

# CORNING

Epic<sup>®</sup>  
system

## **Resonant Waveguide Grating Biosensors for High-Throughput Screening of GPCRs**

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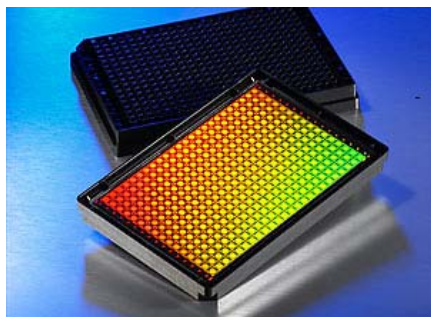
## Abstract

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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 ( $\beta_2$ AR), which is a typical  $G\alpha_s$ -coupled GPCR. We first optimized assay conditions, including buffer composition and DMSO tolerance for a known  $\beta_2$ AR agonist, epinephrine. A431 cells were then screened for  $\beta_2$ AR agonists and antagonists using the LOPAC compound library. The Epic System was able to identify all of the  $\beta_2$ 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.



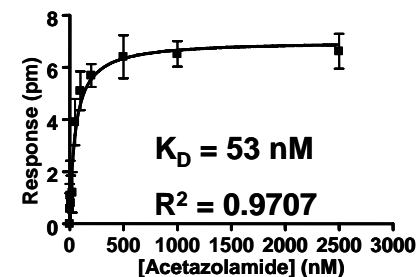
## Microplate

- 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)

