

# GPCR DRUG DISCOVERY

## *Assay Development and Screening*



## OUR CAPABILITIES, SKILLS AND EXPERTISE

### ASSAY DEVELOPMENT

— In depth expertise in GPCR cell line generation and receptor pharmacology ensures optimal assay development for HTS and secondary screening applications

— Access to a comprehensive assay toolbox enables the selection of the best assay format for the chosen target and ensures quick transition through to HTS, accelerating hit identification

— Assay toolbox includes DiscoverX's ground breaking HitHunter™ and PathHunter™ cAMP technology and access to over 300 validated GPCR cell lines

### MEDIUM AND HIGH THROUGHPUT SCREENING

— EVOscreen™: HTS conducted in 384 to 2080 well formats on three proprietary and fully integrated screening systems using, amongst others, our In-sight™ single molecule reader

— All assay technologies supported and tailored to the selected GPCR signalling pathway and pharmacology

— Fragment screening used in a GPCR context allows access to potential new SAR

### HIT CHARACTERISATION

— Full potency determination using primary and secondary assay formats to carry out hit qualification

— Compound analoguing supported by SAR analysis

— Mutagenesis experiments and analysis to support modelling and hit optimisation

### SCREENING LIBRARY

— High quality library consisting of 250,000 compounds for HTS, selected for diversity and containing over 80,000 non-commercially available compounds synthesised by Evotec

From assay development to lead optimisation, Evotec has the key tools and expertise to deliver on GPCR drug discovery. In depth knowledge of GPCR pharmacology and novel computational tools have been combined to maximise chances of identifying high quality hits. Fragment screening and a wide portfolio of assay techniques, tailored to each GPCR target, are applied to enable optimal Structure Activity Relationship (SAR) assignment for further optimisation through our experienced medicinal chemists.

Setup and application of appropriate disease models enables a quick proof of concept of lead compounds.

— 35,000 GPCR focused set with a proven enhanced primary screening hit-rate

— 30,000 fragment collection available

### COMPUTATIONAL CHEMISTRY

— Hierarchical GPCR modelling supported by mutagenesis and *in vitro* analysis

— 3D pharmacophore tools, homology modelling and virtual screening expertise

— Structure-based design and *de novo* ligand design

### MEDICINAL CHEMISTRY

— Evotec has a strong track record in drug discovery: ▶ >125 hit-to-lead and lead optimisation projects ▶ Identified >100 lead series ▶ Identified >20 preclinical candidates ▶ Supported the identification of 15 compounds that have been approved for clinical trials

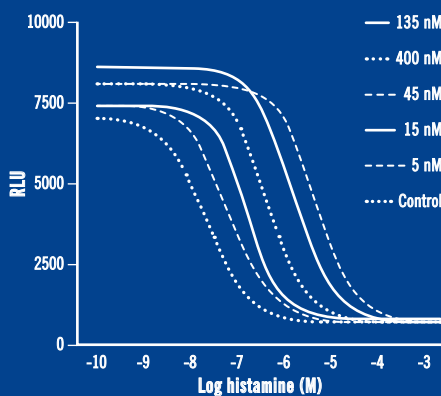
*From target to clinic, Evotec has the key tools and scientific know-how to successfully deliver on GPCR drug discovery programmes*

## Case study: Identification of novel H3 antagonists

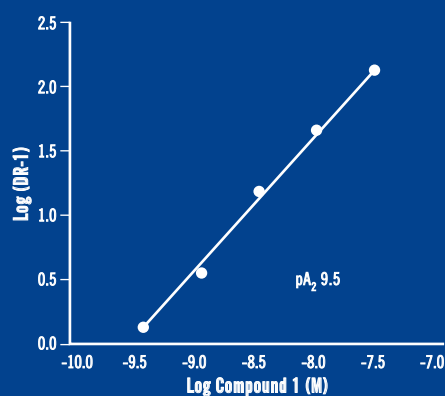
Following a HTS, potent H3 antagonists were identified and further optimised by Evotec. Five novel series were identified and *in vivo* activity in appropriate disease models demonstrated. Multiple, structurally diverse compounds have subsequently been

taken forwards into early development.  
 — An HTS was performed with over 250,000 compounds  
 — High quality *in silico* model of the H3 receptor developed during hit-to-lead phase

