

ACT ONE: A NOVEL REAL-TIME cAMP ASSAY USING THE FLIPR AT THE DOPAMINE D₂ RECEPTOR

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Many G protein coupled receptors signal through changing adenylyl cyclase activity. FLIPR has become a screening platform of choice, allowing real-time measurements in live cells of parameters such as membrane potential and intracellular calcium. ATTO Pharmaceuticals have now developed a novel method for measuring real-time changes in cAMP levels on the FLIPRTM. The ACT: One biosensor uses a modified rat olfactory cyclic nucleotide gated channel (CNG) to sense physiological changes in cAMP levels and reports these as a change in membrane potential. The current study used this approach to examine the pharmacological profile of the G_i coupled human dopamine D₂ receptor.

HEK_293_ASC0083 cells stably expressing the hD₂ receptor and CNG channel (ATTO Pharmaceuticals) were plated at 50,000 cells per well, 24 hrs prior to assay. Cells were subsequently incubated with membrane potential dye (Molecular Devices, UK) for 120 minutes and forskolin/antagonist for 5 minutes prior to addition of agonist for 5 minutes on the FLIPRTM.

Forskolin produced a concentration dependent change in membrane potential with a pEC₅₀ of 4.92±0.07 (n=3). The dopamine receptor agonists quinpirole, bromocriptine and dopamine caused concentration dependent reversals of forskolin stimulated changes in fluorescence in HEK_293_ASC0083 cells with pEC₅₀ values of 8.18±0.08 (n=3), 6.38±0.05 (n=6) and 8.07±0.13 (n=6), respectively, whereas aripiprazole lacked agonist activity. In addition, a variety of typical and atypical antipsychotics reversed quinpirole stimulated inhibition of cAMP levels in a concentration dependent manner (Table 1 with pK_i values from Wood *et al*, 2001).

Table 1. Summary of the activity of antipsychotic drugs

	pK _B	sem	n	pK _i
Haloperidol	8.13	0.18	3	8.8
Olanzapine	7.73	0.05	3	7.9
Clozapine	6.60	0.14	4	7.0
Risperidone	7.44	0.16	3	8.2
Aripiprazole	8.37	0.11	3	7.8
Chlorpromazine	7.63	0.06	3	8.2

These data confirm that the ACT: One technology can report real-time receptor mediated changes in intracellular cAMP levels on the FLIPR. The pharmacology of the dopamine D₂ receptor measured using this approach correlates well with that reported in literature. As such, this assay provides a novel method for analysing the pharmacological profiles of G-protein coupled receptors that couple through the adenylyl cyclase signal transduction cascade.

Wood M.D, Heidbreder C, Reavill C *et al*. (2001) *Drug Dev Res* **54**:88-94.