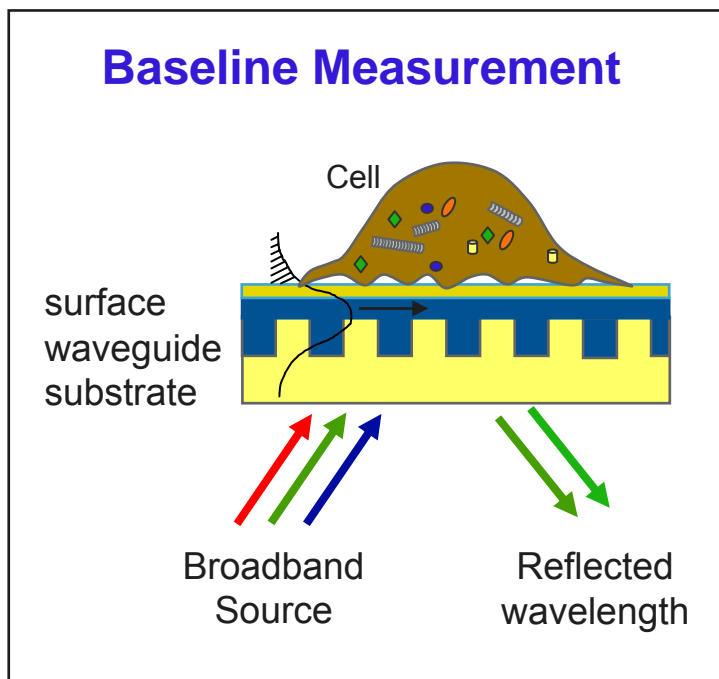


## Binding Data

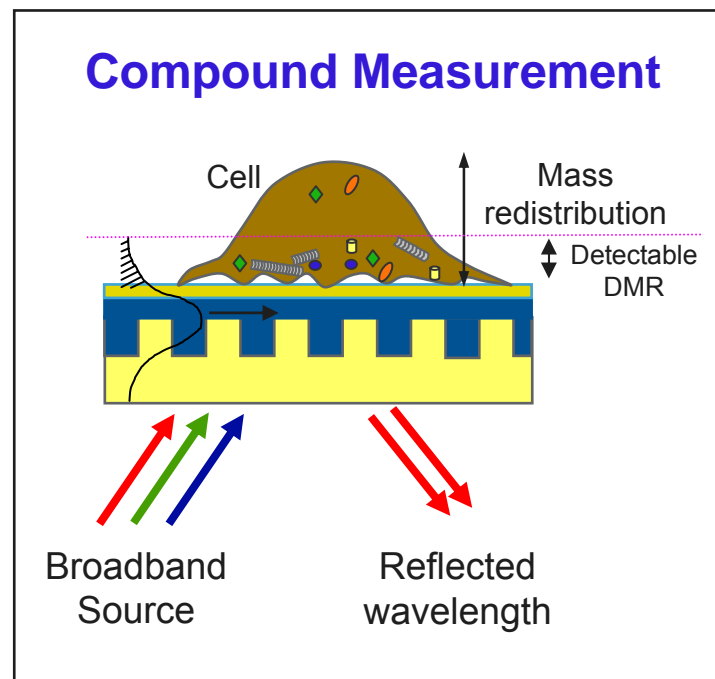
- Manipulated and analyzed by customer

# 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 →



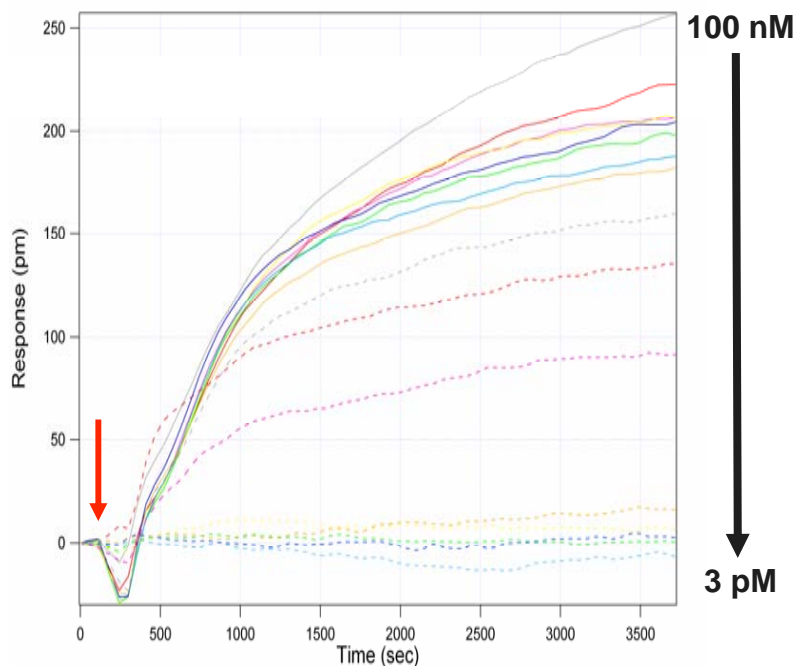
# Cell-based Assays Using the Epic<sup>®</sup> System

## Experimental Overview:

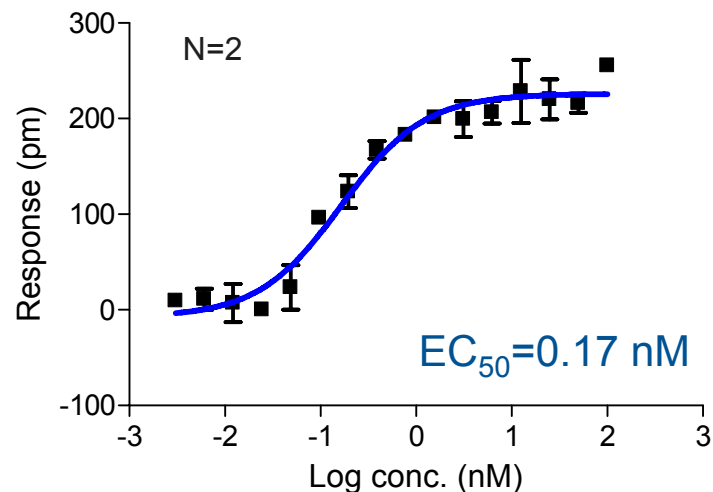
- A431 cells:
  - A431 is a human epidermoid carcinoma cell line.
  - A431 cells endogenously express the  $\beta_2$  adrenergic receptor ( $\beta_2$ AR).
    - $\beta_2$ AR is a prototypical  $G_{\alpha_s}$ -coupled receptor.
    - Epinephrine is a natural agonist for the  $\beta_2$ AR.
- Assay optimization:
  - Media composition
  - DMSO tolerance
  - Cell seeding number
  - Volume of compound addition
- Compound screening using the LOPAC<sup>™</sup> library from Sigma-Aldrich:
  - The LOPAC<sup>™</sup> library is a commercially available library consisting of 1280 pharmacologically active compounds.
  - The LOPAC<sup>™</sup> compounds were screened in order to identify specific  $\beta_2$ AR agonists and antagonists in A431 cells.
  - The LOPAC<sup>™</sup> library contains 7 known  $\beta_2$ AR agonists and 1 known  $\beta_2$ AR antagonist.

