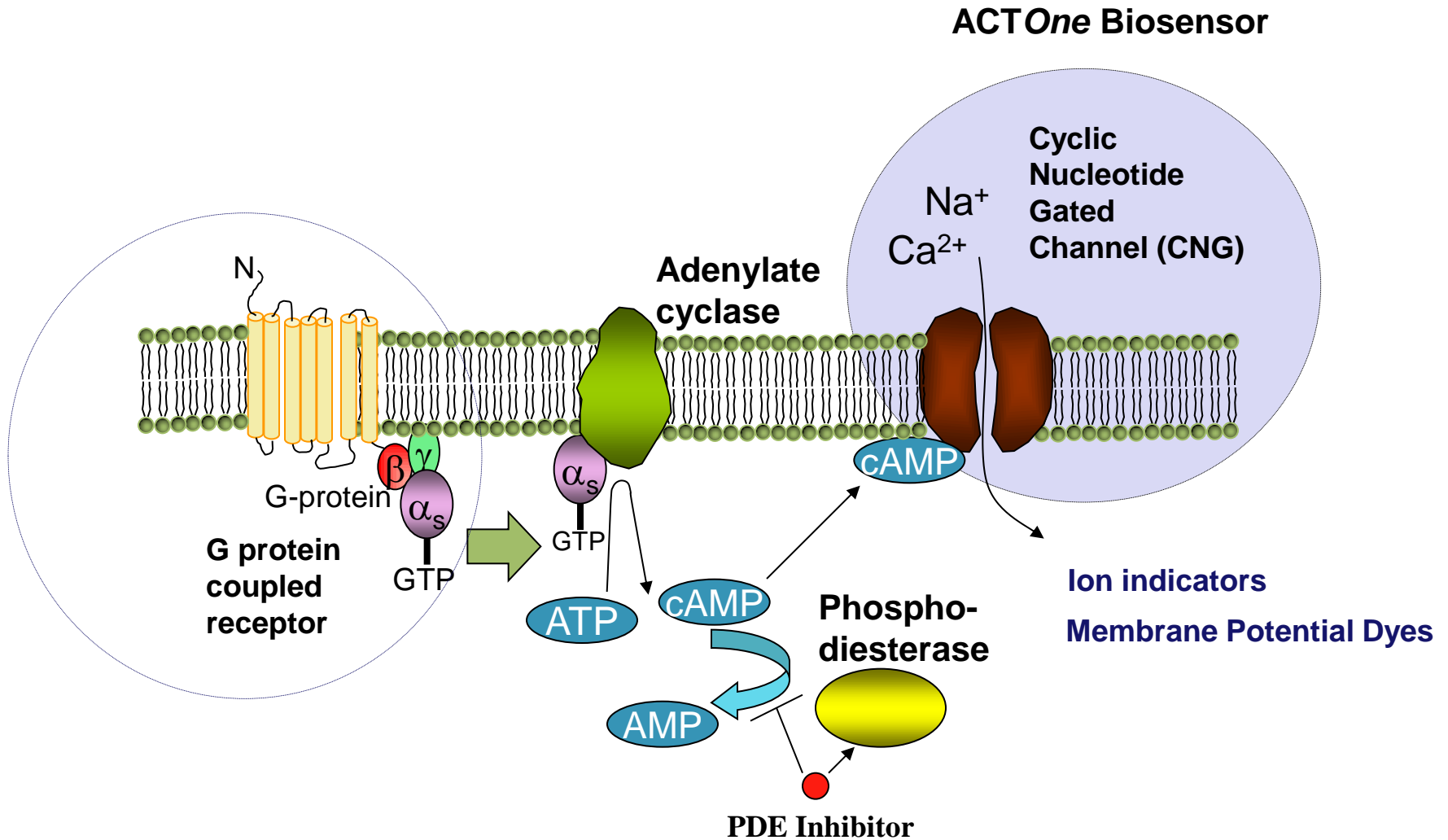


Advanced cAMP Technology (ACTOne) Assay on Phosphodiesterase (PDE)

