# Identification of Potential Pharmacoperones using a Cell-based 1536 Well High Throughput Screening Campaign Capable of Rescuing the Functionality of Mutant Misfolded V2R

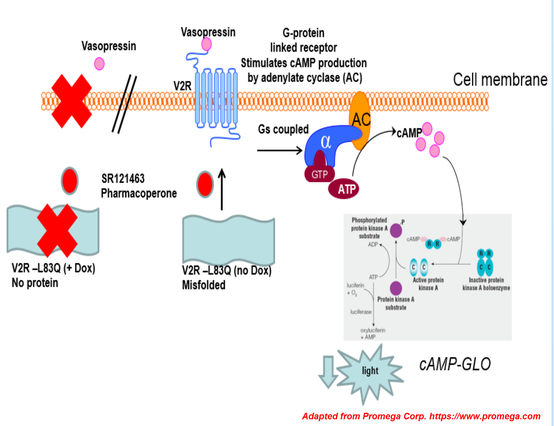

Emery Smith[1], Jody Janovick[2], Louis Scampavia[1], P. Michael Conn[2], Timothy Spicer[1]

[1]The Scripps Research Institute Molecular Screening Center, Scripps Florida, Jupiter, Florida
[2] Texas Tech University Health Sciences Center, Lubbock,Texas


## Abstract

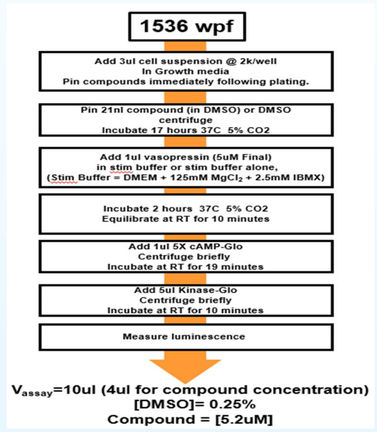

Pharmacoperone drugs correct the structure of misfolded protein mutants and restore function (i.e. "rescue") by correcting the routing of (otherwise) misrouted mutants. Assays specifically for pharmacoperones have not been applied to screen large libraries previously. Currently most pharmacoperones possess intrinsic agonist or antagonist activities since these were identified serendipitously using high throughput screens aimed at discovering direct agonists or antagonists. Here we describe an ultra-high throughput homogeneous luminescence based cAMP detection system designed to specifically identify pharmacoperones of the vasopressin type 2 (V2) receptor (V2R); known to be involved in nephrogenic diabetes insipidus. Development of such assays is important and novel since useful chemical structures with the ability to control cellular trafficking, but lacking intrinsic agonist or antagonist properties, have not likely been identified using existing screens. In the described assay, the level of functional hV2R (mutant) present in each test well is quantitated by stimulation with saturating levels of agonist followed by use of a luminescent-based cyclic adenosine monophosphate (cAMP) assay. This allows the assay to identify compounds which increase the trafficking of mutant hV2R[L83Q] in our model system. We completed a 645K compound HTS campaign including secondary and tertiary assays which yielded 147 compounds with an EC50<5uM. 96 compounds of interest were tested as powders; 83 of which were found active and selective. Here, we describe the process and the results of the V2R HTS campaign.