# Epinephrine Dose-response in A431 cells

## Optical Response



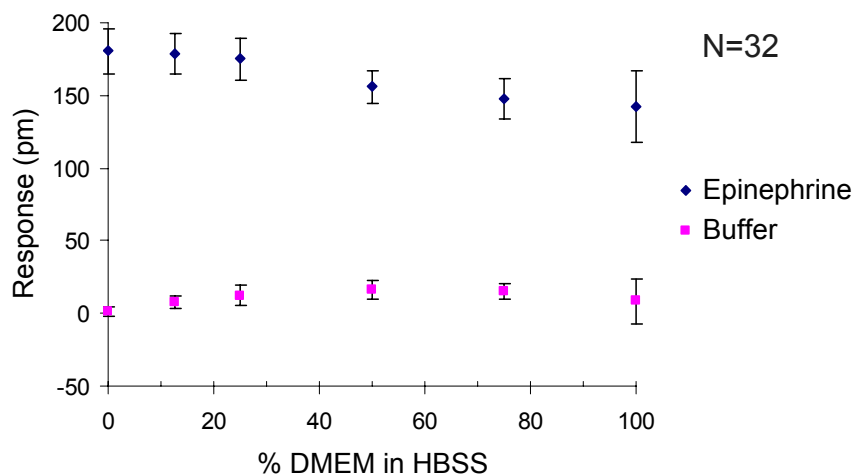
## Epinephrine Dose-Response



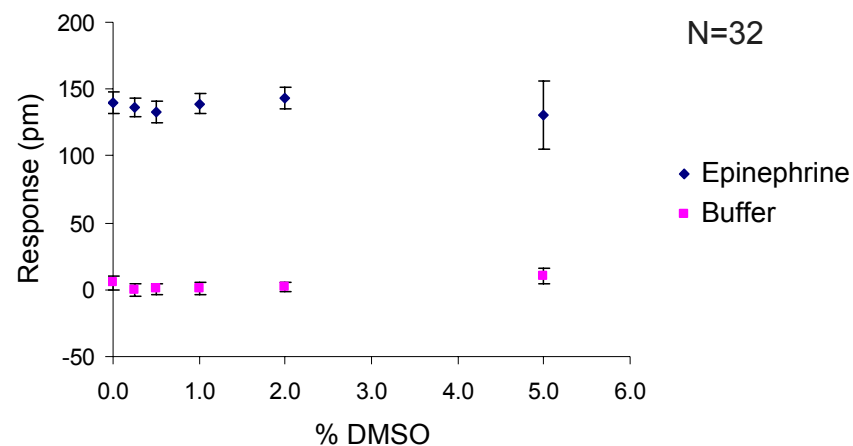
- **Left Panel:** Dose-dependent optical response of epinephrine in A431 cells. Epinephrine was added to A431 cells from 100nM to 3pM. The red arrow indicates the point of epinephrine addition
- **Right Panel:**  $EC_{50}$  estimation using GraphPad Prism. An  $EC_{50}$  value of 0.17nM was calculated.

