

# Use of a high-throughput phosphodiesterase profiling platform to study inhibitors selectivity



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## PLATFORM REPRODUCIBILITY, ROBUSTNESS & RELEVANCE EVALUATION USING REFERENCE INHIBITORS

This table shows the results of three independent profiling runs. Cyclic nucleotide substrate is presented for each PDE. In general, the average IC<sub>50</sub> value of the standard reference inhibitor is in the same range as the literature values. The average Z' presented demonstrates the robustness of our assays.

Enzyme	PDE1B	PDE2A	PDE3A	PDE4A	PDE4B	PDE4D	PDE5		
Typical inhibition concentration response curves									
Substrate	cGMP	cAMP	cAMP	cAMP	cAMP	cAMP	cGMP		
Standard inhibitor	Calmidazolium (●)	EHNA (▲)	Trequinsin (●) / Milrinone (■)	Rolipram (♦)	Rolipram (♦)	Rolipram (♦)	T 1032 (▼) / Zaprinast (♦)		
Calculated IC <sub>50</sub>									
. Run 1	1 μM	1.7 μM	0.36 nM	0.59 μM	41 nM	55 nM	44 nM	7.1 nM	0.63 μM
. Run 2	2.7 μM	2.3 μM	0.20 nM	0.34 μM	87 nM	67 nM	45 nM	2.9 nM	1.4 μM
. Run 3	1.7 μM	1.9 μM	0.39 nM	0.88 μM	53 nM	89 nM	80 nM	14 nM	0.42 μM
. Average IC <sub>50</sub> ± SD	1.8 μM ± 0.85	1.97 μM ± 0.31	0.32 nM ± 0.1	0.6 μM ± 0.27	60 nM ± 24	70 nM ± 17	56 nM ± 20	8 nM ± 5.5	0.82 μM ± 0.52
Published IC <sub>50</sub>	1 μM <sup>1</sup>	1 μM <sup>2</sup>	0.3 nM <sup>2</sup>	0.3 μM <sup>3</sup>	55 nM <sup>4</sup>	140 nM <sup>4</sup>	110 nM <sup>4</sup>	1 nM <sup>5</sup>	0.76 μM <sup>3</sup>
Average Z'	0.67	0.67	0.75	0.58	0.64	0.52	0.62		

Enzyme	PDE6	PDE7A	PDE8A	PDE9A	PDE10A	PDE11A			
Typical inhibition concentration response curves									
Substrate	cGMP	cAMP	cAMP	cGMP	cAMP	cAMP			
Standard inhibitor	Zaprinast (♦)	BRL50481 (■)	Dipyridamol (●)	BAY 736691 (●) / Zaprinast (♦)	Zaprinast (♦) / Papaverin (■)	Dipyridamol (●) / Zaprinast (♦)			
Calculated IC <sub>50</sub>									
. Run 1	0.22 μM	0.28 μM	7.4 μM	1.1 μM	51 μM	42 μM	0.1 μM	0.50 μM	11 μM
. Run 2	0.23 μM	0.46 μM	6 μM	1.5 μM	39 μM	52 μM	0.1 μM	0.59 μM	5.4 μM
. Run 3	0.27 μM	0.44 μM	7.9 μM	1.1 μM	40 μM	47 μM	0.12 μM	0.57 μM	6.6 μM
. Average IC <sub>50</sub> ± SD	0.24 μM ± 0.026	0.39 μM ± 0.099	7.1 μM ± 0.98	1.23 μM ± 0.23	43 μM ± 6.7	47 μM ± 5	0.11 μM ± 0.01	0.55 μM ± 0.05	7.7 μM ± 3
Published IC <sub>50</sub>	0.15 μM <sup>3</sup>	0.26 μM <sup>6</sup>	9 μM <sup>7</sup>	Not found	35 μM <sup>8</sup>	11 μM <sup>9</sup>	18 nM <sup>10</sup>	0.37 μM <sup>11</sup>	12 μM <sup>11</sup>
Average Z'	0.83	0.6	0.62	0.85		0.71		0.69	

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## FULL SELECTIVITY PROFILE OF REFERENCE INHIBITORS & CLINICAL DRUGS

