eurofins | DISCOVERY

CASE STUDY

IDENTIFYING ALLOSTERIC MODULATORS AND BIASED AGONISTS FOR CLINICALLY RELEVANT GPCRS

Case Studies Using Eurofins Discovery's GPCR Product Solutions

ABSTRACT

G-protein-coupled receptors (GPRCs) are a vast protein family involved in nearly every aspect of human disease. Currently, one-third of all FDA approved drugs target GPCRs and the sheer number of understudied and orphan receptors in the family have made them an attractive target for many drug discovery programs. Most GPCRs are able to initiate multiple downstream signaling pathways, motivating pharmaceutical companies of all sizes to explore new biased agonists and/or allosteric modulators that can selectively activate pathways or provide activity dependent modulation. These targeted clinical candidates hold great therapeutic potential and have the potential to minimize side-effects associated with unbiased orthosteric GPCR activation. However, biased agonism and allosterics are immensely complex, in part due to the advanced methods and tools needed to characterize compound pharmacology as well as the need to understand therapeutic relevance. Developing methods to measure signaling bias has proven to be a major barrier in the development of new GPCR therapies – a barrier that Eurofins Discovery has actively worked to deconstruct. By leveraging their pipeline speed, expertise, end-to-end problem solving, and flexible partnerships, Eurofins Discovery has helped a wide number of teams, both large and small, overcome this challenge efficiently and effectively.

By adopting Eurofins Discovery GPCR screening and profiling services, clients were able to:

- Access high-throughput GPCR assays with rapid turnaround times
- Quickly develop and validate exclusive GPCR assays not offered by other CROs
- Solve complex research questions and data analysis challenges
- Work with a collaborative partner with experience in a variety of business models

THE CHALLENGE: DEVELOPING SPECIFIC THERAPEUTIC GPCR MODULATORS WITH BIASED DOWNSTREAM SIGNALING

Without question, G protein-coupled receptors (GPCRs) are the most targeted protein family for drug development. Approximately 34% of all FDA-approved drugs target GPCRs, amounting to ~27% of the global therapeutic market share.[1] GPCRs make up the largest human membrane protein family (~800 total) and – through their prominence on cell surfaces – they play a pivotal role in extracellular stimuli-sensing. This allows them to regulate a multitude of signaling cascades through recognition of hormones and neurotransmitters, as well as growth factors, light, flavors, and odors.[2,3,7] Furthermore, their accessibility on the cell surface makes them particularly well-suited to drug development, as potential therapeutics do not need to traverse the cell membrane to be effective.[1]

For these reasons, many research endeavors are focused on developing new GPCR therapies and identifying additional druggable GPCRs. Historically, GPCR-targeted drugs have been used as analgesics and to treat hypertension, allergy, schizophrenia, and depression. More recently, they have expanded into other therapeutic areas such as oncology, Alzheimer's disease, obesity, and diabetes.[1] Even with these existing drugs, the size of the GPCR family and the diverse number of human disease associations offer significantly more opportunities to develop novel therapeutics.

Traditional approaches for drug development have focused on identifying molecules that bind and activate a specific GPCR target. Considerations for selectivity, off-target activity, and side effects are typically centered on related GPCR family members and liability targets (such as the ion channel, hERG, which is critical for coordinating cardiac muscle contraction).[4] As our understanding of GPCR cell

biology increases, it is becoming apparent that selectivity and side effects are issues that also relate to the target receptor itself as most GPCRs are capable of initiating multiple downstream signaling pathways that can have differing therapeutic consequences.[5,6] G protein-dependent signaling, which modulates the level of secondary messengers like cAMP and calcium, regulates downstream pathways like PKA, PKC, and CaM Kinase.[7] The extent of secondary messenger signaling is regulated by β-Arrestin, which is recruited to the GPCR upon activation, blocking further signaling and mediating receptor recycling via endocytosis.[8] β-Arrestin also directly modulates signaling, like the MAPK cascade, thereby able to regulate cellular responses in a G-protein-independent manner.

