Performance of the Predictor™ Fluorescence Polarization Assay: A tool for High-Throughput Screening of hERG Channel Affinity

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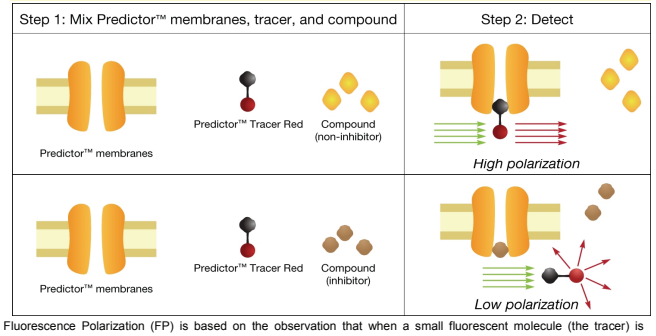
Introduction

The propensity of compounds with widely diverse structures to block the human ether-a-go-go related gene (hERG) potassium channel has remained a challenge in the development of small molecule based therapies. In many cases, hERG channel block induces long QT syndrome (LQT), which can produce the characteristic ventricular arrhythmia Torsade de Pointes that may degenerate into ventricular fibrillation and ultimately sudden death. Patch-clamp electrophysiology remains the functional gold standard for testing the interaction of compounds with the hERG channel, but the cost of these assays remains high. Because many compounds that block the hERG channel interact with two aromatic residues near the inner vestibule of the channel (Mitcheson et al, 2000; Fernadez et al, 2004), radioligand displacement assays have been used routinely as an initial funnel for hERG channel liability at early stages of drug discovery. The efficiency of radioligand displacement assays suffers as these assays remain heterogeneous and the costs associated with the radioligands remains unattractive. Here we describe a high-throughput assay for the rapid determination of hERG channel affinity based on the principles of fluorescence polarization. A library of fluorescent tracers was constructed using scaffolds designed from structures that are known to have high-affinity for the hERG channel. We examined this library to identify the best candidate tracers and subsequently used one to enable a hERG channel fluorescence polarization displacement assay. This assay has identified the IC_{50} values of known hERG channel blockers tightly correlated to and within a narrow range of the published values determined by radioligand binding assays and patch-clamp recordings. Optimization of the assay conditions (buffers, incubation time, and assay plate) has provided a high-throughput assay for the rapid determination of hERG channel affinity.

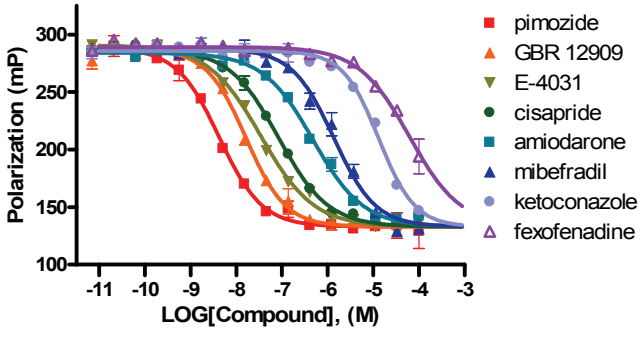
Figure 1 – Predictor™ Fluorescence Polarization Assay Principle



Fluorescence Polarization (FP) is based on the observation that when a small fluorescent molecule (the tracer) is excited with plane polarized light, the emitted light becomes depolarized because the molecule tumbles rapidly in solution during its fluorescent lifetime. If the tracer is bound to a large molecule, the tracer's rotation is slowed and the light remains highly polarized.

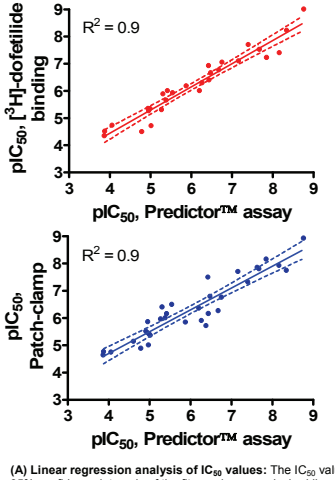
Methods:
Test compound serial dilutions were prepared at 100X in DMSO or water, then diluted to 4X (4% DMSO) in assay buffer. 5 µL of 4X test compounds were dispensed to a 384-well plate (Corning, #3677) using a Biomek FX Laboratory Automation Workstation (Beckman Coulter, Inc.). The Biomek FX was then used to dispense 10 µL of 2X Predictor hERG Membranes, followed by 5 µL of 4X Predictor hERG Tracer Red. Each compound was tested in the absence and presence of 30 µM E-4031 to correct for test compound interference. Plates were incubated for 4 hours prior to measuring fluorescence polarization on a Safire^{2}™ plate reader (Tecan) using excitation at 530 nm, emission at 585 nm (20 nm bandwidth).
Concentration curves were generated from duplicate points and fit to sigmoidal dose response curves (Excel or Prism)

Figure 2 – Pharmacology of Predictor™ hERG Fluorescence Polarization Assay



Concentration-response curves: The Predictor™ fluorescence polarization assay was used to generate IC_{50} data for 38 known hERG channel blockers from duplicate wells. Eight examples are shown (n=2 ± SEM). Some curve fits were constrained to the minimum polarization produced by 30 µM E-4031.