ACTOne Assay on Phosphodiesterase (PDE)

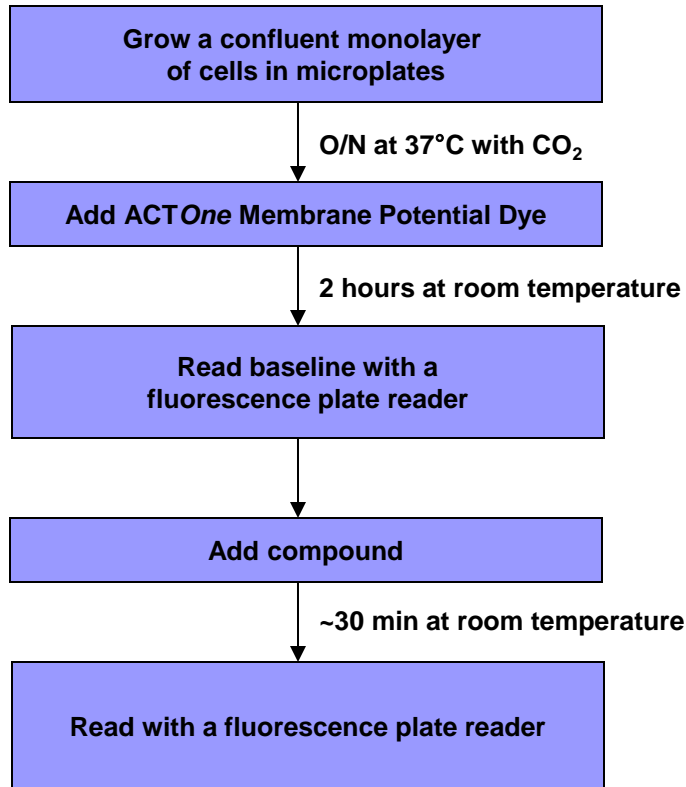
- **PDEs play an essential role in signal transduction by regulating intracellular levels of cAMP and cGMP.**
- **30 PDE genes, 11 subfamilies.**
- **Some PDEs are specific for cGMP or cAMP. The rest use both substrates.**
- **Drug targets for asthma, cardiovascular disease, attention-deficit hyperactivity disorder, Parkinson's and Alzheimer's disease.**

