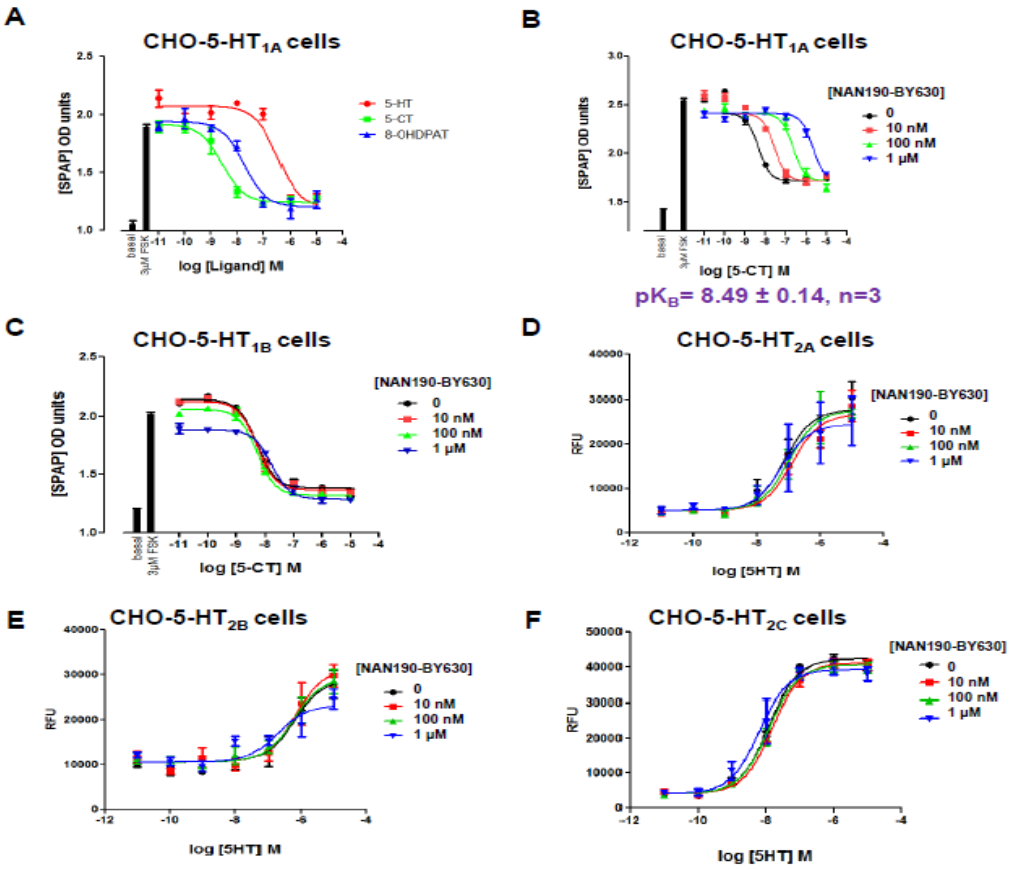
<u>Panel 1: Functional assays in CHO cells stably expressing five different 5-HT receptor subtypes</u> – *The fluorescent ligand CA200992 (NAN190-BY630) is a high-affinity selective antagonist at the 5-HT$_{1A}$ receptor*



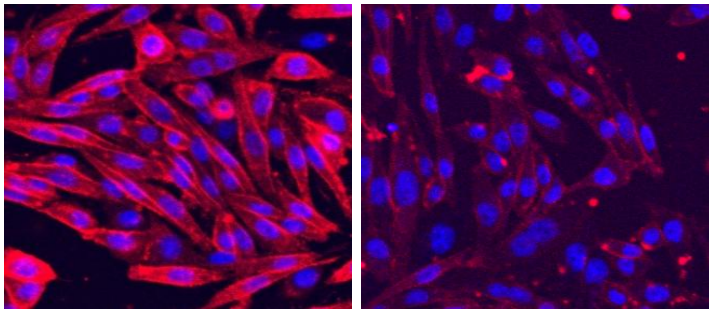
A: Selective and non-selective 5-HT receptor agonists induce decrease in SPAP levels

