Use of a Label-Free Platform in a preclinical Contract Research Organization Environment

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Introduction

Caliper Discovery Alliances & Services (CDAS) is a Contract Research Organization offering comprehensive *in vitro* and *in vivo* preclinical contract research services. Our offering is articulated around different platforms including Target Validation with the creation of transgenic animals, Assay Development, Screening, Safety Pharmacology, Biochemical and Functional cell based profiling, Drug Efficacy in disease models and non-invasive small animal Optical Imaging.
NovaScreen Biosciences, now part of CDAS, pioneered receptor profiling studies in the early 1990's and has established a best in class safety pharmacology platform with a large number of GPCR binding assays. Functional studies are very complementary to affinity-based radioligand assays as they provide very valuable information on the way a drug interacts with a GPCR target. Using CDAS GPCR*Functional*™ programs, you can determine if a drug is an activator or an inhibitor or what its potency and efficacy are for a given GPCR target. Understanding drug functionality is also an important benefit of functional studies and you can for example identify allosteric modulators that are generally difficult to identify using orthosteric ligand-based radioligand binding assays.

We have created GPCRFunctional™ Label Free service programs to offer more relevant assay development, screening, drug activity and receptor characterization studies in a more physiological cellular context.

Problem Statement

The challenge before us was to develop a substantial list of cell-based functional assays for a wide variety of receptor types in a manner that maximized scientific value and provided the broadest range of applications such as receptor de-orphanization, accurate mode of action and pharmacology studies including screening and selectivity. We desired a universal method for all GPCRs that was direct, did not require genetic manipulation, the systematic use of commercially available recombinant cell lines, or that would measure one parameter only such as the accumulation of cAMP or a flux of calcium that can give a partial representation only of multiple signal transduction pathways originating from a single GPCR.

Previous Options

Prior to the availability of the various plate-based SPR or impedance label-free instruments, there were many methods that were required to assay the various types of receptor-based "second messengers". These included Ca flux from intracellular stores (Gq GPCRs), cAMP measurement (Gi and Gs GPCRs), membrane potential (ligand-gated ion channels), ion flux (all ion channels), and GTPgS binding (applicable to all GPCRs, but popularly applied to Gi GPCRs). While these methods can be performed with primary and immortalized cells, in many cases the response measured with such cells gave marginal assay windows and prompted the investment in recombinant cells. Further, these assays are typically laborious, involved the use of radioactivity or antibodies, or were susceptible to artifactual effects. Alternative approaches involving genetic manipulations to the G-protein or addition of reporter genes create larger assay windows and

high throughput screening capability but are burdened with a high buy-in price for organizations such as ours that offer broad target profiling.

Solution

Maintaining all of these various methods and capabilities is far from ideal. A better answer is to incorporate a platform with broad applicability, such as the ability to perform assays on both recombinant and primary cells. With the capability to use recombinant or primary cells, either adherent or in suspension, that express Gi, Gq, Gs, G15/16, receptor tyrosine kinases (RTKs) or ligand–gated ion channels (LGICs), the new plate-based label-free instruments offer a near universal platform for functional cellular responses. The added capability of performing biochemical binding assays on the same platform is a further additional bonus offered by the surface plasmon-resonance based systems like Corning's Epic and the SRU BIND®.

Because cell-based label-free assays are fairly novel and not yet entirely integrated in to the drug discovery industry, we have chosen to initially focus on the use of recombinantly expressed receptors. Combined with the proper selection of the parental cell, there is a minimized chance of interference from other non-target receptor interactions. This greatly increases the certainty that the measured effect is a consequence of an interaction with the expressed recombinant protein and not from other interactions. Of course, the parental cells are also available for testing, should confirmation be needed. CDAS is in the process of pharmacologically characterizing many GPCRs in classical monometric functional assays (cAMP or Ca-flux) and correlating those results with those from the holistic cellular label-free assay. In all cases reviewed to date, the rank orders of potency were the same, though the outright potencies at some receptors changes. These results will be published in the near future.

