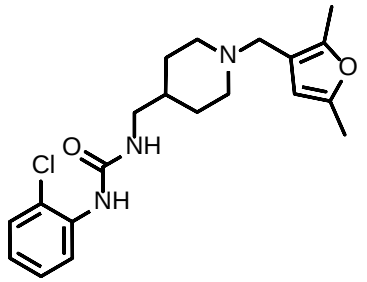
Compound Characterization



VU0530244 (5): ^{1}H NMR (400 MHz, DMSO) δ 7.92 – 7.88 (m, 2H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.34 – 7.32 (m, 1H), 7.27 – 7.21 (m, 2H), 7.19 (s, 1H), 7.01 (dd, *J* = 8.3, 1.6 Hz, 1H), 4.84 (t, *J* = 8.9 Hz, 1H), 4.74 (dd, *J* = 8.9, 6.0 Hz, 1H), 4.49 (t, *J* = 9.5 Hz, 1H), 4.33 (dd, *J* = 9.9, 6.0 Hz, 1H), 4.16 (ddd, *J* = 9.1, 6.0, 3.0 Hz, 1H), 4.09 (s, 3H), 2.41 (s, 3H). Retention time: 0.59 min, m/z = 390.2 (M+H).



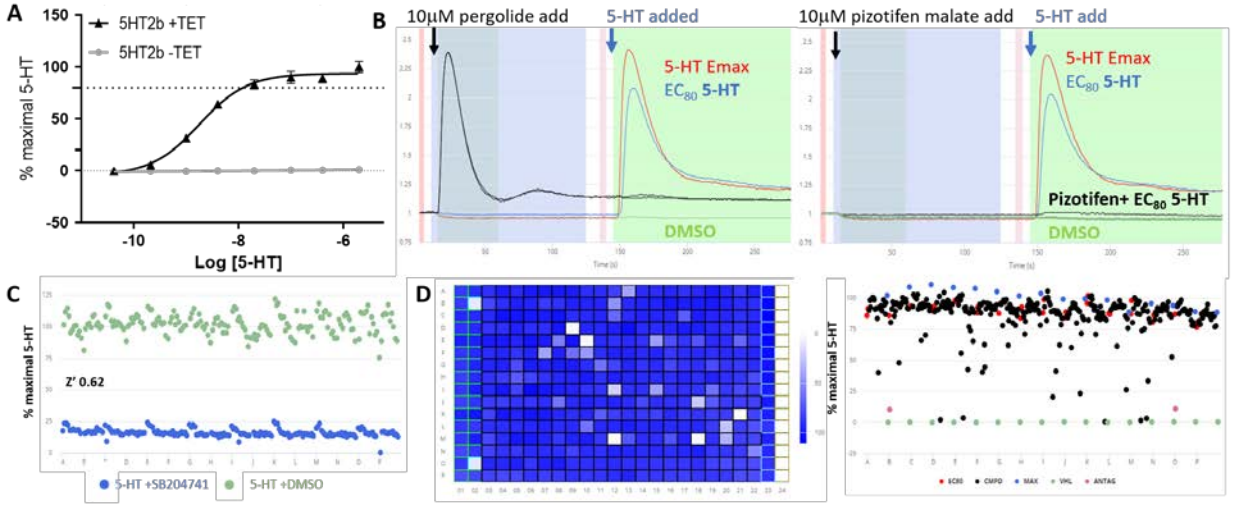
VU0544894 (6): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 – 8.41 (m, 1H), 7.79 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.38 – 7.29 (m, 3H), 6.95 – 6.92 (m, 1H), 6.84 – 6.82 (m, 2H), 5.47 – 5.42 (m, 1H), 4.22 – 4.02 (m, 2H), 3.88 (s, 3H), 3.88 – 3.71 (m, 2H), 2.18 – 2.07 (m, 2H), 1.99 – 1.87 (m, 2H). Retention time: 0.92 min, m/z = 447.2 (M+H).



VU0631019 (7): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.33 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.23 (ddd, *J* = 8.5, 7.4, 1.5 Hz, 1H), 6.96 (ddd, *J* = 8.0, 7.4, 1.6 Hz, 1H), 6.70 (s, 1H), 5.88 (s, 1H), 4.93 – 4.85 (m, 1H), 3.24 (s, 2H), 3.16 (t, *J* = 6.3 Hz, 2H), 2.91 (dt, *J* = 11.9, 3.3 Hz, 2H), 2.21 (s, 3H), 2.19 (s, 3H), 1.94 (td, *J* = 11.7, 2.5 Hz, 2H), 1.71 (d, *J* = 12.9 Hz, 2H), 1.61 – 1.50 (m, 1H), 1.37 – 1.27 (m, 2H). Retention time: 0.55 min, m/z = 376.2 (M+H).

Supplemental Figure 1

Validation Results of 5-HT$_{2B}$ HEK293T cells



A: Increasing 5-HT stimulated calcium response in HEK293T cells expressing 5-HT$_{2B}$ requires induction with tetracycline (+tet). Dose-dependent measurement of 5-HT in triplicate 8 point format provides pre-screening calculations for EC$_{80}$ 5-HT concentrations to be used on each set of screening plates**. B**. FDA drug set (1,181) was used for a pilot screen and shown to be HTS acceptable for hit selections in the calcium assay and 5-HT$_{2B}$ HEK293T cell line. Kinetic measurement of calcium response over time(s) of representative traces of wells showing example agonist pergolide (left), and antagonist pizotifen (right, black) alongside plate controls of maximal concentrations of 5-HT (red), EC$_{80}$ 5-HT (blue) and DMSO (green). Time indicated with arrows for compounds (14s) and 5-HT (147s). Selected compound hits demonstrate different modes of decreasing the EC$_{80}$ 5-HT response, where 10-55s peak of pergolide used to identify putative agonists, compared to the absence of activity in the 10-55s measurement for pizotifen malate. These hit picking criteria were used for our Vanderbilt Discovery Collection screen in search of novel antagonists and NAMs. **C**: Scatterplot of checkerboard alternating 5-HT with DMSO (green, n=176) vs 5-HT with antagonist SB204741 (blue, n=176). Values normalized to Emax 5-HT, Z' of 5-HT +/- SB204741 = 0.62. **D**: Screening layout with test compounds (n=320) in columns 3-22 of 384 well plate shown as values normalized to maximal 5-HT in column 23 (n=16), DMSO in column 24 (n=16). Scatter plot (right) shows values identifying contents as the test compounds in black (CMPD), maximal 5-HT in blue (MAX), antagonist control SB204741 in pink (ANTAG), EC$_{80}$ 5-HT in red and DMSO without 5-HT in green (VHL). Values calculated as the peak response of 5-HT using the maximal values per well (145s-290s) minus minimum values just before 5-HT stimulation (135-140s) and normalized to the plate maximal 5-HT response. The Z' calculation is monitored for every screen plate using the outer wells in columns 1,2,24 of EC$_{80}$ 5-HT v DMSO (Z' = 0.79).