The GPCR mode of action of many currently marketed drugs was not known when they were first discovered, but today a more focused, knowledge-based approach can be taken to GPCR-based drug discovery. Pam Barnacal looks at the activities of some of the companies at the forefront of GPCR research.

GPCR Structure and Function
GPCRs are transmembrane proteins that are expressed in every type of cell in the body and control a wide variety of physiological processes – such as neurotransmission, chemotaxis, inflammation and cell proliferation. The superfamily of GPCRs exhibit a common structure consisting of seven membrane-spanning regions. The binding of a signalling molecule to a GPCR results in G protein activation, which in turn triggers the production of any number of second messenger molecules inside the cell. Second messenger signalling GPCRs carry information within cells via two major pathways: regulation of cAMP levels and increases in intracellular Ca^2+ triggered by inositol (1,4,5) tri-phosphate (IP3). These signalling pathways are activated by the specific G protein associated with the receptor: Gs- and Gi-coupled receptors result in variations of cAMP, while Gq-coupled GPCRs activate phospholipase C (PLC) and trigger the inositol phosphate cascade.

As well as G-protein dependent second messenger activation, GPCR stimulation also results in G-protein independent signalling events, such as the recruitment and internalisation of arrestins; these regulate GPCR activity by blocking GPCR coupling to G proteins, thereby having an impact on both the efficacy and potential side effects of GPCR-targeted drugs.

Through this sequence of events, GPCRs play a critical role in mediating signal transduction, and faulty GPCR signalling is implicated in several disease areas including cancer, inflammation, heart disease and pain. GPCR-based drugs work by either activating or inhibiting the intracellular signalling pathways at the receptor, mediating both beneficial and adverse effects. Of the over 200 GPCRs encoded in the human genome, around 130 have proven intractable to traditional small molecule and antibody drug approaches, and these so-called ‘orphan receptors’ are the subject of extensive research as targets for the next generation of GPCR drugs.

Recent years have seen a revival in GPCR-based research. Several small biopharm companies have been formed on the basis of proprietary technologies for the development of GPCR-based drugs, while reagent providers have made available an extensive range of tools and technologies for use by GPCR researchers. The activities of some of these companies are outlined below.

**Trevena, Inc**
Trevena (King of Prussia, PA, US) is focused on the discovery and development of GPCR ‘biased ligands’. Many GPCR-based drugs have limited efficacy and undesirable adverse effects that can prevent their broader use; moreover, many GPCRs linked to disease cannot be translated into medicines because of specific target-related adverse effects. The reason for this is that nearly all GPCRs are known to be linked to at least two major intracellular signalling pathways, with different biological responses – beneficial and adverse – associated with each pathway. When a biased ligand binds to the receptor, it activates one of these pathways while deactivating the other (in contrast to
the conventional industry approach of activating or deactivating all signalling pathways).

By specifically activating selected receptor signals, Trevena is able to create agents with increased efficacy and/or decreased adverse effects. The company’s expertise lies in understanding which signalling pathways downstream of a GPCR are associated with beneficial versus adverse effects, and then engineering biased ligands that activate only the beneficial pathways. This biased ligand approach builds on research at the laboratories of Robert Lefkowitz, MD, and Howard Rockman, MD, at the Duke University Medical Center (Durham, NC), and has potential across a wide range of receptors and therapeutic areas.

Trevena’s emerging pipeline of biased ligand drugs is currently focused on cardiovascular and CNS diseases that have significant unmet medical need. Its most advanced compound, TRV120027, is a β-arrestin biased ligand of the angiotensin receptor (AT1R) in development for the treatment of acute heart failure. The compound successfully completed a Phase 1 healthy volunteer study in early 2010, and is undergoing Phase 2 studies. The company also has two different opioid receptor biased ligand programmes for pain indications currently in lead optimisation. Other early-stage research activities are focused on inflammation.

**Dimerix Bioscience**

Dimerix (South Yarra, Australia) has developed a proprietary platform capability – known as the GPCR Heteromer Identification Technology (GPCR-HIT) – for the identification of complexes formed by GPCRs known as GPCR heteromers. Research has demonstrated that a number of GPCRs are capable of forming heteromers from a combination of two or more individual GPCR subunits. The ligand-binding properties and intracellular signalling pathways of GPCR heteromers usually show elements from both parent receptors, but they may also produce quite unexpected pharmacological effects, making them an important focus for the discovery of novel GPCR-based drugs.

The Dimerix platform enables the identification and development of optimal lead compounds that target GPCR heteromers, and has applications at multiple stages of the drug development process – the end result being the creation of drugs with improved efficacy and safety profiles. The GPCR-HIT platform includes a library of novel GPCR heteromer targets, screening and profiling capabilities, and knowledge of biased intracellular signalling mediated through GPCR heteromers.

Dimerix has identified over 30 novel GPCR heteromers that have not been disclosed in the literature, enabling it to follow certain commercial drug development and re-positioning strategies. By leveraging its GPCR-HIT assay, together with its library of novel targets, the company is developing a pipeline of lead products for oncology, metabolic, cardiovascular and inflammatory diseases with highly specific activity and a reduced risk of off-target effects.

As well as in-house drug development, Dimerix also partners with other biopharma companies to enable the discovery of heteromer-optimised drugs, and out-licenses its GPCR-HIT platform to undertake library screening and drug candidate profiling.