## 1. V2R cAMP Pharmacoperone Assay Principle



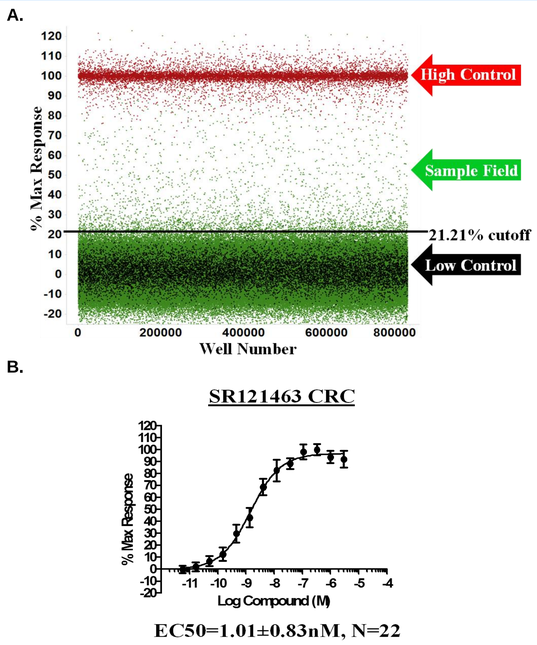

Schematic diagram of cAMP production in cells and the Promega cAMP-Glo™ Max Assay. Binding of vasopressin to V2R alters the conformation of the associated heterotrimeric G protein, causing dissociation of the Gα and Gβγ subunits and initiating a cascade of cellular events. The alpha subunit is categorized into one of several groups: αs, αi/o, αq and α12/13. Gαs activates adenylate cyclase, while Gαi/o inhibits adenylate cyclase activity. As the concentration of cAMP increases, cAMP binds to protein kinase A, and the regulatory subunits undergo a conformational change to release the catalytic subunits. The free catalytic subunits then catalyze the transfer of the terminal phosphate of ATP to a protein kinase A substrate, consuming ATP in the process. The level of remaining ATP is determined using the luciferase-based Kinase-Glo® Reagent. Luminescence is inversely proportional to cAMP levels. Thus, as V2R pharmacoperones cause cAMP concentration to increase, luminescence decreases.

## 2. V2R cAMP Pharmacoperone Automated 1536 well Protocol



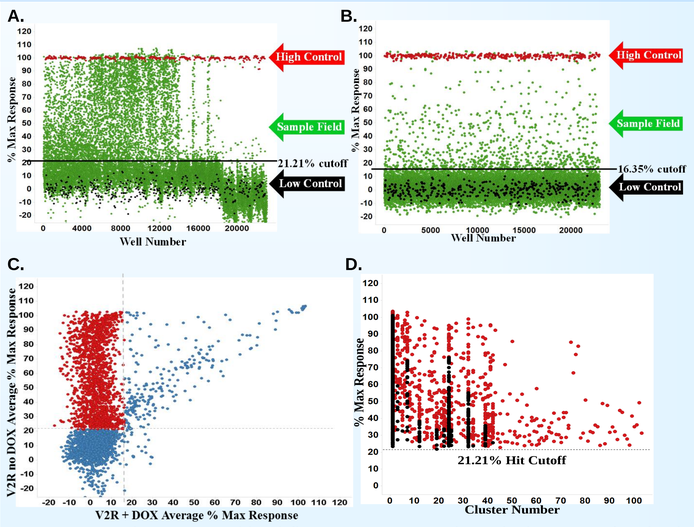

HeLa cells stably expressing L83Q hV2R under the control of a tetracycline-controlled transactivator were cultured in growth media (1× DMEM + 10% FBS and 1 mg/L gentamicin). The cells were cryogenically frozen to maintain the same state of the cells for all batches tested. The frozen cells were thawed on the day of the assay and seeded as described. The counterscreen utilizes the same cells but treated with doxycycline to eliminate receptor expression altogether. The control compound does not respond in the counterscreen (not shown).

## 3. V2R cAMP Pharmacoperone Primary HTS



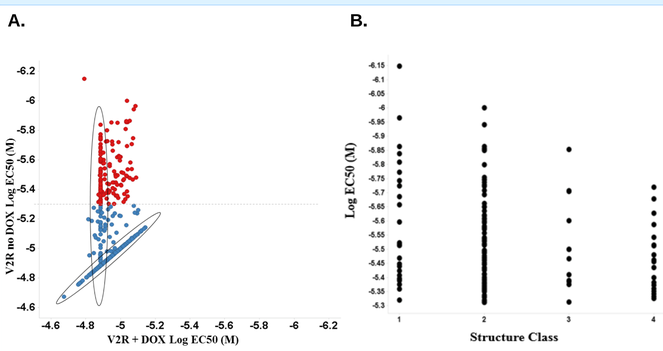

644,951 were screened in singlicate at 5.22uM. The results of the primary HTS are shown in **(A)** with compound wells tested (green dots), and control wells (red dots-SR121463 EC100 high control in 5uM vasopressin and black dots-DMSO in 5uM vasopressin). **(B)** The control compound SR121463, a known antagonist of V2R, was tested during HTS and had EC50 data as shown.