B: CA200992 right-shifts agonist (5-CT) concentration-response curve in a parallel fashion in 5-HT$_{1A}$ receptor-expressing cells

C, D, E and F: CA200992 has no effect in CHO cells expressing each of the other receptors. Assays in D, E and F quantified intracellular calcium levels (Relative Fluorescence Units, RFU)

<u>Panel 2: Imaging CA200992 (red) binding in CHO-5-HT$_{1A}$ receptor-expressing cells</u> - *CA200992 displays superior photophysical properties*

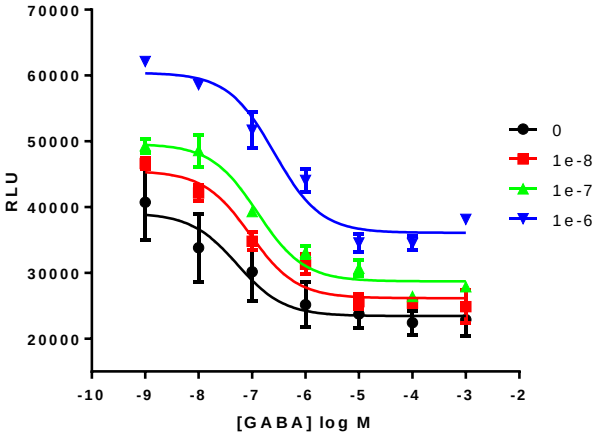
G **H**



G: CA200992 (10 nM) binds intensely to the CHO-5-HT$_{1A}$ receptor on the cell membrane

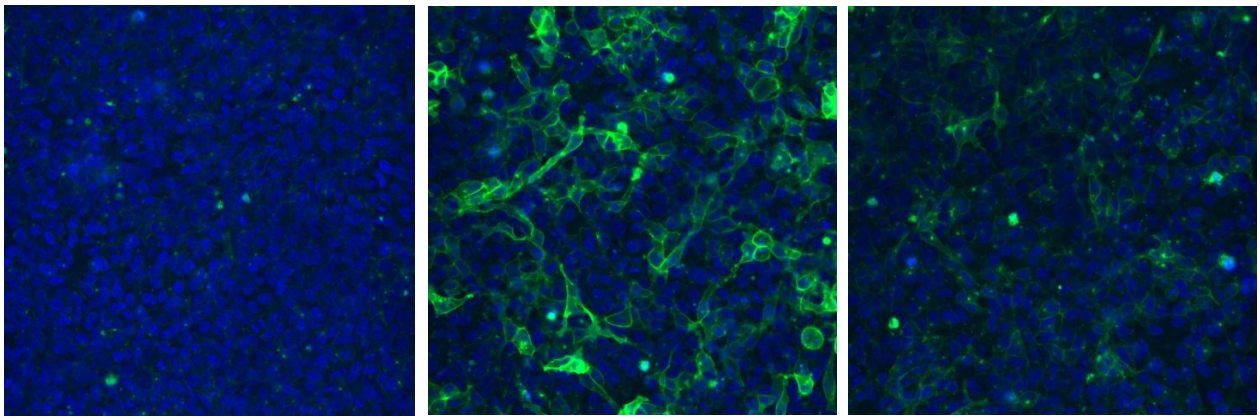
H: Binding of CA200992 is displaced by an unlabelled competitor, NAN-190 (1 µM)

A: <u>Functional assay</u> - *CA200935 antagonises $GABA_B$ receptor-mediated GABA-induced decrease in cAMP levels (Relative Luminescence Units, RLU) in HEK-Glo cells transiently expressing the receptor, in a manner that suggests the receptor is constitutively active*



B-D: <u>Imaging CA200935 (green) binding to the $GABA_B$ receptor</u> – *CA200935 displays excellent imaging properties*

B **C** **D**



B: CA200935 does not bind to the cell membrane in non-$GABA_B$ receptor-expressing (mock-transfected) cells

C: CA200935 (100 nM) binds intensely to the $GABA_B$ receptor in $GABA_B$ receptor-expressing cells

D: CA200935 binding to the $GABA_B$ receptor in $GABA_B$ receptor-expressing cells is displaced by an unlabelled $GABA_B$ receptor antagonist, CGP 54626 (100 nM)