# 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<sup>®</sup> System

### Cell Seeding Procedure:

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

### Compound Source Plate Preparation:

1. For β<sub>2</sub>AR agonist screen:
  - The LOPAC<sup>™</sup> 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 β<sub>2</sub>AR 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<sup>™</sup> 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<sup>®</sup> microplate.
- All four microplates exhibited similar EC<sub>50</sub> values.
- These values are consistent with the literature value.

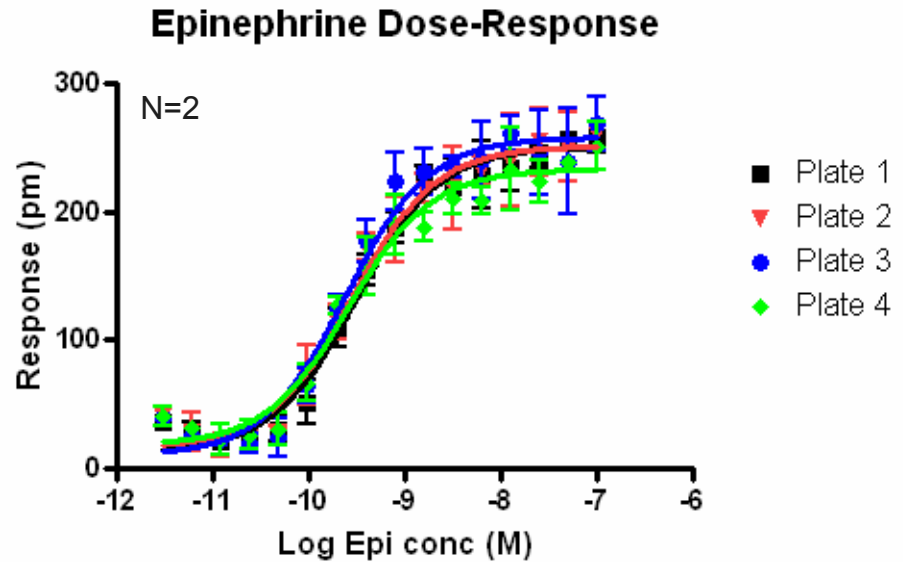


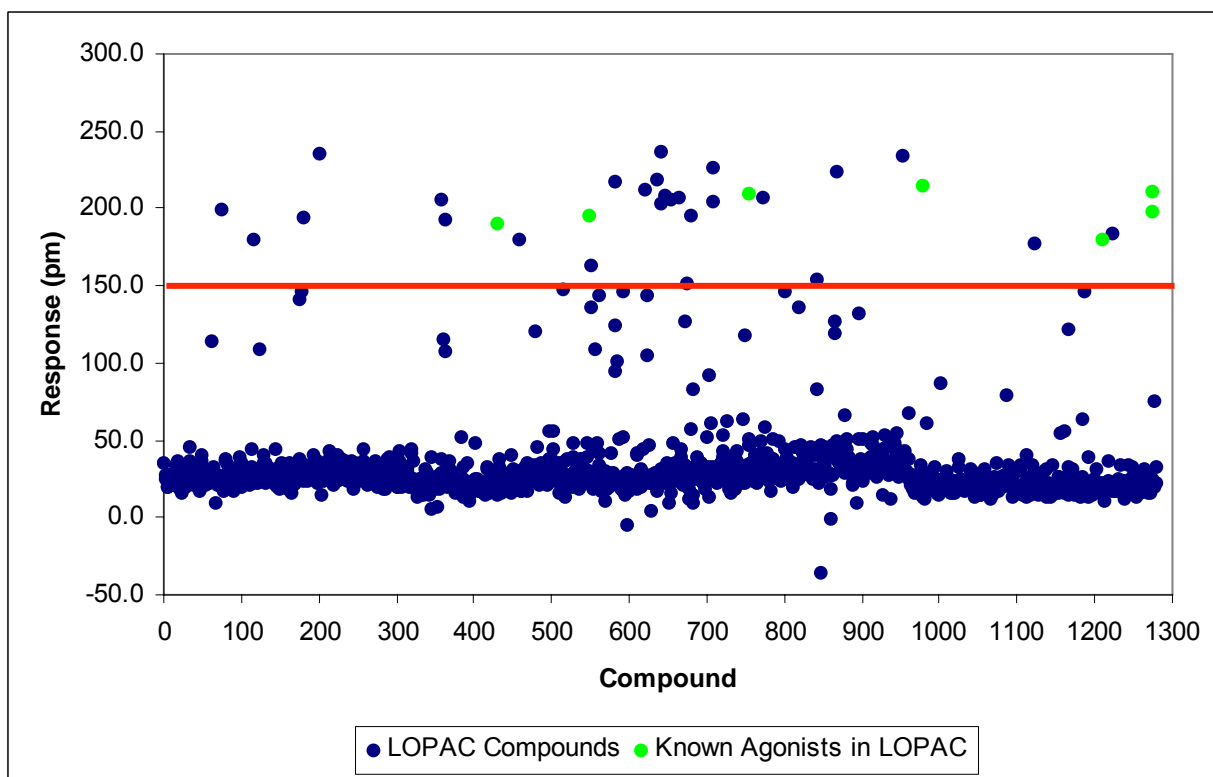
	Plate 1	Plate 2	Plate 3	Plate 4
EC <sub>50</sub>	0.31 nM	0.28 nM	0.24 nM	0.26 nM