## 4. V2R cAMP Pharmacoperone Confirmation and Counterscreen

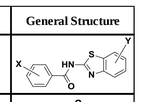
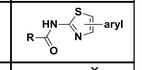
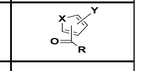



3,734 compounds were tested in triplicate at 5.22uM as shown in the scatterplots for the V2R confirmation **(A)** and V2R counterscreen **(B)**. The active compounds from these screens were compared in a correlation plot **(C)** with 1,694 compounds selectively active in V2R (red dots). These (1,694) were clustered based on structure similarities (D) and the top 640 compounds based on cluster rank analysis are highlighted (red dots).

## 5. V2R cAMP Pharmacoperone Titration Assays




C.

| Structure Class | Class Name | General Structure |
|---|---|---|
| 1 | Aminobenzothiazole benzamide |  |
| 2 | Aryl Aminothiazole Amide |  |
| 3 | Heteroaryl Amide |  |
| 4 | Other | |

664 compounds were tested in 10 point 1:3 dilution titration assays at 13.4uM starting concentration. **(A)**. EC50s of all dose responses tested. Dashed lines represent activity cutoff parameters for each assay tested. V2R selectivity is demonstrated (red dots). Some compounds (circled) do not reach EC50 even at highest concentration tested. **(B+C)** 147 Selective compounds can be divided into 4 structural classes. **(B)** The EC50s of the compounds in each class. **(C)** The basic scaffolds of each class.

## 6. V2R cAMP Pharmacoperone HTS Project Statistics

| Step | Screen type | Target | Number of compounds tested | Selection criteria | Number of selected compounds (Hit rate ) | Assay statistics Z' | Assay statistics S/B |
|---|---|---|---|---|---|---|---|
| 1 | Primary screen | V2R | 644,951 | 21.21% [a] | 3,472 (0.54%) | 0.73 ±0.09 | 4.83 ±0.84 |
| 2a | Confirmation screen | V2R | 3,734 | 21.21%[b] | 1,930 (51.69%) | 0.80 ±0.03 | 5.75 ±0.14 |
| 2b | Counterscreen screen | V2R + DOX | 3,734 | 16.35 %[c] | 256 (6.86%) | 0.84 ±0.03 | 7.08 ±0.32 |
| 3a | Dose Response Screen | V2R | 664 | EC50<5uM | 147 (22.14%) | 0.84 ±0.02 | 7.64 ±1.42 |
| 3b | Dose Response Counterscreen | V2R + DOX | 664 | EC50<5uM | 0 (0%) | 0.79 ±0.04 | 5.86 ±0.99 |
| 4a | Powders Dose Response | V2R | 96 | EC50<5uM | 83 (86%) | 0.79 ±0.04 | 8.28 ±0.22 |
| 4b | Powders Dose Response | V2R + DOX | 96 | EC50<5uM | 0 (0%) | 0.81 ±0.01 | 8.38 ±0.64 |

[a] The primary screen hit-cutoff was calculated using the Interval based cutoff.
[b] The primary hit cutoff was used
[c] The counterscreen hit cutoff was calculated by using the average + 3 standard deviations of the DMSO sample field plates, n=6 plates.

## 7. V2R cAMP Pharmacoperone Conclusions and Next Steps

- The V2R cAMP pharmacoperone assay was screened against the Scripps Drug Diversity Library (SDDL). 147 compounds were found to selectively activate the receptor and, in turn, allow vasopressin to activate the receptor to trigger the cAMP pathway.
- The 147 selective compounds break into 4 distinct classes that will be studied further.
- 96 of the compounds are being pursued in secondary assays to confirm pharmacoperone activity
- Previous V2R pharmacoperones show antagonist activity in the wild type V2R receptor. The same 96 compounds will be tested for their antagonist response, targeting compounds that have little to no antagonist traits.

## 8. V2R cAMP Pharmacoperone References


1. Conn PM, Smith E. High Throughput Screen for Pharmacoperones of the Vasopressin Type 2 Receptor. J. Biomol Screen. 2013 Sep; 18(8):930-7.
2. Conn PM, Spicer TP, Scampavia L, Janovick JA. Assay Stratagies for Identification of Therapeutic Leads That Target Protein Trafficking. Trends Pharmacol Sci. 2015 Aug: 36(8): 498-505.


## 9. V2R cAMP Pharmacoperone Acknowledgements


- Funding through NIH grant ID#5R01DK099090-04
- Thanks to Justin Shumate, Pierre Baillargeon and Lina Deluca for the assistance with robotics and compound management.


- *This poster can be viewed online by scanning the following QR Code*