cyprotex discovery

hERG Safety

experts in **TOXICOLOGY**

Background Information

'The impressive list of drugs, already on the market or still under development that have been reported to adversely prolong repolarisation, makes it imperative to investigate any new chemical entity for this potential side effect before its first use in man.'

[1]Haverkamp W, Breithardt G, Camm AJ, Janse MJ, Rosen MR, Antzelevitch C, Escande D, Franz M, Malik M, Moss A and Shah R. (2000) *Eur Heart J* **21** **(15)**; 1216-31.

- The human ether-a-go-go related gene (hERG) encodes the inward rectifying voltage gated potassium channel in the heart (I_{Kr}) which is involved in cardiac repolarisation.
- Inhibition of the hERG current causes QT interval prolongation resulting in potentially fatal ventricular tachyarrhythmia called *Torsade de Pointes*.
- A number of drugs have been withdrawn from late stage clinical trials due to these cardiotoxic effects, therefore it is important to identify inhibitors early in drug discovery.
- Cyprotox hERG Safety assay employs the Ionworks™ HT System (Molecular Devices) as an automated patch clamp electrophysiology measurement.
- The Ionworks™ HT system delivers high quality, accurate and sensitive data which is comparable with the traditional single cell patch clamp method.

Protocol

Instrument
Ionworks™ HT System (Molecular Devices)

Analysis Method
Electrophysiology

Cell Line
CHO-hERG cells

Perforating Agent
Amphotericin B

Test Compound Concentration
1 µM for single point;
0.008, 0.04, 0.2, 1, 5, 25 µM for IC_{50}
(different concentrations available)

Final DMSO Concentration
0.25 %

Number of Replicates
8 replicates (single point),
4 replicates per concentration (IC_{50})

Quality Controls
0.25 % DMSO (negative control)
Quinidine (positive control)
Seal Resistance must be > 50 MOhms
Pre-compound current must be ≥ 0.1 nA

Compound Requirements
100 µL of 10 mM solution

Data Delivery
% Inhibition (single point)
or IC_{50} determination

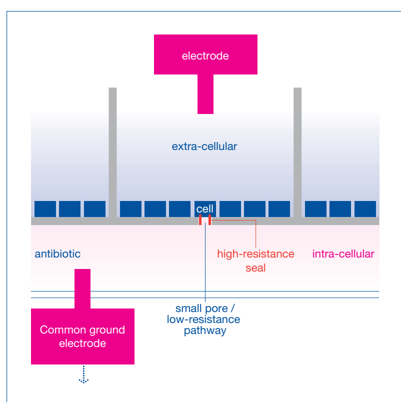
To find out more contact sales@cyprotex.com

To date, electrophysiology remains the 'gold standard' method with which to characterise ion channel properties, as binding, flux and fluorescence assays only indirectly measure ion channel properties[2].

Cyprotox hERG Safety

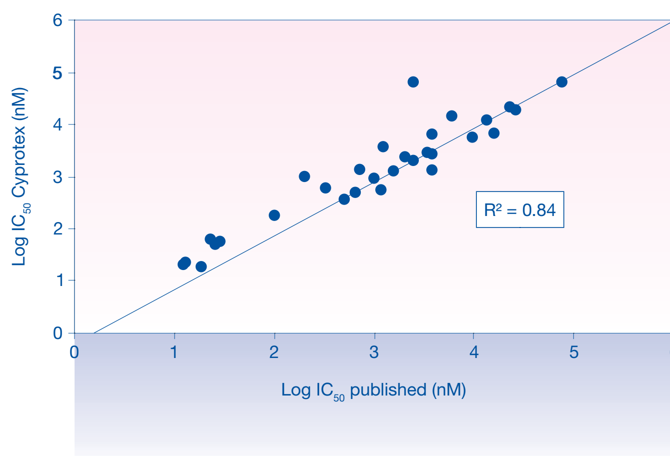
For the validation, a literature search was performed to identify a selection of compounds which were known to inhibit the hERG current with a range of potencies.

Figure 1
Cyprotox hERG safety experimental approach.



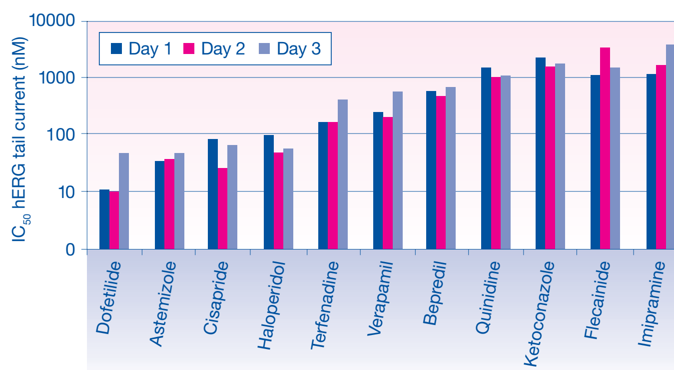
A single hERG-expressing cell is positioned by negative pressure over a pore in the bottom of each well of a specially designed patch plate containing 384 wells. The aperture separates two isolated fluid filled upper and lower chambers. The positioned cells form stable seals over the apertures impeding electrical flow between the two chambers. A cell membrane pore-forming agent (Amphotericin B) is introduced into the lower chamber creating an electrical pathway through the portion of the cell membrane exposed via the small aperture in each well. An electronics head containing 48 electrodes is positioned in the upper chamber clamping the cell membrane potential and subsequently recording ionic currents from up to 48 cells in parallel. Current is monitored before and after test compound addition.

Figure 2
Comparison of Cyprotox hERG Safety data with published traditional patch clamp data.



The graphs illustrates that the Cyprotox hERG Safety assay using the Ionworks HT system generates data comparable with traditional single cell patch clamp measurements.

Figure 3
Cyprotox hERG Safety IC_{50} data generated for a set of compounds over 3 separate days.



The data illustrate that good consistency is achieved over a number of different days for compounds with a range of different potencies. The method used in the Cyprotox hERG Safety assay has also been extensively validated by other groups: Kiss *et al*., 2003; Schroeder *et al*., 2003.

References

[1] Haverkamp W *et al.* (2000) *Eur Heart J* **21 (15)**; 1216-31.

[2] Finlayson K *et al.* (2004) *European Journal of Pharmacology* **500**; 129-142.

[3] Kiss L *et al.* (2003) *Assay and Drug Development Technologies* **1**; 127-135.

[4] Schroeder K *et al.* (2003) *Journal of Biomolecular Screening* **8** (1); 50-64.