

CORNING

Epic[®]
system

**Application of the Corning[®] Epic[®]
System for Label-Free Cell-based
Screening Using the LOPAC[™]
Library**

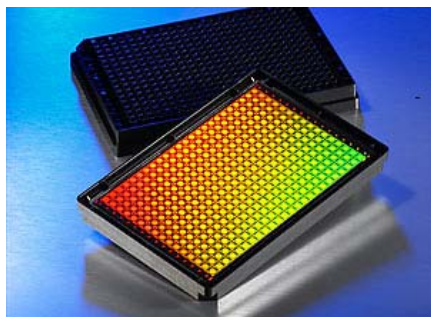
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Abstract

G protein-coupled receptors (GPCRs) are the largest family of cell surface receptors and the largest class of drug targets. The activation of GPCRs is known to lead to the dynamic translocation of multiple signaling molecules or molecular assemblies during its signaling cycle, and in many cases cytoskeletal reorganization. Such a movement and/or reorganization results in dynamic redistribution of cellular contents, dynamic mass redistribution (DMR), which can be monitored online in living cells using the Corning® Epic® system – a label-free and non-invasive biosensor system that is centered around resonant waveguide grating biosensors. The resultant DMR signal offers a novel and functional optical signature for studying GPCR signaling and screening of GPCR drug compounds. The human epidermoid carcinoma cell line, A431, was used as a model system for screening the response of an endogenously expressed GPCR. A431 cells endogenously express the beta2 adrenergic receptor (β_2 AR), which is a typical $G\alpha_s$ -coupled GPCR. We first optimized assay conditions, including buffer composition and DMSO tolerance for a known β_2 AR agonist, epinephrine. A431 cells were then screened for β_2 AR agonists and antagonists using the LOPAC compound library. The Epic System was able to identify all of the β_2 AR agonists (7) and antagonists (1) in the LOPAC library. Z' analysis suggests that the Epic System is suitable for cell-based high throughput screening.

Corning® Epic® System

The Corning Epic System is a high-throughput, label-free detection platform that consists of SBS-standard 384-well microplates with optical sensors inside each well, an HTS-compatible microplate reader and a set of label-independent assay protocols. The Epic System is applicable to both biochemical and cell-based assays, and enables high-throughput screening of “intractable” targets.



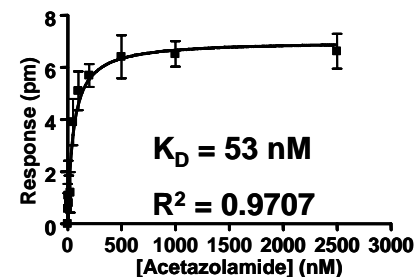
Microplate

- 384-well format
- Optical biosensor in each well
- Surface chemistry



Microplate Reader

- Compatible w/ HTS automation
- $\geq 40,000$ wells/8hrs
- Sensitivity of $5\text{pg}/\text{mm}^2$
(300Da drug to 75kDa target)