	Primary target	PDE1B	PDE2A	PDE3A	PDE4A	PDE4B	PDE4D	PDE5	PDE6	PDE7A	PDE8A	PDE9A	PDE10A	PDE11A
Calimidazolium	PDE1													
EHNA	PDE2													
Trequinsin	PDE3													
Levosimendan														
Anagrelide														
Milrinone	PDE4													
Rolipram														
Zaprinast	PDE5													
Sildenafil (Viagra)														
Tadalafil (Cialis)	PDE7													
BRL50481														
Papaverin	MULTI-TARGET													
Dipyridamole														
Ethaverine	NON PDE													
Moricizine														
Berberine														
Dequalinium														
Rosuvastatin														
Rotlerin														

□ No IC<sub>50</sub> calculated □ IC<sub>50</sub> > 10 μM □ 1 μM < IC<sub>50</sub> < 10 μM □ 100 nM < IC<sub>50</sub> < 1000 nM □ IC<sub>50</sub> < 100 nM

A representative panel of compounds was profiled on all PDEs. Results (average of 2-3 experiments) are shown as a hit map. Results are in agreement with the known properties of those compounds<sup>2</sup>.

## MATERIALS AND METHODS

### ENZYME SOURCE

All human recombinant phosphodiesterase enzymes were from BPS Bioscience except PDE5 and PDE6 respectively extracted from human platelets and bovine retina.

### ASSAY PROTOCOL

#### HTRF®

In a 96-half well plate, 2 μL inhibitor and 8 μL PDE were added to the well, followed by the addition of 10 μL substrate cAMP or cGMP depending on the specificity. 20nM cAMP and 120nM cGMP were used in order to fit within the linear part of each assay kit calibration curve. After 30 min incubation at room temperature, 10 μL of each HTRF reagent (cNMP-d2 and anti cNMP-cryptate) were added to the assay plate. After 1h at room temperature, the signal was measured on RUBYstar (BMG, LABTECH).

#### SPA (PDE6 only)

In a 96-well plate, 10 μL inhibitor and 50 μL buffer (containing [<sup>3</sup>H] cGMP and cGMP) were added to the well, followed by the addition of 40 μL enzyme. After 60 min incubation at room temperature, 30 μL of SPA beads were added to the assay plate. After 20min at room temperature, the signal was measured on Top count (Packard).

## CONCLUSION

We have demonstrated that our new phosphodiesterase profiling platform is:

- ROBUST: Homogeneous results, acceptable z'
- REPRODUCIBLE: Consistent IC<sub>50</sub> over several independent experiments
- RELEVANT: Confirmed target specificity of compounds, coherent IC<sub>50</sub> values when compared to literature

## INTRODUCTION

Cyclic nucleotide phosphodiesterases (PDEs), ubiquitously distributed in mammalian tissues, play a major role in cell signaling by hydrolyzing cAMP and/or cGMP. Due to their diversity and specific distribution at cellular and subcellular levels, PDEs can selectively regulate various cellular functions. The PDE superfamily represents 11 gene families (PDE1 to PDE11) and increased understanding of their function at the cell and molecular level provides an impetus for the development of isoenzyme selective inhibitors for the treatment of various diseases. Examples are PDE3 inhibitors for congestive heart failure, PDE4 inhibitors for inflammatory airways disease and the most well known, PDE5 inhibitor for erectile dysfunction (Viagra).

As PDEs are expressed in a variety of tissues, selectivity is a prerequisite for a therapeutically applicable PDE inhibitor. As an example, high selectivity for PDE5 inhibitors is important for treatment of erectile dysfunction to minimize the possibility of side effects that arise as a result of inhibition of other PDEs. Possible side effects include heart rate increase and vasodilation that are attributed to inhibition of PDE1 and PDE3, or blue-green vision disturbances that are attributed to inhibition of PDE6.

In this context, we have designed a high throughput profiling platform to determine the inhibitory activity and selectivity of compounds on the PDE superfamily. To validate the robustness, reproducibility and relevance of this platform, we determined the potency and selectivity of a broad panel of commercially available reference inhibitors and known clinical drugs in three independent experiments.