# Z' Analysis

- Z' was calculated twice for each Epic<sup>®</sup> 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<sub>2</sub>AR Agonist Screen



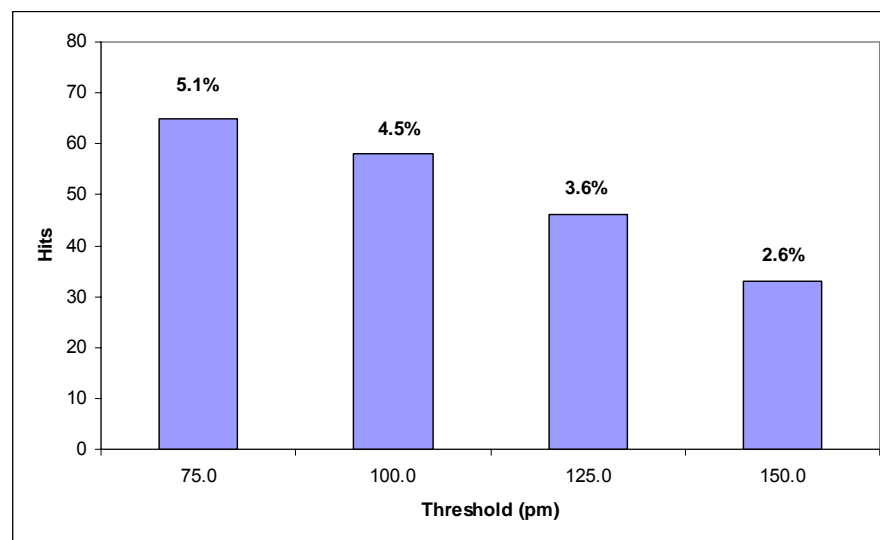
Threshold set at 150pm

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

## $\beta_2$ 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  $\beta_2$ 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