Phosphodiesterase & ACTOne Biosensor

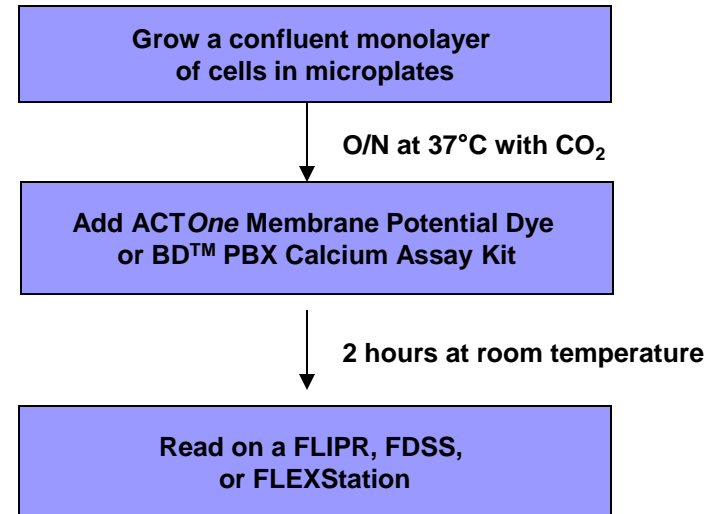


ACTOne PDE Assay Protocol

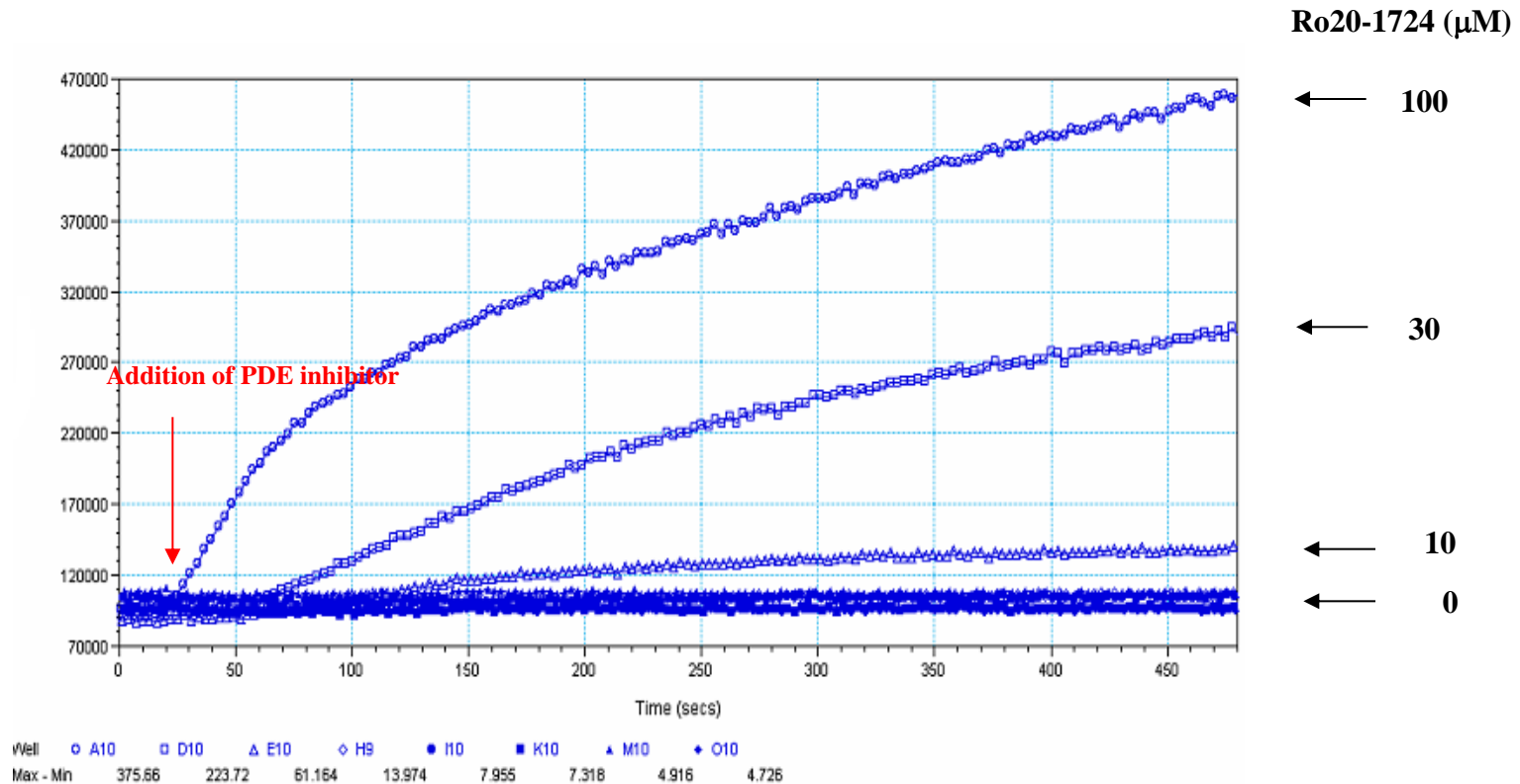
End Point Assay



Kinetic Assay

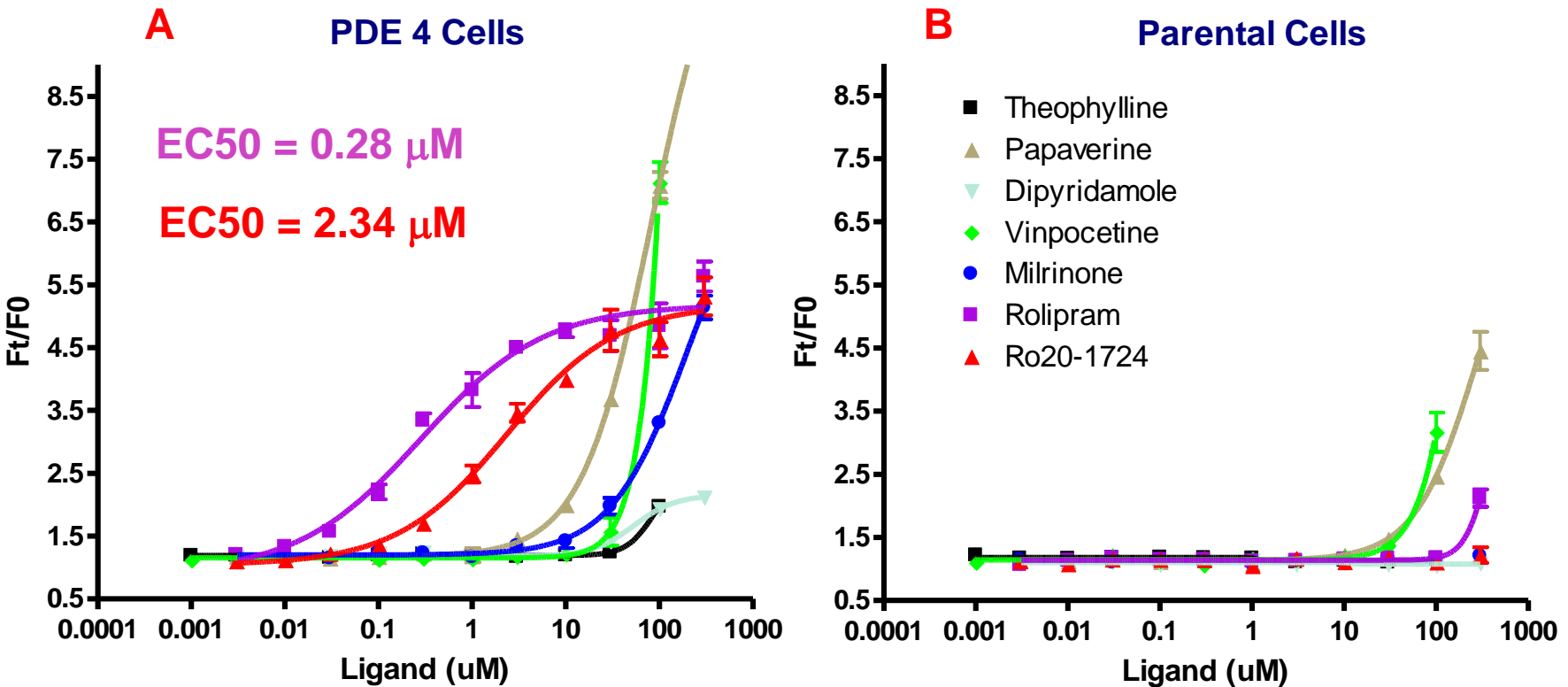


Detect Rapid Response in PDE4 Cells After Specific Inhibitor Treatment



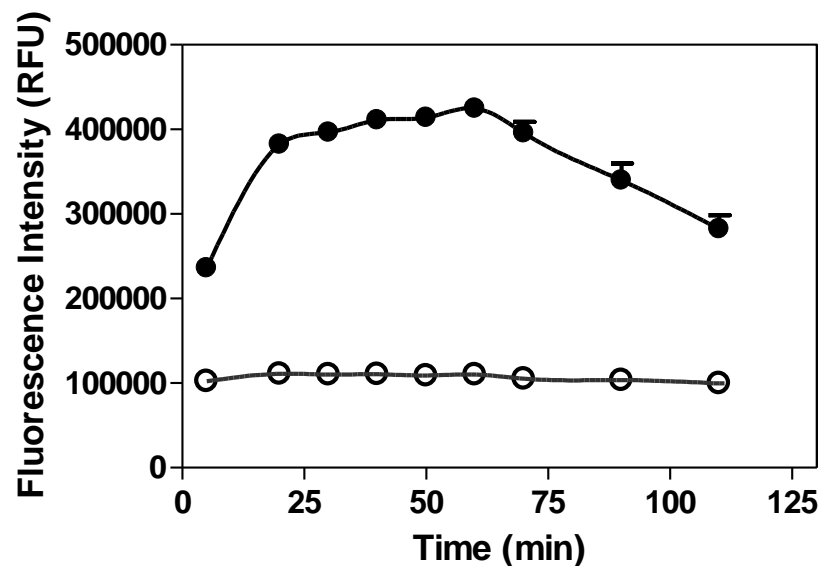
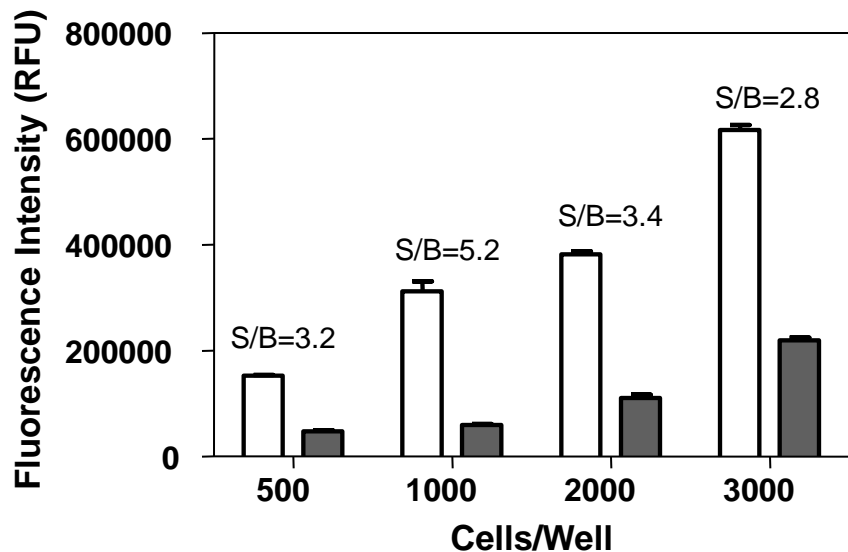
Kinetic response of ACTOne PDE4 cells to Ro20-1724 using ACTOne Membrane Potential Dye Kit. ACTOne PDE4 cells were plated overnight in 100 μl