

ION CHANNEL DRUG DISCOVERY

Assay Development and Screening



OUR CAPABILITIES, SKILLS AND EXPERTISE

ION CHANNEL MOLECULAR BIOLOGY

- More than 100 stable cell lines established over the last 3 years
- Constitutive and inducible expression of target ion channels in target cell lines
- Biohazard levels S1 + S2
- Several virus transduction systems established (e.g. retro and lenti virus) for rapid and efficient cell line generation
- 60 off-the-shelf validated assays for selectivity screening

HIT IDENTIFICATION

- High throughput electrophysiology using the IonWorks® Quattro™ platform for primary screening or hit profiling of up to 20,000 ion channel hits
- Fluorescence-based assays including proprietary assay principle based on membrane potential-sensitive dye and FLIPR™ type assays (Ca flux / membrane potential)
- Virtual screening

SCREENING LIBRARY

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

- 10,000 ion channel focused set with a proven enhanced primary screening hit-rate

HIT CHARACTERISATION

- Portfolio of available ion channels for screening including stable / transiently transfected cell lines and primary cells
- Comprehensive hardware platform including: ▶ Automated patch-clamp robots IonWorks® Quattro™ (MDS), QPatch™ HTX (Sophion) and Patch Liner® 8 (Nanion) ▶ Planar semi-automated Port-A-Patch® (Nanion) ▶ Dynaflo™ Pro II - system (Collectricon) ▶ Manual rigs

MEDICINAL CHEMISTRY AND COMPUTATIONAL CHEMISTRY

- Evotec's medicinal chemists have successfully prosecuted hit-to-lead and lead optimisation programmes for both voltage-gated and ligand-gated ion channel targets: ▶ Experience with developing SAR based on data from complex

Evotec's ion channel drug discovery platform brings together:

- A successful track record in ion channel drug discovery
- Validated state-of-the-art technologies for hit identification and optimisation
- In-depth electrophysiology and ion channel pharmacology expertise

This leading technology platform and scientific know-how will dramatically increase your probability of success and make Evotec your partner of choice for ion channel research

assay protocols ▶ Optimising selectivity against related family members ▶ Ensuring selectivity against hERG

- Computer aided ion channel ligand design through: ▶ Substructure, 2D similarity, 3D pharmacophore and shape analysis ▶ *in silico* predictive modelling including a highly predictive 3D model utilising the hERG channel structure

TRACK RECORD IN ION CHANNEL DRUG DISCOVERY

- Expertise in identifying channel openers and blockers for: ▶ Voltage-gated ion channels: K_v, Ca_v and Na_v families ▶ Other potassium ion channels: KCa (SK, BK, IK), Inward rectifiers (e.g. Kir2.1) and Tandem pore domain (e.g. TREK, TASK) ▶ Ligand-gated ion channels: P2X and TRP families, Glutamate (NMDA, AMPA) and GABA_A receptors ▶ Other channels: CRAC and chloride channels (e.g. CFTR)

In depth electrophysiology and ion channel pharmacology expertise drives success

Case study: Discovery, characterisation and optimisation of selective inhibitors of a voltage-gated ion channel target

K_v1.3: TARGET-TO-LEAD DISCOVERY

— Evotec has carried out, using its fully integrated drug discovery platform: hit identification, medicinal chemistry and electrophysiological profiling

HIT IDENTIFICATION

— High throughput screening of 1.1 million compounds using a proprietary membrane potential sensitive dye in a cell-based assay
— Multiple tractable series advanced into hit-to-lead

MEDICINAL CHEMISTRY

— Medicinal chemistry optimisation activities on-going on multiple compounds series in parallel
— Potent (<10 nM) and selective (>30-fold) compounds identified

HIT AND LEAD CHARACTERISATION

— IC₅₀ determination on voltage-gated potassium channels by automated electrophysiology

