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In-vitro hERG & NaV1.5 cardiotoxicity assay

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Nick Lynch^{1,2}

¹Curlew Research; ²ASAP Discovery Consortium

ASAP Discovery



Nick Lynch

Curlew Research, ASAP Discovery Consortium

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We use this protocol and it's working

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Abstract

In vitro hERG and NaV1.5 cardiotoxicity assays are crucial for evaluating the potential of new drugs to cause cardiac arrhythmias. These assays involve measuring the effects of compounds on the hERG (human ether-à-go-go-related gene) and NaV1.5 (sodium channel) ion channels, which play vital roles in cardiac repolarization and action potential propagation.

Safety warnings

- ⚠ Always wear appropriate PPE for this protocol
Refer to Material Safety Data Sheets for additional safety and handling information.

Summary

- 1 The human ether-a-go-go related gene (hERG) potassium channel is a voltage-gated ion channel found in the heart, essential for cardiac repolarisation. The channel has a large inner cavity making it sensitive to drug binding. Inhibition of the hERG ion channel results in decreased function and produces Long QT Syndrome (LQTS) leading to potentially fatal cardiac arrhythmias. Cardiovascular safety issues are a major cause for the attrition of drug development candidates and testing cardiac risk via the hERG ion channel is recommended during the drug discovery process.

This covers hERG and NaV1.5 assays

Sample Preparation

- 2 Compounds were solubilised to 10mM in DMSO and subsequently diluted in HBPS to 30mM.

Assay

- 3 6-Point concentration-response curves were generated using serial dilutions from the top test concentration. Electrophysiological recordings were made from a Chinese Hamster Ovary cell line stably expressing the full-length ion channel.
- 4 Single cell ionic currents were measured in whole-cell configuration at room temperature (25°C) using a QPatch II (Sophion Bioscience).
- 5 The internal solution for hERG contained (mM): 120 KCl, 20 KF, 10EGTA, 10 HEPES and was buffered to pH 7.3.
- 6 The internal solution for hNaV1.5 contained (mM): 140 CsF, 10 NaCl, 10 HEPES, 1 EGTA buffered to pH7.3.
- 7 The external solution (HEPES-buffered saline, HBPS) contained (mM): 138 NaCl, 4.5 KCl, 1.8 CaCl₂, 1.0 MgCl₂, 10 HEPES, 10 glucose, buffered to pH7.4.
- 8 The voltage protocol for each assay is given below (Table 1). The test pulse was applied every 10 seconds.

Compounds were incubated for 120 seconds to allow stabilization of the parameters.



Data Analysis

- 9 Concentration-response curves were generated by cumulative addition of compound with concentrations low to high. In all cases, steady-state inhibition was achieved before the next concentration of compound was added. For voltage-gated sodium channels, to investigate any use-dependence, an 8-pulse voltage protocol is applied at a frequency of 3Hz.

Inhibition is compared at pulse 1 (P1) and pulse 8 (P8). Any difference in the potency of a compound at P8 versus P1 would be typical of use-dependent block.

	Protocol	Holdin g potenti al (mV)	Step (mV, msec)	Pulse interval (sec)	Comment
	hERG	-80	40, 1500/-40, 1500	10	
	hNaV1.5 peak	-110	0, 10	10	8-pulse protocol at 2.5Hz

Table 1. Voltage protocols used.

- 10 IC₅₀ values were obtained from a 4-parameter logistic fit of the concentration-response data. Greater or less than values indicate where the potency estimates could not be fully resolved due to the concentration range tested.

In such cases, % inhibition at the top test concentration is reported. Compounds with activity less than 30% inhibition at the top test concentration were considered inactive.



Protocol references

https://cdn.prod.website-files.com/659db4dde7f13f395f49157c/65bdebd69760d72632532847_Concept%20Life%20Sciences_LE_hERG_INHIBITION.pdf

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