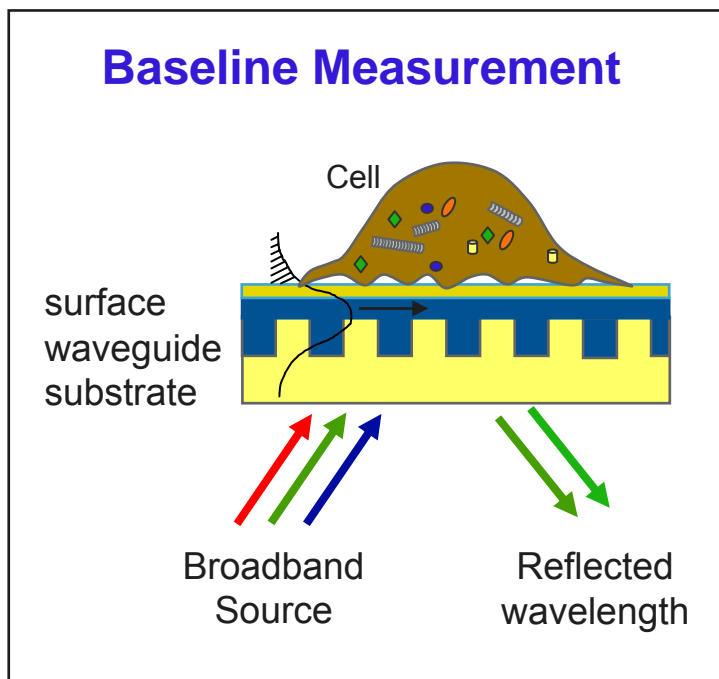


Binding Data

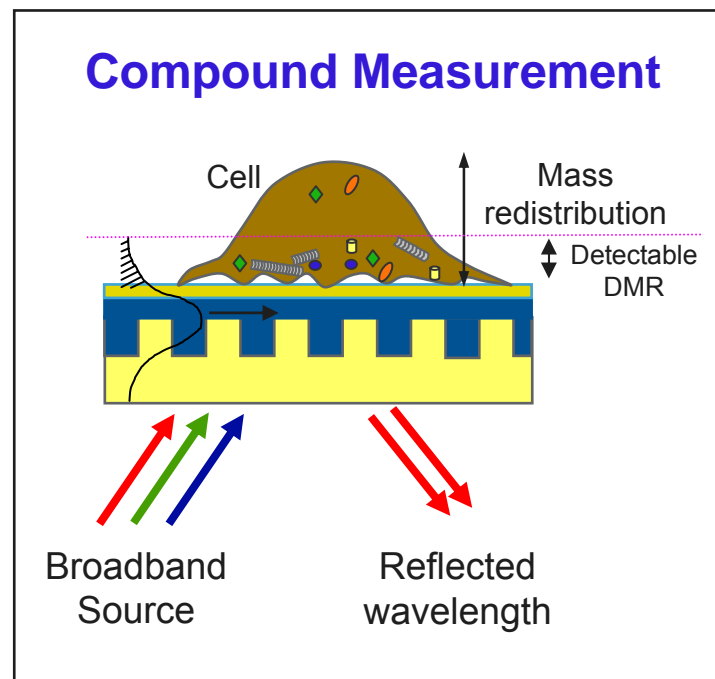
- Manipulated and analyzed by customer

Operating Principle: Cell-based Assays

- Measures changes in local index of refraction resulting from the ligand-induced dynamic mass redistribution (DMR) within the bottom region (~150nm) of the cell monolayer.
- Change in index is manifested by a shift in resonant wavelength



Stimulation →



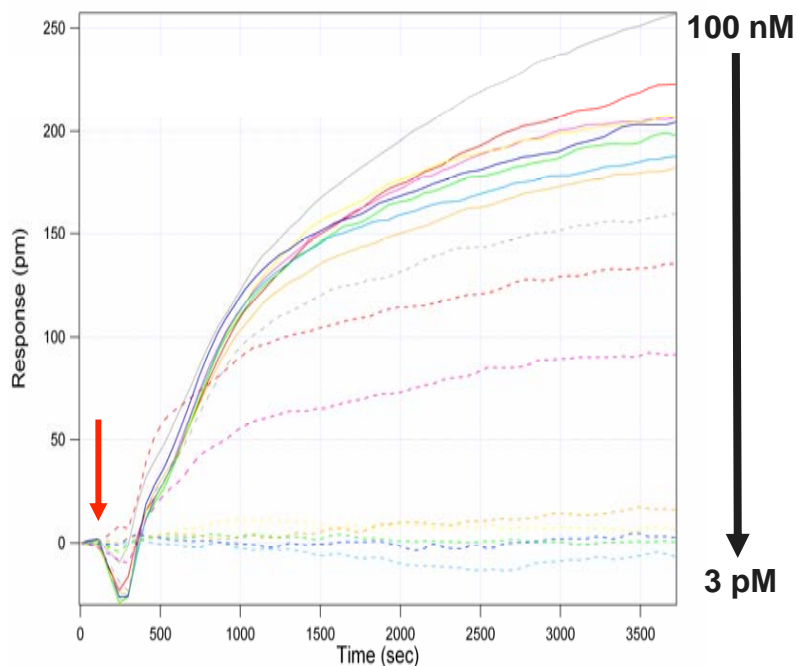
Cell-based Assays Using the Epic[®] System

Experimental Overview:

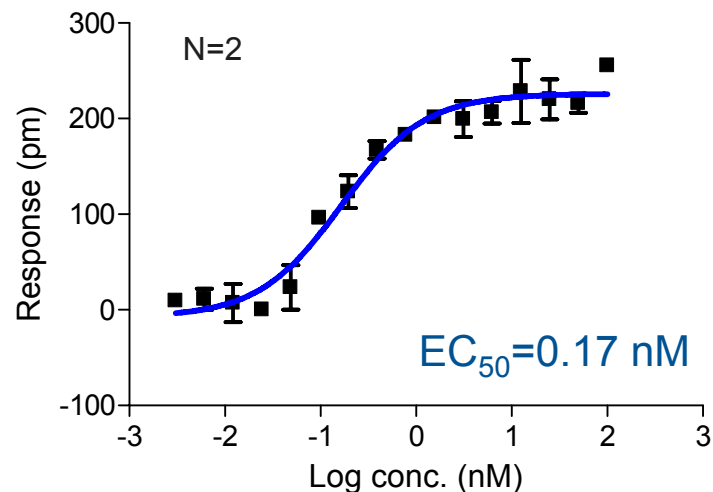
- A431 cells:
 - A431 is a human epidermoid carcinoma cell line.
 - A431 cells endogenously express the β_2 adrenergic receptor (β_2 AR).
 - β_2 AR is a prototypical G_{α_s} -coupled receptor.
 - Epinephrine is a natural agonist for the β_2 AR.
- Assay optimization:
 - Media composition
 - DMSO tolerance
 - Cell seeding number
 - Volume of compound addition
- Compound screening using the LOPAC[™] library from Sigma-Aldrich:
 - The LOPAC[™] library is a commercially available library consisting of 1280 pharmacologically active compounds.
 - The LOPAC[™] compounds were screened in order to identify specific β_2 AR agonists and antagonists in A431 cells.
 - The LOPAC[™] library contains 7 known β_2 AR agonists and 1 known β_2 AR antagonist.

Epinephrine Dose-response in A431 cells

Optical Response



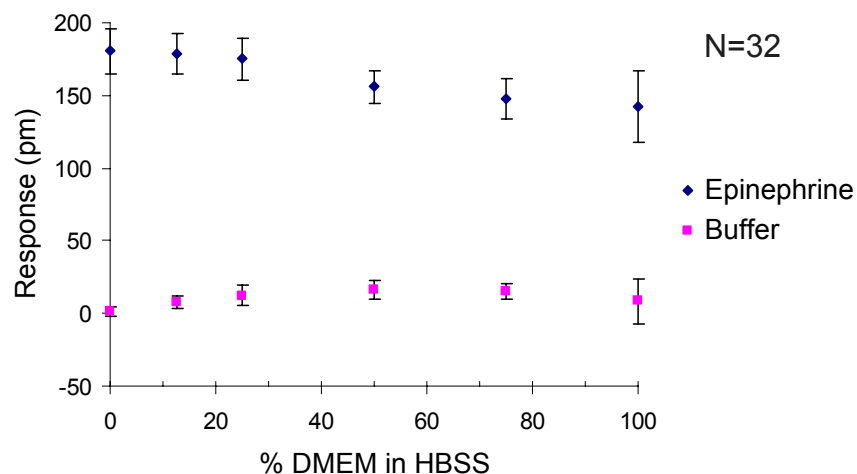
Epinephrine Dose-Response



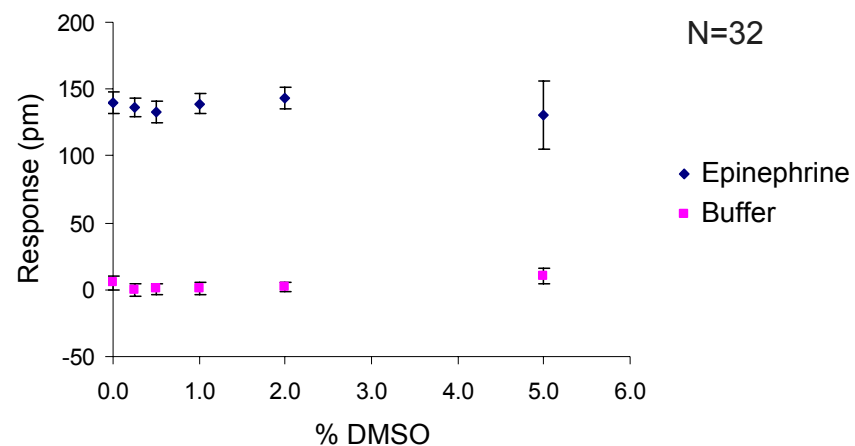
- **Left Panel:** Dose-dependent optical response of epinephrine in A431 cells. Epinephrine was added to A431 cells from 100nM to 3pM. The red arrow indicates the point of epinephrine addition
- **Right Panel:** EC₅₀ estimation using GraphPad Prism. An EC₅₀ value of 0.17nM was calculated.