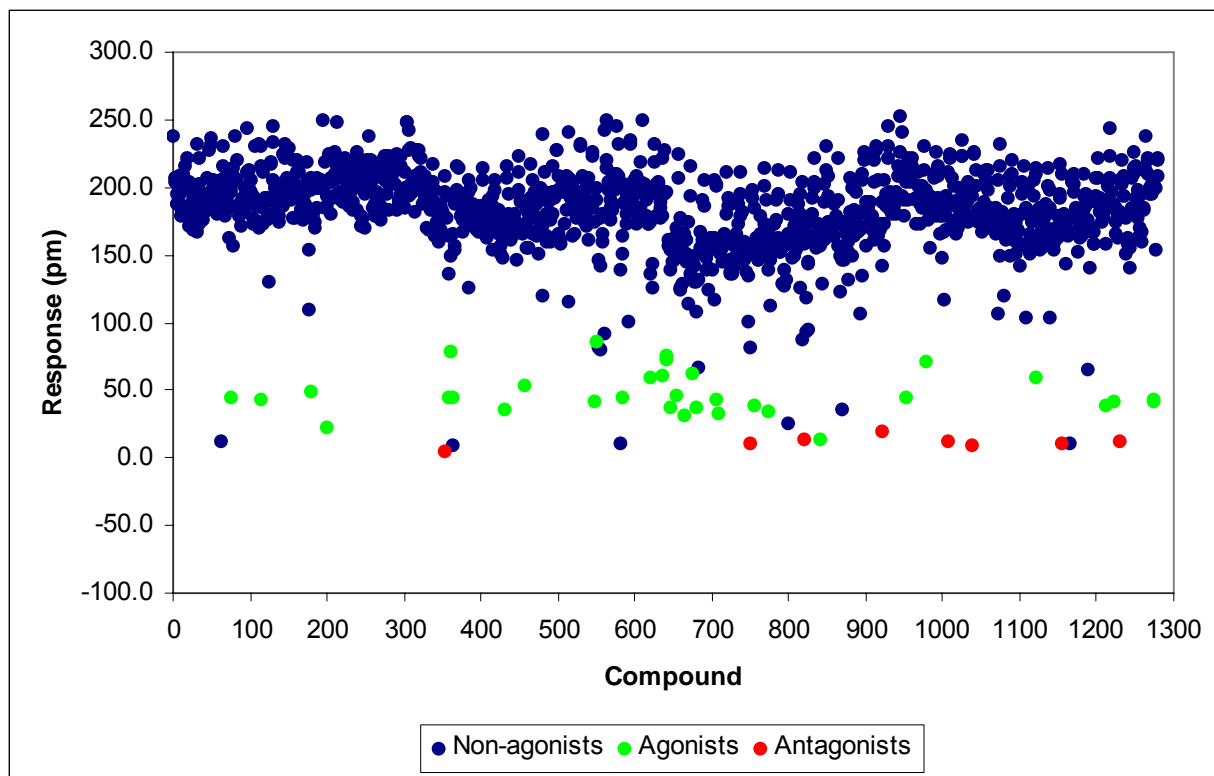


Theshold (pm)	75.0	100.0	125.0	150.0
Total Hits	65	58	46	33
% of Library	5.1%	4.5%	3.6%	2.6%