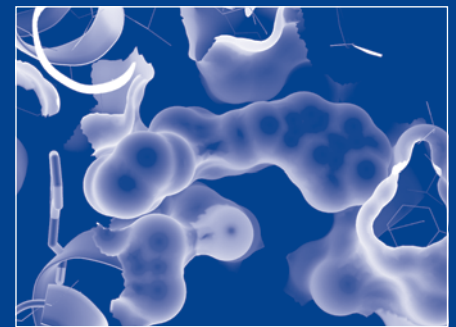
— Model used to inform lead optimisation activities and aid in the identification of potential novel scaffolds  
 — Extensive profiling of advanced leads performed and supported the nomination of multiple development candidates



Human H3 receptor binding assessment of Compound 1 in Evotec's CHO cell *in vitro* assay



Schild plot analysis: Demonstration of competitive H3 receptor binding for Compound 1



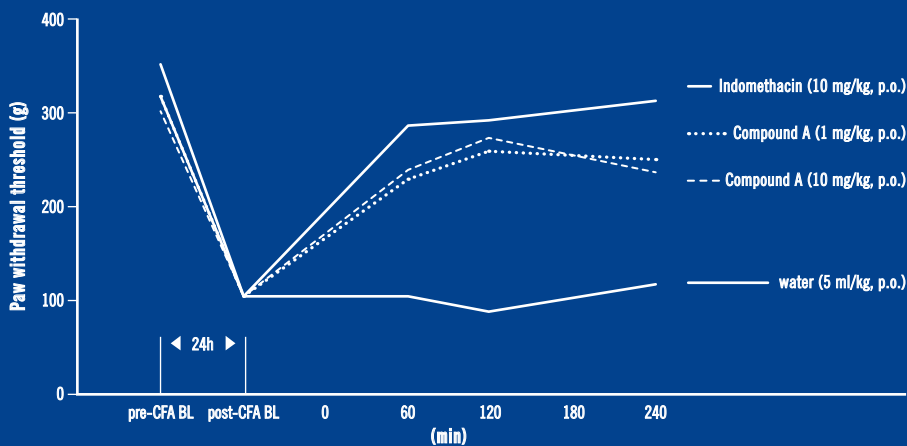
Compound 1 docked into Evotec's Human Histamine H3 receptor model

## Case study: Development of Bradykinin B1 antagonists

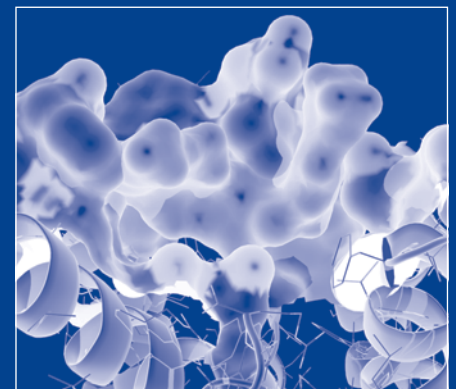
*In silico* analysis of known B1 antagonists provided a pharmacophoric description of the human B1 receptor. This model was then used to rank novel scaffolds suggested by Evotec's medi-

cal chemists prior to synthesis. Using this technique, several novel series were identified and after further optimisation, potent and orally active compounds have been identified.

— High quality homology models of human and rat B1 receptors developed during hit-to-lead phase  
 — Models used to inform lead optimisation and to understand interspecies differences in pharmacology



Effect of Compound A on mechanical hyperalgesia in the CFA model in rat



Compound B docked into Evotec's B1 model