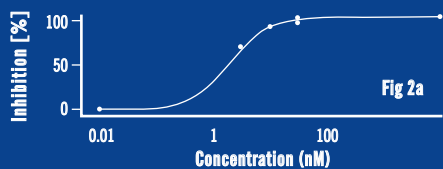
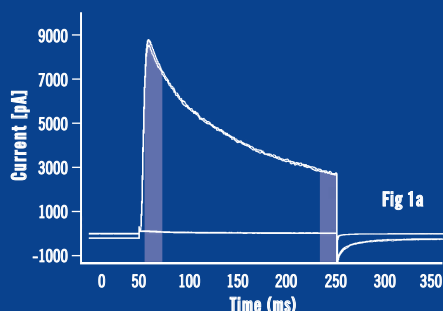


Figure 1) Compound A (30 nM) produces complete block of whole cell currents mediated by potassium channel K_v1.3 (a) but shows good selectivity over closest homologue K_v1.5 (b)

— Broad panel selectivity screens established in-house
— Rapid turnaround times to support medicinal chemistry program

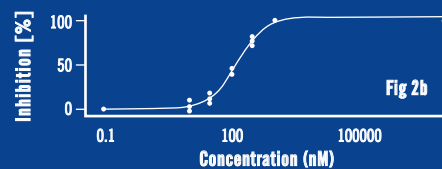
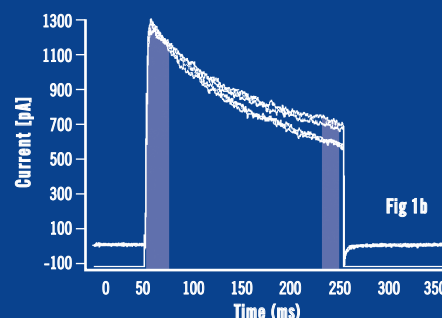
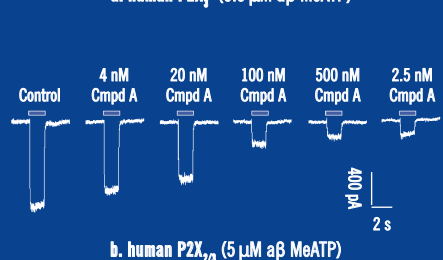
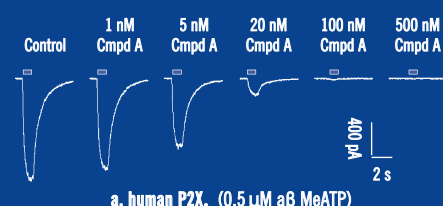


Figure 2) Compound A shows 65-fold selectivity for K_v1.3 over K_v1.5, having an IC₅₀ value of 2 nM and 130 nM at K_v1.3 (a) and K_v1.5 (b), respectively.

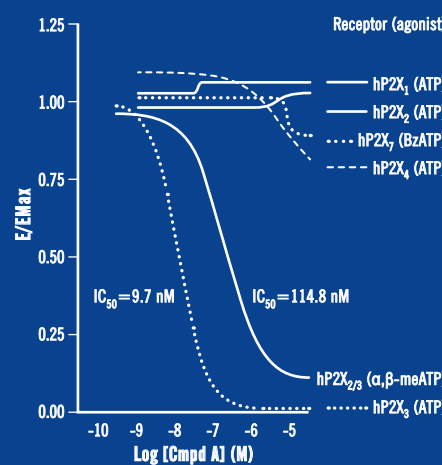
Case study: First in class potential for a challenging ligand-gated ion channel target

P2X_{2/3}: PROPRIETARY SCAFFOLD DESIGN

— Evotec undertook a rational design approach around purinergic scaffolds to identify allosteric modulators of the P2X_{2/3} target
— Electrophysiology used for hit and lead identification and characterisation
— Promising compounds have been identified: ▶ Excellent potency, selectivity, ADMET properties ▶ P2X₃ and P2X_{2/3} selectivity ▶ Orally active in pain models
— Industry leading position with multiple, published patent applications
— Best and first-in-class potential



Selective block by Compound A of whole cell currents mediated by (a) human P2X₃ homomeric receptors and (b) human P2X_{2/3} heteromeric receptors



Compound A selectively blocks calcium influx, measured by FLIPR™, mediated by human P2X₂ and P2X_{2/3} receptors, and does not show activity at other P2X receptor subtypes