# β<sub>2</sub>AR Agonist Hits

<u>Name</u>	<u>Class</u>	<u>Action</u>	<u>Selectivity</u>	<u>Description</u>
L(-)-Norepinephrine bitartrate	<b>Adrenoceptor</b>	Agonist	alpha, beta1	Adrenergic neurotransmitter; vasoconstrictor
Phenylephrine hydrochloride	<b>Adrenoceptor</b>	Agonist	alpha1	alpha1 Adrenoceptor agonist; mydriatic; decongestant
R(-)-Isoproterenol (+)-bitartrate	<b>Adrenoceptor</b>	Agonist	beta	Sympathomimetic amine acting almost exclusively on beta adrenoceptors
Isotharine mesylate	<b>Adrenoceptor</b>	Agonist	beta	beta-Adrenoceptor agonist; bronchodilator
(±)-Isoproterenol hydrochloride	<b>Adrenoceptor</b>	Agonist	beta	Sympathomimetic amine acting almost exclusively on beta adrenoceptors; bronchodilator
Nylidrin hydrochloride	<b>Adrenoceptor</b>	Agonist	beta	beta Adrenoceptor agonist; peripheral vasodilator
(-)-Isoproterenol hydrochloride	<b>Adrenoceptor</b>	Agonist	beta	beta-Adrenoceptor agonist; increases cytosolic cAMP
Terbutaline hemisulfate	<b>Adrenoceptor</b>	Agonist	beta	beta-Adrenoceptor agonist; bronchodilator
Tulobuterol hydrochloride	<b>Adrenoceptor</b>	Agonist	beta	beta-Adrenoceptor agonist related to structurally to terbutaline; bronchodilator
Dobutamine hydrochloride	<b>Adrenoceptor</b>	Agonist	beta1	beta1 Adrenoceptor agonist
Fenoterol hydrobromide	<b>Adrenoceptor</b>	Agonist	beta2	Beta2-adrenoceptor agonist; bronchodilator
Formoterol	<b>Adrenoceptor</b>	Agonist	beta2	beta2-Adrenoceptor agonist
Metaproterenol hemisulfate	<b>Adrenoceptor</b>	Agonist	beta2	beta2-Adrenoceptor agonist
Ritodrine hydrochloride	<b>Adrenoceptor</b>	Agonist	beta2	beta2-Adrenoceptor agonist; relaxes uterine muscle contractions
Albuterol hemisulfate	<b>Adrenoceptor</b>	Agonist	beta2	beta Adrenoceptor agonist
Salbutamol	<b>Adrenoceptor</b>	Agonist	beta2	beta2-Adrenoceptor agonist
Salmeterol xinafoate	<b>Adrenoceptor</b>	Agonist	beta2	beta2 Adrenoceptor agonist
BRL 37344 sodium	<b>Adrenoceptor</b>	Agonist	beta3	Selective beta3 adrenoceptor agonist
(±)-Norepinephrine (+)bitartrate	<b>Adrenoceptor</b>	Agonist		Adrenergic neurotransmitter
(-)-Epinephrine bitartrate	<b>Adrenoceptor</b>	Agonist		Endogenous hormone and neurotransmitter
(±)-Epinephrine hydrochloride	<b>Adrenoceptor</b>	Agonist		Adrenoceptor agonist
(-)-alpha-Methylnorepinephrine	<b>Adrenoceptor</b>	Agonist		Active enantiomer; adrenoceptor agonist; vasoconstrictor; antihypertensive
Labetalol hydrochloride	<b>Adrenoceptor</b>	Antagonist	beta	Competitive beta-adrenoceptor antagonist
S(+)-Isoproterenol (+)-bitartrate	<b>Adrenoceptor</b>		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

# $\beta_2$ AR Antagonist Screen



## $\beta_2$ 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  $\beta_2$ 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  $\beta_2$ 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.

# β<sub>2</sub>AR Antagonist Hits

<u>Name</u>	<u>Class</u>	<u>Action</u>	<u>Selectivity</u>	<u>Description</u>
(±)-Propranolol hydrochloride	<b>Adrenoceptor</b>	Antagonist	beta	<b>beta Adrenoceptor antagonist</b> ; cardiac depressant (anti-arrhythmic)
S(-)-Timolol maleate	<b>Adrenoceptor</b>	Antagonist	beta	<b>beta Adrenoceptor antagonist</b> ; antihypertensive; antiarrhythmic; antiglaucoma agent
ICI 118,551 hydrochloride	<b>Adrenoceptor</b>	Antagonist	beta2	<b>Highly selective beta2 adrenoceptor antagonist</b>
(S)-Propranolol hydrochloride	<b>Adrenoceptor</b>	Blocker	beta	<b>Active beta-adrenoceptor receptor blocking enantiomer</b> ; 5-HT <sub>1</sub> serotonin receptor antagonist
(±)-Pindobind	<b>Adrenoceptors</b>	Ligand	beta	Analog of pinolol which contains a bromo-acetyl group capable of alkylation of <b>beta-adrenoceptors</b>
(±)-CPP	Glutamate	Antagonist	NMDA	Potent and selective NMDA glutamate receptor antagonist; anticonvulsant
Propafenone hydrochloride	K <sup>+</sup> Channel	Blocker	hKv1.5	Blocks hKv1.5 and ATP-sensitive K <sup>+</sup> channels; <b>beta-adrenoceptor antagonist</b>
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

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- A431 cells were used to screen the LOPAC™ library for  $\beta_2$ 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  $\beta_2$ 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  $\beta_2$ 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 EC<sub>50</sub> values were observed for each Epic® microplate.
  - EC<sub>50</sub> values ranged from 0.24-0.32nM for the epinephrine-induced response.