Optimization of Cell-based Assay

Optimization of Assay Medium



DMSO tolerance



- **Left Panel:** Optimization of assay medium. HBSS was supplemented with 0% to 100% DMEM. 2nM Epinephrine was added to A431 cells in the indicated assay medium. 100% HBSS (0% DMEM) was identified as the optimal assay buffer.
- **Right Panel:** DMSO tolerance. 2nM epinephrine was added to A431 cells in assay buffer containing 0% to 5% DMSO. Epinephrine response in A431 cells was tolerant up to 5% DMSO.

Compound Screening on the Epic[®] System

Cell Seeding Procedure:

1. Seed an Epic microplate with A431 cells (20000 cells/well).
2. Incubate cells overnight at 37°C/5% CO₂.
3. Wash cells once with serum-free culture medium.
4. Incubate cells overnight in serum-free culture medium at 37°C/5% CO₂.
5. Wash cells once with assay buffer.
6. Incubate cells in 30µL assay buffer.

Compound Source Plate Preparation:

1. For β₂AR agonist screen:
 - The LOPAC[™] compounds were diluted to 4µM in assay buffer (0.6% DMSO).
 - The final assay concentration for each compound was 1µM (0.6% DMSO).
2. For β₂AR antagonist screen:
 - Epinephrine was diluted in assay buffer to 10nM (0.6% DMSO).
 - The final assay concentration of epinephrine was 2nM (0.6% DMSO).

Epic Instrument Procedure:

1. Take a baseline scan (~5 min).
2. Add 10µL of the LOPAC[™] compound solution to the Epic microplate (Agonist screen).
3. Incubate Epic microplate for 1 hour.
4. Take a final scan (~5 min).
5. Add 10µL of epinephrine (Antagonist screen).
6. Incubate Epic microplate for 1 hour.
7. Take final scan (~5 min).

A431 Cell Performance

- Each microplate contained an epinephrine dose-response control.
- This control monitored the performance of the A431 cells on each Epic[®] microplate.
- All four microplates exhibited similar EC₅₀ values.
- These values are consistent with the literature value.