culture medium on a 96 well Biocoat poly-D lysine coated plate. The next day, cells were dye-loaded with 100 μl /well of ACTOne Membrane Potential Dye. After 2 hour of incubation at room temperature, dilutions of Ro20-1724 were added to the plate by a FlexStation (Molecular Devices), and the data was recorded simultaneously.

Dose Response Curves of Various PDE Inhibitors in PDE4 Cells



Response of PDE4 cells and parental cells to different PDE inhibitors. Cells were plated overnight in 20 μ l culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 μ l/well of ACTOne membrane potential dye. After 2 hour of incubation at room temperature, baseline was recorded using a FlexStation (Molecular Devices). 10 μ l of PDE inhibitors at various concentrations were added to the cell plate, and the data was recorded 30 minutes after drug addition. Dose response curves were generated by Prism (Graphpad). **A.** PDE4 cells. **B.** Parental cells.

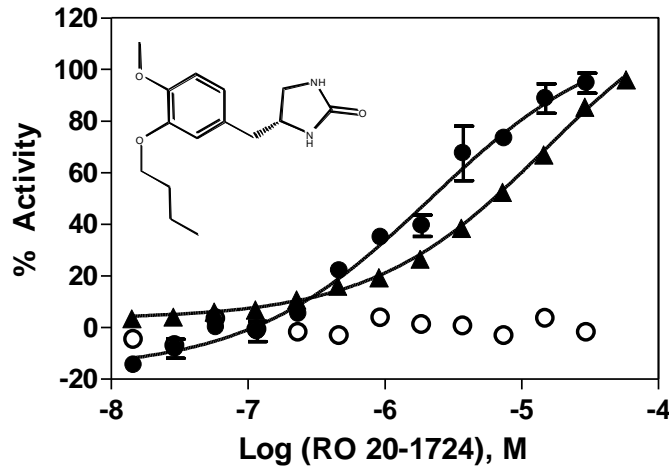
The Assay Can Be Miniaturized to 1536-Well Format