It is believed that drug development efforts that are able to achieve GPCR specificity and/or biased signaling are likely to minimize off-target side effects. An important example is the pharmacological use of Opiates for pain control. Despite their well-documented properties as analgesics, this class of drug exhibits major side effects, such as constipation and respiration depression. The effects can be so severe that dosage is often reduced, thereby compromising the ability to alleviate acute pain.[9,10,11] These negative results are largely driven via β-arrestin mediated signaling pathways and are often associated to opioid receptors that are localized distal (such as the gut and lungs) to the pain site.[4] Both allosteric and biased ligand molecules offer the potential to minimize side effects. Since allosteric molecules act only when the receptor is also activated by a ligand, they enhance the action of natural endorphins released in response to pain, whilst having no impact on other regions of the body where opioid receptors are expressed.[10] Biased ligands that preferentially signal via the G_i-coupled cAMP pathway also offer the potential for reduced side effects. Whilst these molecules will activate opioid receptors systemically, the absence of signaling via β-Arrestin mediated pathways will minimize the associated side effects in gut and lung localized receptors.[11]

As such, drug development programs that can identify biased agonists and/or allosteric modulators offer great potential, despite significant challenges in developing such molecules. Combining signaling bias with typical therapeutic considerations, such as potency, selectivity, and pharmacokinetics, adds a significant level of complexity to discovery projects. Thus, as research endeavors expand their focus on the development of additional GPCR-based treatments there is significant need for screening and profiling platforms with the capability to rapidly identify novel chemotypes in existing chemical libraries that exhibit biased or allosteric properties, without inflating drug development timelines and costs.

THE APPROACH: EUROFINS DISCOVERY GPCR PLATFORM

To accommodate this unmet need, Eurofins Discovery has developed a suite of GPCR product solutions. These offerings, which include PathHunter® screening and profiling services, approach the challenge of the GPCRome through broad coverage, relevant assay development, and an industry leading number of GPCR assays (>1600) that can access GPCR signaling through six discrete mechanisms:

- cAMP signaling
- Calcium flux
- GTP binding
- β-Arrestin signaling
- Receptor internalization
- Cellular trafficking

Through the application of Eurofins Discovery's GPCR platform, the program provides clients with unmatched speed, field expertise, end-to-end solutions, and a flexible partnership to address their challenges. Below are several case studies that demonstrate the process of partnering with Eurofins Discovery to identify new GPCR-based therapies.

UNMATCHED SPEED IN GPCR SCREENING AND LEAD OPTIMIZATION REDUCES DEVELOPMENT TIMELINES

Given the interest in GPCR therapies across the pharmaceutical community, ensuring rapid data collection and assessment is crucial to success. Recently, Eurofins Discovery partnered with a GPCR-focused pharmaceutical client that sought to identify novel chemotypes for an emerging GPCR subfamily. To accomplish their goals, this company sought to assess GPCR signaling across eight distinct experimental assays. Eurofins Discovery already had six of the eight assays available for immediate use, whereas competing CROs did not have any. Naturally, this advantage offered a rapid start-up time for the collection of GPCR screening data.

Furthermore, as Eurofins was developing the remaining two assays, the company simultaneously performed high-throughput screening (HTS) using the existing six methods. Specifically, they screened a 100,000-compound library in just six days per target (Table 1) with an average hit rate of 0.43%

Criteria	Metrics
Compounds per screen	100,000
Average hit rate	0.43%
Average turnaround	6 days
Average Z prime	0.59
Number of screens	6
Time to completion for program	6 weeks

Table 1: High-throughput GPCR screening turnaround and assay quality.

(430 hits/100K screen) and an excellent Z′ average (0.59). This remarkable rate drove the completion of all six screens in a total of six weeks, compared with ~90 days at other CROs. Eurofins Discovery views assay validation as an integral part of the service and therefore emphasized rapid validation in parallel. Through this, they were able to validate an assay within 10 days – four times faster than the norms for other CROs.