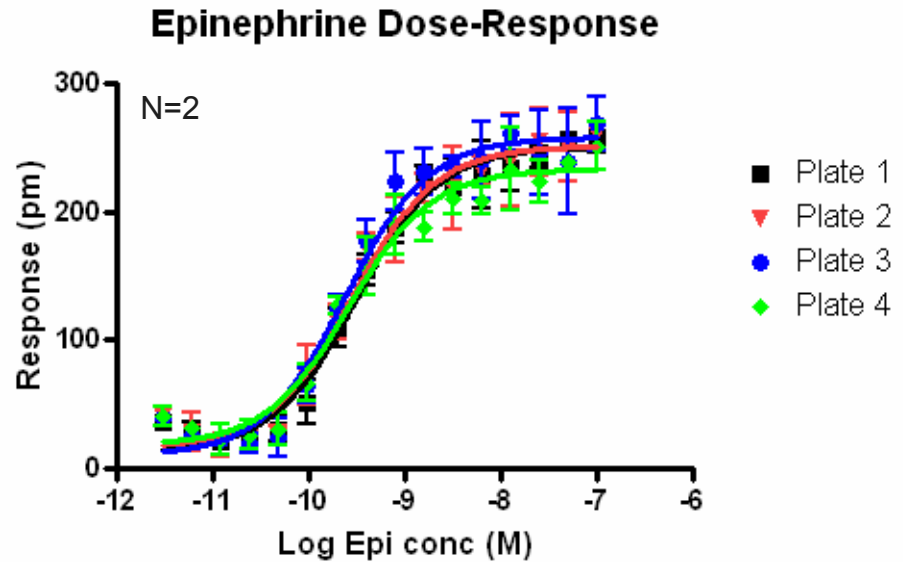


	Plate 1	Plate 2	Plate 3	Plate 4
EC ₅₀	0.31 nM	0.28 nM	0.24 nM	0.26 nM

Z' Analysis

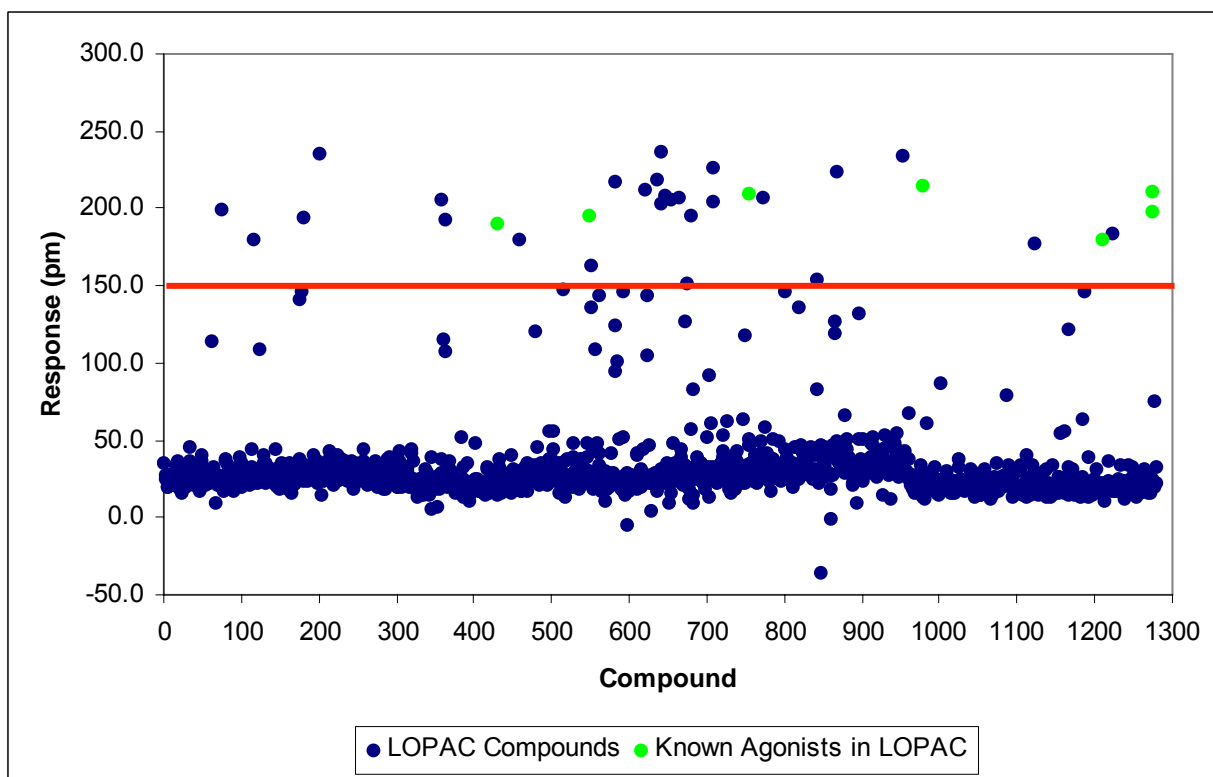
- Z' was calculated twice for each Epic[®] microplate.
 - Once for the Agonist screen (addition of LOPAC compounds)
 - Once for the Antagonist screen (addition of 2nM epinephrine)

- Z' analysis for Agonist screen:
 - Positive controls: 1μM Epinephrine (N=16)
 - Negative controls: Buffer (N=16)

- Z' analysis for Antagonist screen:
 - Positive controls: 2nM Epinephrine (N=8)
 - Negative controls: Buffer (N=8)

	Z'			
	Plate 1	Plate 2	Plate 3	Plate 4
Agonist screen	0.72	0.82	0.70	0.70
Antagonist screen	0.72	0.80	0.72	0.63