**Anchor Therapeutics**

Anchor Therapeutics (Cambridge, MA, US) designs and develops peptide modulators of GPCRs, called pepducins; these modulate GPCR activity via an intracellular allosteric mechanism that is well-suited for a wide variety of targets, including intractable and orphan receptors. Whereas most currently marketed GPCR-based drugs act at the receptor’s extracellular binding site (orthosteric site), pepducins are deliberately designed to modulate GPCRs through their intracellular domain (allosteric site), altering the GPCR’s downstream signalling pathways. Pepducin technology thus represents a new paradigm in GPCR-based drug research and, when applied to previously intractable GPCRs, could greatly expand the number of therapeutically accessible GPCR-related disease pathways.

Pepducins for over 15 different GPCRs have been produced, and several of these have shown impressive activity in preclinical in vivo models. One of Anchor’s advanced pepducins activates C-X-C chemokine receptor type 4 (CXCR4), a GPCR that helps recruit stem cells to sites of injury. The receptor activates two pathways, and the pepducin triggers only one of the two, making it a biased ligand. One possibility for this pepducin would be to attract stem cells to ischaemic tissue following a heart attack. In collaboration with its pharma partners, Anchor is developing other pepducin-based treatments in the areas of regenerative medicine, diabetes, inflammation and cancer.

**Heptares Therapeutics**

Heptares (Cambridge, UK) was founded in 2007 as a spin-out from the MRC Laboratory of Molecular Biology at Cambridge to develop the pioneering work of the founding scientists Richard Henderson and Chris Tate, together with a wider group of MRC scientists. Despite the importance of GPCRs as drug targets, only six novel GPCR targets have been drugged with small molecules in the past 10 years. A major reason for this is that GPCRs are very unstable, and lose their highly organised structure and activity when taken out of the cell membrane. To
overcome this problem, Heptares has developed a GPCR stabilisation technology – StaR®. A StaR® is a stabilised GPCR containing a small number of point mutations that greatly improves its thermostability without disrupting its pharmacology. The technology is transferrable across GPCR families and allows the selection of stable, functionally relevant, purified conformations of target GPCRs that retain their expected drug-binding characteristics.

The StaR® stabilisation technology provides an unprecedented insight into GPCR biology – enabling the design of novel drugs that selectively modulate clinically important, yet historically intractable, GPCR drug targets. The approach is expected to generate new chemical templates for GPCRs that will hopefully overcome issues such as low selectivity, poor pharmacokinetic profiles or toxicity that are often present in existing chemotypes identified by other means – opening up new possibilities for both small molecule and antibody therapeutics across the GPCR target universe.

To capitalise on the opportunity presented by StaR® technology, Heptares has established a fully integrated in-house lead discovery and optimisation capability. The company is also applying its StaR® technology to GPCR antibody discovery using in vitro phage display and in vivo immunisation approaches. In addition to its NCE programmes, Heptares has established four major collaborations: with AstraZeneca for the development of small molecule and antibody candidates targeting specific GPCRs linked to CNS/pain, CV/metabolic and inflammatory disorders; with Shire for a novel small molecule adenosine A2A antagonist with potential in the treatment of CNS disease; with Takeda for a GPCR linked to CNS disorders; and with the Novartis Option Fund for a single GPCR target of strategic interest to Novartis.

Euroscreen
Founded in 1994 as a spin-off from the Université Libre de Bruxelles (Belgium), Euroscreen (Gosselies, Belgium) was one of the first companies to be involved in the development and commercialisation of GPCR reagents. Since then, it has leveraged its GPCR expertise to move across into drug candidate development on various, new GPCR targets and concepts – as evidenced by its pipeline of small molecule drug candidates for the treatment of metabolic, endocrine and inflammatory diseases. The company now operates a dual business strategy, combining its internal drug discovery programmes with a full business unit providing GPCR custom testing services through its FAST Business Unit.

Euroscreen has a pipeline of preclinical drug candidates across a number of therapeutic areas, with its own programmes mainly targeting applications for areas of unmet medical need. Lead compounds currently in development include: SN-JJ, a proprietary target implicated in metabolic disease states and being developed in partnership with Ortho-McNeil-Janssen for the treatment of diabetes; and ESN-364, an NK3 modulator in development for the treatment of non-malignant disorders such as benign prostate hyperplasia and endometriosis. To complement its internal development programmes, the company also has drug discovery and licensing partnerships with several pharmaceutical companies including Boehringer-Ingelheim, Cephalon, Grunenthal, HGS, Ortho-McNeil-Janssen, Monogram Biosciences, Novartis and Pfizer.

DiscoveRx
DiscoveRx (Fremont, CA, US) is dedicated to the development and commercialisation of innovative solutions for the study of GPCRs, kinases and other major drug target classes. The company provides over 400 functional cell-based assays designed to detect GPCR signalling for both known and orphan GPCRs, based on three technology formats: second messenger signalling, arrestin binding and receptor internalisation.

The measurement of compound activity using multiple signalling read-outs for the same GPCR enables high content compound analysis and provides a deeper understanding of the effects of a compound on overall GPCR activation. This allows researchers to interrogate all GPCR signalling pathways and determine the potency of lead candidates for therapeutic target(s) of interest, as well as their cross-reactivity. Importantly, this approach can lead to the identification of functionally selective compounds, while providing valuable information on lead therapeutic candidates in a high biological context.

Conclusion
Today, GPCR-based drug discovery is on a very different footing from the earlier days of drug R&D, when GPCRs weren’t even known to be implicated in the activity of many newly-developed drugs. A number of important advances over the past decade have made a significant contribution to present-day understanding of the importance of GPCRs in drug discovery. New research concepts and techniques mean that GPCR-based drugs can now be developed from first principles, with the aim of directly affecting a specific target and producing a desired therapeutic effect. Whether this knowledge-based approach will fulfil the hopes for innovative GPCR-based therapies – particularly in areas of unmet medical need – only time will tell.

Note
StaR® is a registered trademark of Heptares Therapeutics.