Broad Receptor Applicability

The promise of these instruments of broad receptor applicability was put to the test early on. Native cell lines, which are known to have certain receptor expression from various other sources, were panned by a substantial small molecule agonist library. The library consisted of 90 known agonists, with multiple agonists for a number of receptor families. The combination of a measured response with the kinetic profile of that response indicated when certain receptors or receptor families were activated, and what G-protein or proteins were involved in the signal transduction. The library included nicotine, an agonist at both the well-known ligand-gated ion channel (nicotinic cholinergic) and a newly discovered GPCR (GPR109a).
Below is a bar chart of the early responses from a single early time point (5 min) of the kinetic read in a human neuroblastoma cell line. Note the upward responses are likely Gq while the downward responses to opioid agonists indicate their typical Gi coupling. Agonists that are known to interact with Gs GPCRs caused responses that were more fully developed in the 30 minute time frame than in this early time frame.
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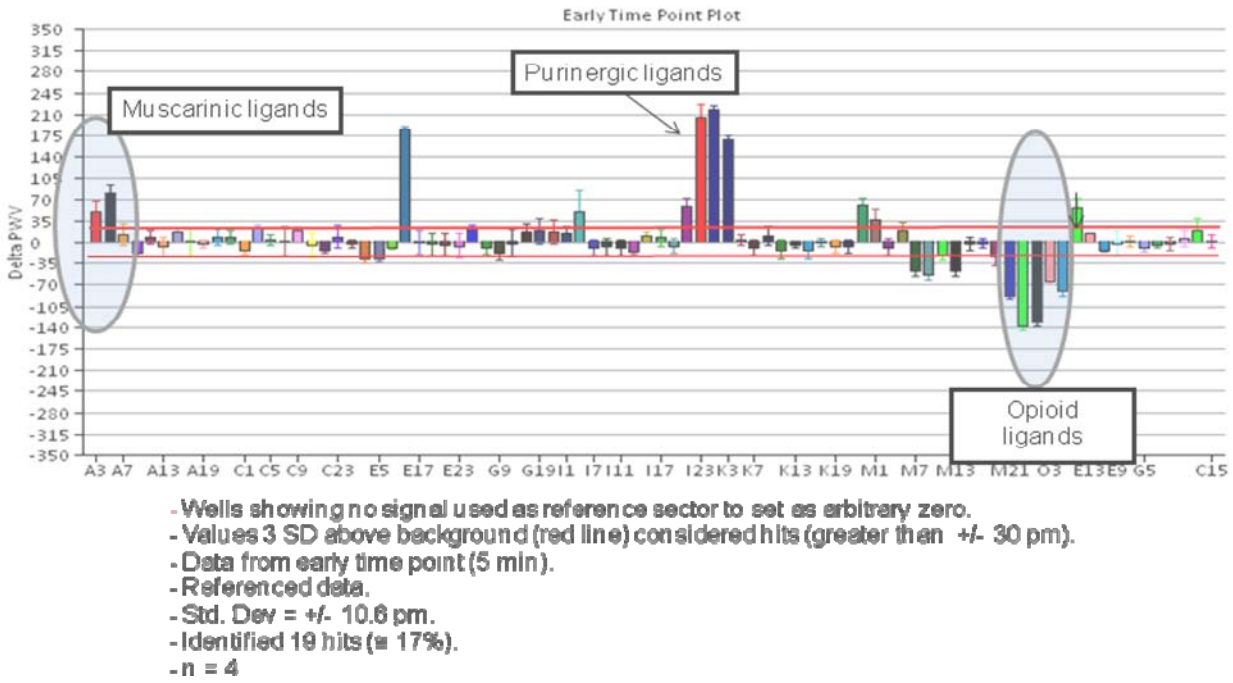

Singular Platform from Assay Development to HTS

With its fast plate reading time of < 1 minute for 384-well plates and a very accessible plate tray, the BIND instrument is easily incorporated into a high throughput screening (HTS) application. It is readily integrated with various liquid handling systems and plate feeders to more fully maximize its screening potential. At the same time, the instrument control software also provides a means to adjust what wells are read, and how often, so that assay development is readily accomplished using the same instrument and plates that are used for HTS. Thus, the process from assay development to HTS is short and direct.

Implementation

We are establishing a panel of functional receptor assays using recombinantly expressed cell lines so that we provide data with a high degree of confidence. This approach minimizes the chance of off-target effects being misinterpreted as on-target responses. Multiple agonist and antagonist responses are characterized for each receptor using known reference agents. These responses are compared to the results from other methods that are determined in house, from publications, or both.

CDAS is now able to offer functional assays for most of the GPCR targets on our popular General Side Effect Profiling panels (GEN SEP). Those safety pharmacology panels have become industry standards for drug profiling at early stages to identify possible liability issues, off-target effects or to provide relevant SAR information prior to submission of NDE applications to the FDA. The enhancement of follow-on functional assays (many using the same tissue source as the binding assay) further strengthens our safety pharmacology programs.

Additionally, we continue to gain experience with human derived primary and immortalized cell lines, and offer assays based on such endogenously expressed targets on a custom basis. These assays attain an even greater degree of physiological relevance while adding some risk of false positives from off-target interactions. Further pharmacological experiments can be performed at

CDAS by using appropriate selections of our over 1000 optimized in vitro assays to further refine and define the results from such assay systems.

Summary

The panel of available functional GPCR assays at CDAS is growing rapidly, largely due to the incorporation and strengths of the BIND® platform. The system provides a high degree of physiological relevance to our cellular functional assays along with artifact-free results, near-universal application regardless of the second messengers involved, and true HTS capability. Such features allow us to offer a substantial increase in value and breadth of services to our customers.

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