β_2 AR Agonist Screen



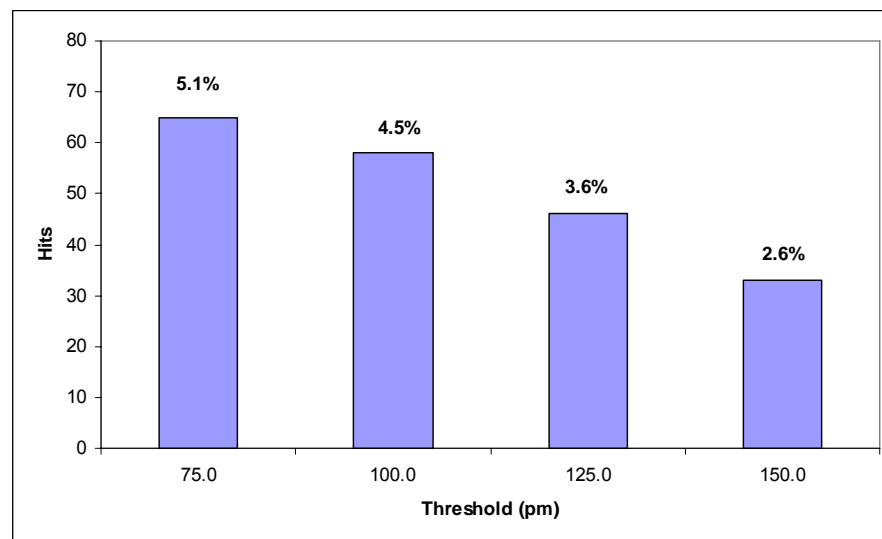
Threshold set at 150pm

Total Hits	33	
Selectivity	Hits	% of Hits
Adrenergic	24	72.7%
Adenosine	3	9.1%
Dopamine	1	3.0%
Others	5	15.2%

β_2 AR Agonist Hits

- Most of the compounds did not elicit a response when added to A431 cells.
- 33 agonist hits were identified with a threshold set at 150pm.
 - 2.6% of the LOPAC™ library
- All 7 known β_2 AR agonists were identified (green points).
 - No false negatives
- 73% of the agonist hits were specific for adrenergic receptors.
- 3 adenosine agonists and 1 dopamine agonist were identified.
 - A431 cells are known to express adenosine receptors.
- 5 additional compounds were identified as agonists.
- An orthogonal screening approach is being run to validate these hits (cAMP analysis).