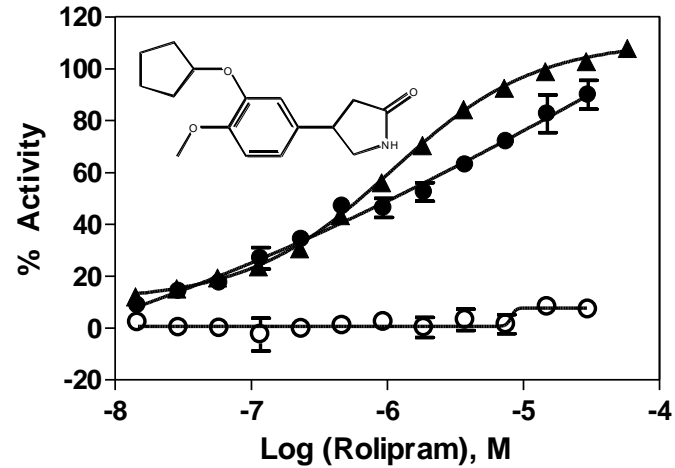
The assay was used to measure endogenous PDE4 activity

Dose Response Curves of PDE4 Inhibitors Obtained from 1536-well Plate

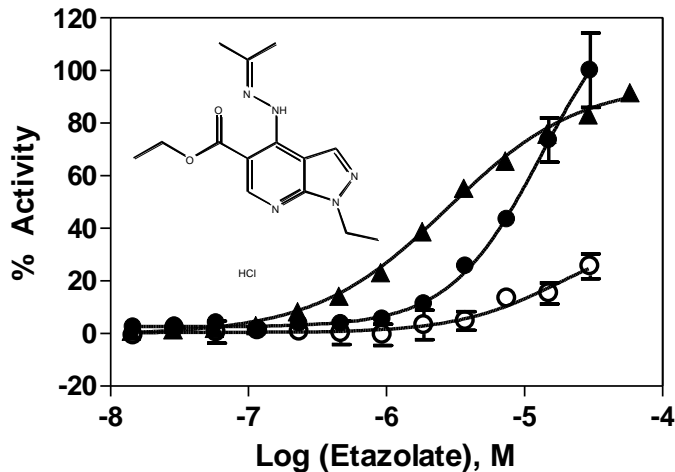
a. RO 20-1724



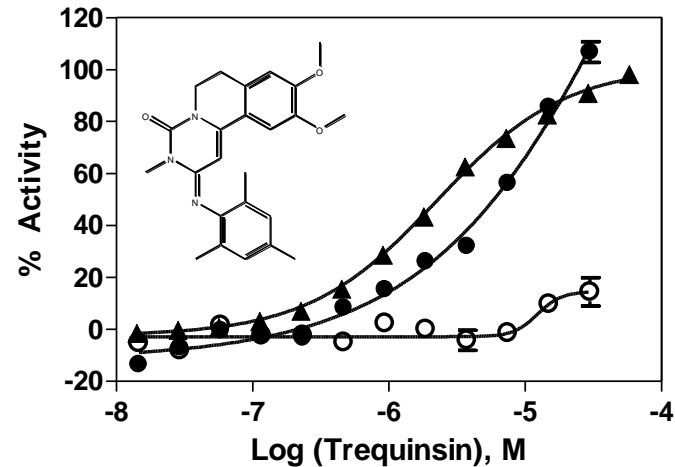
b. Rolipram



c. Etazolate



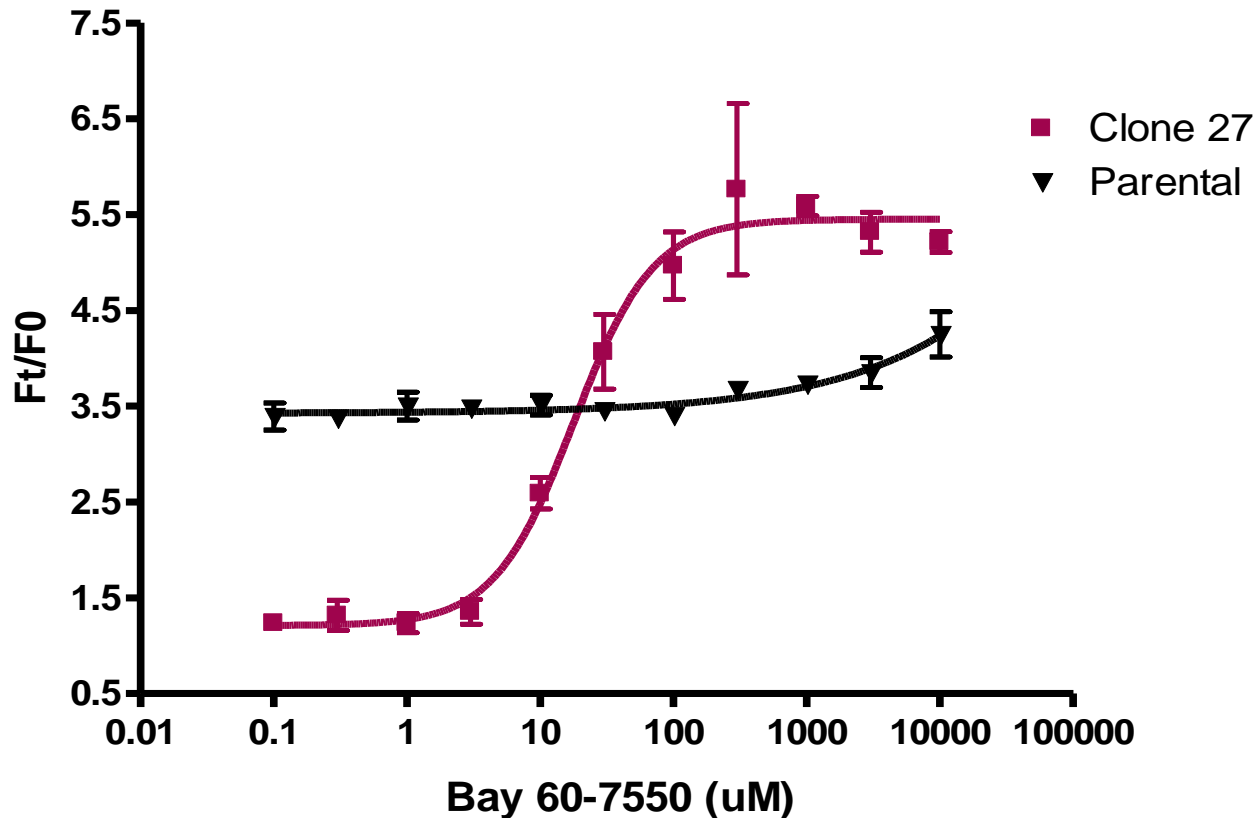
d. Trequinsin



Screening Result

- 9205 compounds were screened in 7 or 15 concentrations in 1536-well plate format.
- One third of the compounds are known compounds. The rest are compounds with unknown functions.
- **All known PDE4 inhibitors have been identified from the screening.**
- 195 specific novel hits were obtained.

ACTOne Assay on Recombinant PDE2A



Parental Cell contains endogenous PDE4. Its activity was blocked by 10 μ M Ro20-1724

Clone 27 contains both PDE4 and PDE2A. The assay was performed in the presence of 10 μ M Ro20-1724

Summary on PDE Project

- **The assay used a constitutively active GPCR (Gs) as a driving force for cAMP production and a cyclic nucleotide-gated cation channel as a biosensor**
- **Cell-based PDE inhibitor assays are much closer to physiological conditions**
- **The assay can be miniaturized into 1536-well format and has big assay window**
- **The assay can be performed both kinetically and in endpoint mode**
- **It can be used for both endogenous and recombinant PDEs**