While speed is obviously a critical feature for large screening endeavors, drug developers also need rapid lead optimization. As an example of this, a large, publicly traded US pharmaceutical company partnered with Eurofins to rapidly characterize each lead compound's function against key GPCR targets. Given the diversity of Eurofins' GPCR assays, the client did not need to contract other CROs, streamlining the process. This company also requested rapidly collected functional data to guide and inform their chemists' syntheses and library development, while minimizing delays during new molecule development. While Eurofins offers lead optimization with rapid turnaround (five days vs 10 at other CROs), they were able to reduce this turnaround to an average of three days for this particular client through sample delivery coordination, advanced scheduling, and a high first-time pass rate of 93% (Table 2). With flexible coordination, Eurofins was able to streamline the lead optimization pipeline for its client, allowing them to more rapidly work towards a clinical candidate.

Criteria	Metrics
Sample submission	Weekly
Required turnaround	5 days
Average turnaround	3 days
On-time delivery	>95%
First pass rate	93%
Z prime	0.75
Project deliverables	Antagonist second messenger dose response
Length of program	12 months to date

Table 2: Lead optimization timelines and success rates

EUROFINS GPCR SCIENTIFIC EXPERTISE ENABLES CUTTING-EDGE APPROACHES TO GPCR DRUG DEVELOPMENT

Given the enormous body of GPCR research and its rapidly expanding directions, there is obvious value to working with experts in the field. Rather than simply providing skilled labor, Eurofins offers overarching scientific expertise that can deliver solutions-focused insights into challenging experimental problems. An example of this is Eurofins' recent partnership with a team from a major US research group to study allosteric regulators of GPCRs. The use of allosteric modulators is one of the more cutting-edge areas of GPCR research.[1,12]

As current GPCR research centers are focused on controlling the signaling output at particular GPCRs, allosteric modulation offers opportunities to regulate agonism and signaling bias through allosteric sites, rather than canonical binding pockets. The ability to control specific signaling pathways and/or enhance agonism through allosteric regulation allows researchers to use existing unbiased GPCR agonists to achieve biased signaling. However, investigating multi-site binding, especially when one of those sites is a non-competitive allosteric switch for the receptor, adds experimental complexity to data output.

Despite this challenge, Eurofins Discovery was able to work with allosteric modulators and apply advanced data analysis, such as EC_{50} shift analysis, to provide quality output without deviating from agreed upon timelines. Even with the additional considerations brought on through allosterics, Eurofins Discovery maintained a seven-day turnaround (Table 3), with a 95% first pass rate and robust assay performance (Z′ > 0.6). Thanks to the quality and pace of the allosteric data collection and analysis delivered by Eurofins experts, this client has continued their partnership for over two years.

Criteria	Metrics
Sample submission	Weekly with flexible delivery
Required turnaround	7 days
Average turnaround	7 days
On-time delivery	>98%
First pass rate	95%
Z prime	>0.6
Project deliverables	Allosteric modulation second messenger dose response
Length of program	>24 months to date

Table 3: Eurofins' GPCR expertise enables rapid turnaround, even with complex research challenges and data analysis

EUROFINS DISCOVERY POWERS END-TO-END SUPPORT IN GPCR THERAPEUTIC DEVELOPMENT

Eurofins Discovery is able to combine its rapid pace and technical expertise to provide end-to-end solutions through collaborative partnerships. In line with this, a major international pharmaceutical company reached out to Eurofins to construct a complete signaling pathway portfolio, including measuring cAMP signaling, receptor internalization, and β-Arrestin signaling to assess signaling bias for a large compound library. To accomplish this feat, they needed a specific reporter cell line able to look at two critical pathways simultaneously. This would enable rapid identification of biased agonists with the potential to avoid in vivo side effects. The Eurofins Discovery team was able to develop an innovative cell line that could measure cAMP signaling and β-Arrestin recruitment as well as validate the necessary HTS assays. The resulting HTS campaign allowed Eurofins to complete HTS (Table 4) with an average hit rate of 0.77% in the 100,000 compounds screened.