Threshold Analysis for Agonist Screen

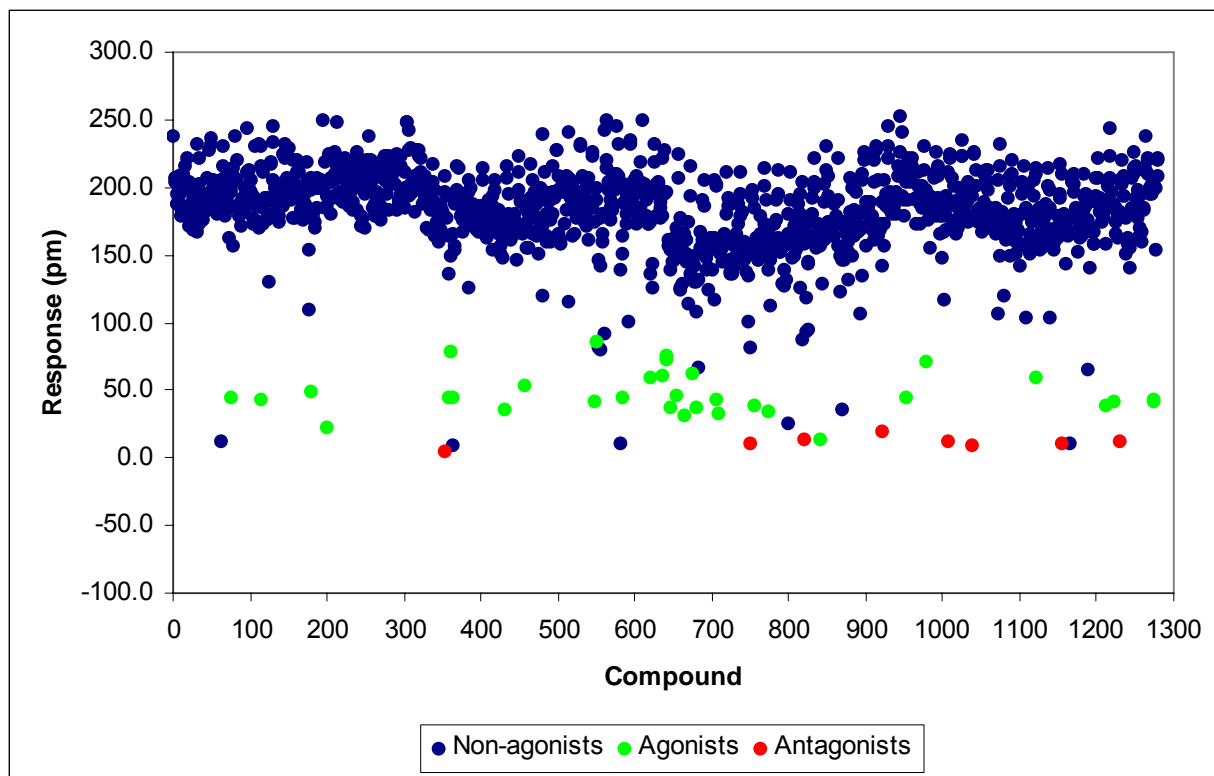


Theshold (pm)	75.0	100.0	125.0	150.0
Total Hits	65	58	46	33
% of Library	5.1%	4.5%	3.6%	2.6%

β₂AR Agonist Hits

<u>Name</u>	<u>Class</u>	<u>Action</u>	<u>Selectivity</u>	<u>Description</u>
L(-)-Norepinephrine bitartrate	Adrenoceptor	Agonist	alpha, beta1	Adrenergic neurotransmitter; vasoconstrictor
Phenylephrine hydrochloride	Adrenoceptor	Agonist	alpha1	alpha1 Adrenoceptor agonist; mydriatic; decongestant
R(-)-Isoproterenol (+)-bitartrate	Adrenoceptor	Agonist	beta	Sympathomimetic amine acting almost exclusively on beta adrenoceptors
Isotharine mesylate	Adrenoceptor	Agonist	beta	beta-Adrenoceptor agonist; bronchodilator
(±)-Isoproterenol hydrochloride	Adrenoceptor	Agonist	beta	Sympathomimetic amine acting almost exclusively on beta adrenoceptors; bronchodilator
Nylidrin hydrochloride	Adrenoceptor	Agonist	beta	beta Adrenoceptor agonist; peripheral vasodilator
(-)-Isoproterenol hydrochloride	Adrenoceptor	Agonist	beta	beta-Adrenoceptor agonist; increases cytosolic cAMP
Terbutaline hemisulfate	Adrenoceptor	Agonist	beta	beta-Adrenoceptor agonist; bronchodilator
Tulobuterol hydrochloride	Adrenoceptor	Agonist	beta	beta-Adrenoceptor agonist related to structurally to terbutaline; bronchodilator
Dobutamine hydrochloride	Adrenoceptor	Agonist	beta1	beta1 Adrenoceptor agonist
Fenoterol hydrobromide	Adrenoceptor	Agonist	beta2	Beta2-adrenoceptor agonist; bronchodilator
Formoterol	Adrenoceptor	Agonist	beta2	beta2-Adrenoceptor agonist
Metaproterenol hemisulfate	Adrenoceptor	Agonist	beta2	beta2-Adrenoceptor agonist
Ritodrine hydrochloride	Adrenoceptor	Agonist	beta2	beta2-Adrenoceptor agonist; relaxes uterine muscle contractions
Albuterol hemisulfate	Adrenoceptor	Agonist	beta2	beta Adrenoceptor agonist
Salbutamol	Adrenoceptor	Agonist	beta2	beta2-Adrenoceptor agonist
Salmeterol xinafoate	Adrenoceptor	Agonist	beta2	beta2 Adrenoceptor agonist
BRL 37344 sodium	Adrenoceptor	Agonist	beta3	Selective beta3 adrenoceptor agonist
(±)-Norepinephrine (+)bitartrate	Adrenoceptor	Agonist		Adrenergic neurotransmitter
(-)-Epinephrine bitartrate	Adrenoceptor	Agonist		Endogenous hormone and neurotransmitter
(±)-Epinephrine hydrochloride	Adrenoceptor	Agonist		Adrenoceptor agonist
(-)-alpha-Methylnorepinephrine	Adrenoceptor	Agonist		Active enantiomer; adrenoceptor agonist; vasoconstrictor; antihypertensive
Labetalol hydrochloride	Adrenoceptor	Antagonist	beta	Competitive beta-adrenoceptor antagonist
S(+)-Isoproterenol (+)-bitartrate	Adrenoceptor		beta	Sympathomimetic amine acting almost exclusively on beta adrenoceptors
5'-N-Ethylcarboxamidoadenosine	Adenosine	Agonist	A1/A2	Potent adenosine receptor agonist with equal affinity at A1 and A2 receptors
5'-(N-Cyclopropyl)carboxamidoadenosine	Adenosine	Agonist	A2	Potent A2 adenosine receptor agonist
Metrifudil	Adenosine	Agonist	A2	Adenosine receptor agonist which displays some selectivity for the A2 receptor type
N-Methyldopamine hydrochloride	Dopamine	Agonist		Dopamine receptor agonist
Felodipine	Ca2+ Channel	Blocker	L-type	L-type calcium channel blocker
Idazoxan hydrochloride	Imidazoline	Ligand	I1 / I2	I2-imidazoline agonist; I1-imidazoline antagonist; alpha2-Adrenergic antagonist
L-N6-(1-Iminoethyl)lysine hydrochloride	Nitric Oxide	Inhibitor	iNOS	Selective inducible nitric oxide synthase (iNOS) inhibitor.
NG-Nitro-L-arginine	Nitric Oxide	Inhibitor	NOS	Potent nitric oxide synthase inhibitor
ML-7	Phosphorylation	Inhibitor	MLCK	Selective myosin light chain kinase (MLCK) inhibitor

