Invitrogen’s Predictor™ hERG Fluorescence Polarization Assay Using BioTek’s Synergy™ 4 Hybrid Microplate Reader

Non-Radioactive High Throughput Screening Assay for hERG Binding

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The hERG channel is one of a family of ion channels shown to be important in the regulation of cardiac rhythm. Compounds that block this ion channel have been shown to increase the QT interval and lead to cardiac arrhythmias. Because there is no way to predict from a compound’s structure whether or not it will block the hERG ion channel, potential drug compounds are screened as early as possible in the R&D process. Here we describe measuring Invitrogen’s fluorescence polarization based Predictor™ assay with a Synergy 4 Hybrid Microplate Reader from BioTek.

Introduction

The hERG is the human homolog of the Ether-a-go-go gene found in Drosophila in the 1960s by William Kaplan [1]. This potassium channel consists of 4 identical subunits, each containing six transmembrane domains. Abnormalities in this channel have been associated with both Long QT and Short QT syndrome, both potentially fatal cardiac arrhythmias, depending on the abnormality. The hERG channel has been shown to be the target for class III antiarrhythmic drugs such as amiodarone, which reduce the risk of re-entrant arrhythmias by prolonging the AP duration and refractory period without slowing conduction velocity in the myocardium. This channel is also quite sensitive to drug binding, without any predictability based on structure, resulting in elongated QT intervals. Due to the potential for complications with increased QT intervals, regulatory agencies have issued recommendations for the evaluation of potential drugs in the preclinical stages of development. Typically, potential drug compounds are tested using a patch clamp electrophysiology test.

Fluorescence polarization (FP) is a fluorescence detection technique first described in 1926 by Perrin [2]. It is based on the observation that fluorescent molecules in solution, when excited by polarized light, emit polarized light, albeit the plane of emitted light will be different than that of the excitatory light due to molecular rotation. A molecule’s polarization is inversely proportional to the molecule’s rotational speed, which is influenced by solution viscosity, absolute temperature, molecular volume and the gas constant. If one keeps viscosity and temperature constant, then the key variable for rotational speed differences is molecular volume or molecular weight.

The Predictor™ hERG kit from Invitrogen is a homogeneous fluorescent assay that uses a simple add-and-read format.

The assay is based on the principle of fluorescence polarization where a red fluorescent tracer is displaced from the hERG channel by compounds that bind to the channel. Assay performance is validated using established hERG channel blockers.

Materials and Methods

Predictor hERG Assay test kits were obtained from Invitrogen Carlsbad, CA). The binding assay was carried out according to the kit instructions. Briefly, reagents were thawed and Predictor hERG membrane preparations were sonicated. Working 4X tracer was prepared by diluting the 250 nM stock provided in the test kit to 4 nM with hERG FP assay buffer also provided. Dilutions of test compounds were prepared as a 4X stocks from their final intended concentrations. Assays were performed in 384-well microplates. Aliquots (5 µL) of each concentration of test compound were pipetted into the appropriate wells of the microplate. As required, 10 µL of (2X) membrane preparation was then dispensed. Working Tracer solution (5 µL) was then added and the plate was allowed to incubate at room temperature for at least one hour.
prior to measuring fluorescence polarization. Fluorescence polarization measurements were made using a Synergy™ 4 Hybrid Microplate Reader from BioTek Instruments (Winooski VT). Measurements were made from the top using the tungsten light source. Both parallel and perpendicular fluorescence were measured using the same 530/25-excitation and a 590/35-emission filters along with a 570 nm cut off dichroic mirror. The PMT sensitivity setting was set automatically such that the positive control well had a raw fluorescence value for the parallel signal of 50,000 relative fluorescent units (RFU). Polarization values were calculated automatically using Gen5™ Data Analysis Software (BioTek Instruments).

Results

Increasing concentrations of the hERG-binding compound, astemizole, demonstrates a marked change in fluorescence polarization (Figure 2). Astemizole binds to the hERG ion channel and displaces the fluorescent tracer molecule. As more tracer is displaced and free to rotate freely in solution the polarization decreases. Using a semilog plot a sigmoidal shaped response curve is observed (Figure 2).

Comparison of compounds to known hERG binding molecules can provide information as to whether or not prospective drugs will have this side effect. As demonstrated in Figure 3 a dose comparison of E410 with astemizole, a known hERG binding molecule, has a 10 fold greater EC50. While this indicates that this compound is less likely to bind than astemizole, the fact that it displaces the tracer indicates that it is a hERG-binding molecule.

Discussion

These data demonstrate the ability of the Synergy 4 to provide the fluorescence polarization measurements necessary for the Predictor™ hERG Binding assay. The Synergy 4 is a modular reader that combines a highly sensitive fluorescence capability with light polarizers. All of the necessary Gen5 Data Analysis Software automatically subtracts the blank wells and calculated polarization values form the parallel and perpendicular fluorescence measurements.

References


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