The HTS data was confirmed using orthogonal methods and pushed towards a lead optimization program using the validated cell line and assay (Table 5). All data was seamlessly incorporated into the client's data management system to allow direct comparisons to other data and for future R&D endeavors. As the project developed, there were opportunities to improve turnaround by addressing compound logistics. The Eurofins Discovery team arranged for direct delivery of samples from a contract compound manufacturer located in China, saving approximately three weeks in the overall data delivery cycle.

The Eurofins Discovery team's ability to meet their partner's diverse challenges at a variety of steps, demonstrates their flexibility and end-to-end service. For this reason, this partnership has remained strong for 3 years.

Criteria	Metrics
Data points screened	100,000
Average hit rate	0.77%
Turnaround	3 days
Average Z prime	0.64
Number of assays	1
Time to completion for program	6 weeks

Table 4: GPCR HTS turnaround and assay quality

Criteria	Metrics
Sample submission	Weekly with flexible delivery
Required turnaround	5 days
Average turnaround	3 days
On-time delivery	>95%
First pass rate	95%
Project deliverables	Cell line development HTS SAR / Lead Op
Length of program	3 years

Table 5: End-to-end support provides rapid problem solving and high success rate

EUROFINS DISCOVERY APPROACHES AS A TRUE COLLABORATIVE PARTNER

In addition to the rapid workflows, GPCR expertise, and start-to-finish service, Eurofins also offers clients a unique benefit: genuine partnership. Eurofins shares a passion for GPCR research, given the tremendous opportunities it holds for humankind. As a result of this belief, Eurofins can wear any hat to help their partners succeed. Recently, Eurofins partnered with a small, virtual GPCR drug discovery company to help them investigate biased ligands in a critically important therapeutic area where Eurofins has significant experience through their GPCR PathHunter® cell offerings.[10,13] While this startup had technical knowledge and virtual capabilities, they lacked a compound library for exploring ligand bias. Eager to work with this promising company, Eurofins took on the logistical tasks of sourcing a compound library and housing it on-site, eventually performing HTS and generating up to 500,000 data points over the course of the program. Eurofins maintained their usual rapid turnaround time (Table 6), then moved forward to develop the cell lines and assays needed for confirmation, along with SAR and lead optimization studies (Table 7). Despite the unique needs of their partner, Eurofins was able to manage large-scale logistics in addition to their products, services, and problem-solving expertise.

Criteria	Metrics
Data points screened	200,000
Average hit rate	0.34%
Turnaround	8 days
Average Z prime	0.62
Number of assays	4

Table 6: HTS on novel compound library sourced for the client

Criteria	Metrics
Sample submission	Weekly with flexible delivery
Required turnaround	10 days
Average turnaround	8 days
On-time delivery	>95%
First pass rate	95%
Project deliverables	Cell line development HTS SAR / Lead Op
Length of program	2 years

Table 7: Lead optimization following novel library screening

ACCELERATING GPCR SCREENING WITH EUROFINS DISCOVERY'S SPEED, EXPERTISE, END-TO-END SOLUTIONS AND PARTNERSHIPS.

Eurofins' GPCR platform offers incredible speed to clients seeking rapid solutions and data collection. Surprisingly, the company successfully pairs this with unmatched flexibility, in part due to the quality of their experienced GPCR scientific and management teams. In addition, partnering with the Eurofins Discovery team enables end-to-end problem solving and collaboration, which helps maintain the company's attitude toward developing a true sense of partnership. Despite the continued challenges associated with studying this enormous protein family and developing biased GPCR signaling, Eurofins continues to deliver for their partners, opening up avenues towards improved scientific understanding, new drug targets, and emerging therapeutics.

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