Supplementary Materials

(-)-Stepholidine is a potent pan-dopamine receptor antagonist of both G protein- and β-arrestin-mediated signaling

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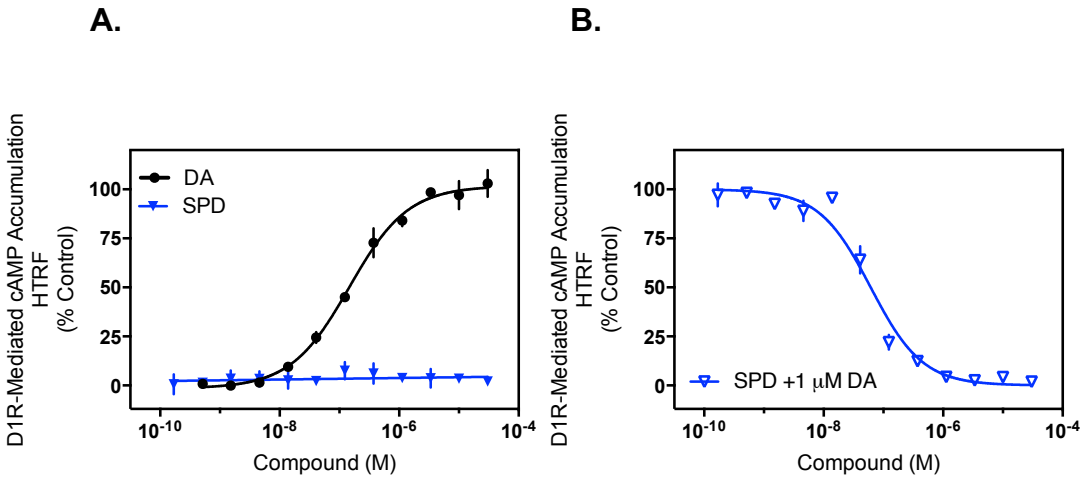
Cisbio HTRF cAMP accumulation assay

In these experiments, cAMP was measured using an HTRF method from Cisbio Assays (Bedford, MA). HEK293 cells stably expressing the human D1R were rapidly thawed at 37°C immediately prior to use and suspended in 9 mL warm Opti-MEM reduced serum media (Gibco 31985-070). Cells were collected at 500 × g for 5 min, the supernatant removed, and the cells re-suspended in 2-4 mL Opti-MEM for cell counting. 10 μL of cell suspension was added to a white bottom 384-well culture plate (Perkin Elmer 6007688) to yield a total of 5,000 cells/well. The plate was centrifuged for 30 seconds at 100 × g and then incubated for 1 hr at 37°C. Dopamine (Sigma H8502) was diluted in Opti-MEM media containing 0.1% ascorbic acid (Sigma A0278) and 2 mM IBMX (Sigma I5879). Compounds were diluted to appropriate concentrations in Opti-MEM media and then added to the plates. Cyclic AMP accumulation was stimulated by the addition of an EC_{80} concentration of dopamine (500 nM) followed by incubation at room temperature for 1 hr. The assay was quenched by the addition of d2-labeled

cAMP and Cryptate-labeled anti-cAMP antibody in lysis buffer according the manufacturer's instructions. Assay plates were centrifuged for 30 sec at 100 × g and then incubated for a minimum of 1 hr at room temperature. The fluorescent signal was measured using a Synergy 4 microplate reader (BioTek Instruments, Winooski, Vermont).



Supplemental Figure 1. HEK293 cells stably expressing the human D1R were assayed for cAMP accumulation using HTRF technology. **A.** Cells were incubated with the indicated concentrations of dopamine (DA) or (-)-stepholidine (SPD) to determine agonist activity. No measurable agonist activity was seen with (-)-stepholidine whereas dopamine gave a robust response (EC_{50} 0.14 ± 0.01 μM, n=3). **B.** Cells were incubated with the indicated concentrations of (-)-stepholidine in the presence of an EC_{80} concentration of dopamine (1 μM) to determine antagonist activity. (-)-Stepholidine potently inhibited the dopamine response exhibiting an IC_{50} value of 58 ± 19 nM (n=4). Data are normalized to the percentage of stimulation seen with the EC_{80} concentration of dopamine and are means ± SEM of three independent experiments, each performed in triplicate.