SAFETY

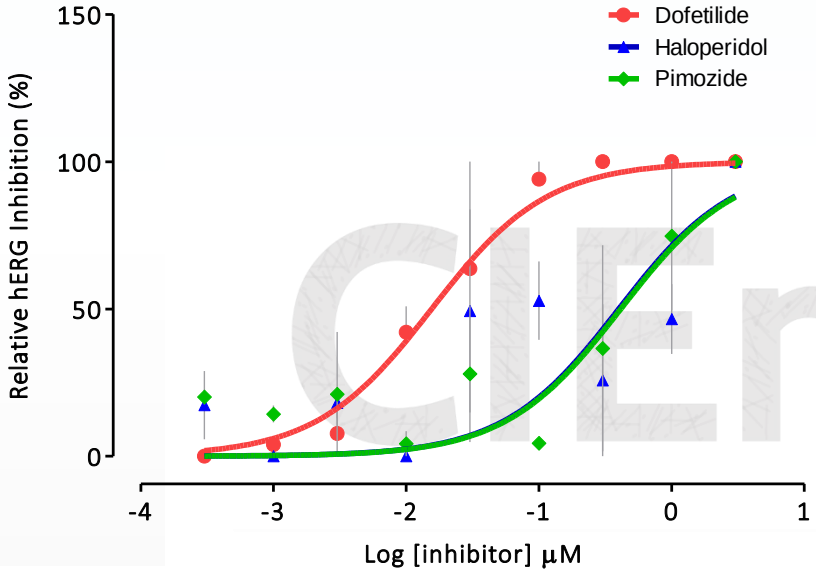
Inhibition of hERG (human ether-a-go-go-related) channel

hERG potassium channels are essential for normal electrical activity in the heart. hERG channel dysfunction may cause long QT syndrome (LQTS), characterized by delayed repolarization and prolonged QT interval, which increases the risk of ventricular arrhythmias and sudden death. This side effect is a common reason for drug failure in preclinical safety tests[1]. The potassium channel assay (FLIPR® Potassium Assay) is a screening of potassium channel activity that allows rapid and robust screening, based on the principle of potassium channel permeability to thallium. The validation of the test produces results equivalent to those obtained through electrophysiology by patch clamp, demonstrating that it is also an appropriate methodology to evaluate the safety of new compounds on the hERG channel[2,3].

Test system: HEK-293 – transfected Kv11.1 (hERG human)
Experimental number: Three wells per group in triplicate
Reference Item: Dofetilide, Pimozide, Haloperidol
Main Read-outs: Fluorescence intensity and IC_{50}

Validation Data

A



B

Compound	IC_{50} (µM)
Dofetilide	0.015 (0.0089 - 0.026)
Pimodize	0.41 (0.16 - 1.05)
Haloperidol	0.39 (0.13- 1.22)

Figure: Evaluation of hERG channel inhibition using the Potassium Assay Kit in HEK-293 cells transfected with the human hERG channel. (A) The graph represents concentration response curves of representative compounds that block hERG channel activities. (B) IC_{50} values obtained from 3 independent assays. Data were expressed as Mean ± Standard Error of Mean (SEM). The IC_{50} was evaluated by non-linear regression of the data generated from the fluorescence intensity values using the GraphPad® software. RFU: Relative Fluorescent Unit.

To avoid bias and allow reproducibility and reliability of all *in vitro* experiments we follow the "Guidance on Good Cell Culture Practice"[3]. All *in vitro* experiments are performed in triplicate wells for each condition and repeated at least three times.

References:

[1] Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. Nature. 23;440(7083):463-9, 2006.
[2]Schmalhofer WA, Swensen AM, Thomas BS, Felix JP, Haedo RJ, Solly K, Kiss L, Kaczorowski GJ, Garcia ML. A pharmacologically validated, high-capacity, functional thallium flux assay for the human Ether-à-go-go related gene potassium channel. Assay Drug Dev Technol. 2010 Dec;8(6):714-26.
[3]Yu H, Li M, Wang W, Wang X. High throughput screening technologies for ion channels. Acta Pharmacol Sin. 37(1): 34–43, 2016.
[4]Coecke S; Balls M; Bowe G; *et al*. Guidance on good cell culture practice: a report of the second ECVAM task force on good cell culture practice. Altern Lab Anim. 2005, 33(3):261-87.

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