β_2 AR Antagonist Screen



β_2 AR Antagonist Hits

- Most of the compounds did not inhibit the epinephrine-induced response (blue points).
- Compounds that were identified as hits in the agonist screen inhibited the epinephrine-induced response due to receptor desensitization (green points).
- 8 compounds were identified as β_2 AR antagonists (red points).
 - These compounds did not elicit a response during the agonist screen.
 - These compounds completely inhibited the epinephrine-induced response during the antagonist screen.
 - 0.6% of LOPAC™ library
- The one specific β_2 AR antagonist in the LOPAC™ library was positively identified (ICI 118,551).
 - No false negatives
- 3 compounds are known beta-adrenergic receptor antagonists.
- 1 compound is an active beta-adrenergic receptor blocking enantiomer.
- 1 compound is known to alkylate beta-adrenergic receptors.

β₂AR Antagonist Hits

<u>Name</u>	<u>Class</u>	<u>Action</u>	<u>Selectivity</u>	<u>Description</u>
(±)-Propranolol hydrochloride	Adrenoceptor	Antagonist	beta	beta Adrenoceptor antagonist ; cardiac depressant (anti-arrhythmic)
S(-)-Timolol maleate	Adrenoceptor	Antagonist	beta	beta Adrenoceptor antagonist ; antihypertensive; antiarrhythmic; antiglaucoma agent
ICI 118,551 hydrochloride	Adrenoceptor	Antagonist	beta2	Highly selective beta2 adrenoceptor antagonist
(S)-Propranolol hydrochloride	Adrenoceptor	Blocker	beta	Active beta-adrenoceptor receptor blocking enantiomer ; 5-HT ₁ serotonin receptor antagonist
(±)-Pindobind	Adrenoceptors	Ligand	beta	Analog of pinolol which contains a bromo-acetyl group capable of alkylation of beta-adrenoceptors
(±)-CPP	Glutamate	Antagonist	NMDA	Potent and selective NMDA glutamate receptor antagonist; anticonvulsant
Propafenone hydrochloride	K ⁺ Channel	Blocker	hKv1.5	Blocks hKv1.5 and ATP-sensitive K ⁺ channels; beta-adrenoceptor antagonist
Phorbol 12-myristate 13-acetate	Phosphorylation	Activator	PKC	Activates protein kinase C in vivo and in vitro; strong NO promoter; promotes expression of iNOS in cultured hepatocytes; T lymphocyte activator

Summary of Results

- A431 cells were used to screen the LOPAC™ library for β_2 AR agonists and antagonists.
- No false negatives were observed during the agonist screen or the antagonist screen.
- 33 hits were identified during the agonist screen.
 - All 7 known β_2 AR agonists were positively identified.
 - 24 of the agonist hits were specific for adrenergic receptors.
- 8 hits were identified during the antagonist screen.
 - The one specific β_2 AR antagonist was positively identified.
 - 4 of the hits are known beta-adrenergic receptor antagonists.
- Robust assay performance was observed during the screen.
 - Z' values ranged from 0.63-0.82.
- Consistent EC₅₀ values were observed for each Epic® microplate.
 - EC₅₀ values ranged from 0.24-0.32nM for the epinephrine-induced response.