Figure 3 – Correlation of Predictor™, [^{3}H]-dofetilide and patch-clamp data



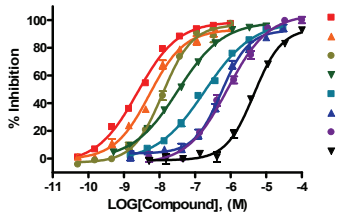
B

Compound	Assay specific IC_{50} (µM)			
	Radioligand binding		Predictor™	Patch-clamp
	[^{3}H]-astemizole	[^{3}H]-dofetilide		
astemizole	0.004	0.001	0.002	0.001
pimozide	0.019	0.006	0.004	0.018
E-4031	0.075	0.020	0.039	0.048
terfenadine	0.127	0.030	0.021	0.016
dofetilide	0.035	0.040	0.007	0.012
GBR 12909		0.060	0.014	0.007
cisapride	0.158	0.080	0.068	0.020
haloperidol	0.300	0.090	0.174	0.174
droperidol		0.120	0.370	0.032
bepridil	0.782	0.170	0.208	0.550
domperidone		0.220	0.329	0.162
amiodarone	0.752	0.400	0.361	0.700
thioridazine	1.139	0.510	0.536	1.250
mibefradil		0.660	1.318	1.430
verapamil	4.936	0.990	3.802	0.706
propafenone		1.000	0.621	0.440
clozapine		1.200	2.833	0.320
quinidine	13.16	1.310	4.877	0.400
fluoxetine	11.99	2.230	4.083	0.990
imipramine	14.06	4.480	11.38	3.400
flecainide	38.49	4.540	10.09	3.910
pyrilamine		5.000	5.322	1.100
desipramine		5.530	10.96	1.390
sparfloxacin		18.80	86.61	18.00
ketoconazole	19.05	19.500	9.479	4.360
diltiazem		31.20	131.0	17.30
fexofenadine	166.7	32.00	16.13	13.10
spironolactone		45.70	134.5	23.00
grepafloxacin		68.90	>500	50.00
erythromycin	328.5	89.20	>500	152.7
nifedipine			>500	>50
glyburide	318.5		>500	74.00
sertindole			0.023	0.014
flunarizine			0.411	1.950
tamoxifen	0.914		1.131	
amitriptyline	15.55		10.53	10.00
maprotiline			12.17	3.100
papaverine			24.51	7.300
Mean FOLD	0.46	1.82		3.31
STDEV FOLD	0.27	1.23		3.21
n=	19	28		33

(A) Linear regression analysis of IC_{50} values: The IC_{50} values are tightly correlated with a slope near unity. The 95% confidence intervals of the fit are shown as dashed lines. Pearson's correlation coefficient (R^2) = 0.9 for both.
(B) Comparison of IC_{50} values: Data generated by the Predictor™ assay are compared to data generated by patch-clamp recordings and reported or summarized in the literature (Diaz et al, J Pharm Toxicol Methods, 2004).

Figure 4 –1536 Automation of Predictor™ hERG Fluorescence Polarization Assay

A



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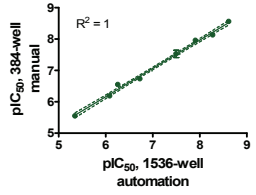

C

Compound	IC_{50} (nM)	
	Automated 1536-well	Manual 384-well (n=3)
Astemizole	2.4	2.8 ± 0.46
Pimozide	5.2	7.5 ± 0.76
Dofetilide	12.2	11 ± 2
Terfenadine	31.3	30 ± 7
Haloperidol	185	189 ± 28
Bepridil	552	285 ± 33
Thioridazine	811	656 ± 41
Fluoxetine	4470	2862 ± 195

(A) Concentration-response curves: The Predictor™ fluorescence polarization assay was automated to generate IC_{50} data for 8 known hERG channel blockers in (n=4 wells ± SEM).
(B) Comparison of IC_{50} values: Data generated by the manual Predictor™ assay in 384-well format (3 separate experiments) are compared to data generated on automation in 1536-well format.
(C) Linear regression analysis of IC_{50} values: The IC_{50} values are tightly correlated with a slope near unity. The 95% confidence intervals of the fit are shown as dashed lines. Pearson's correlation coefficient R^2 = 1.

Methods:
Compounds (80 nL, 100% DMSO) were dispensed to the assay plate with an Echo® 550 (LabCyte).
Predictor hERG membranes (4 µL) were dispensed to the assay plate with a Biomek® FX (Beckman Coulter).
Predictor hERG tracer red (2 µL) and assay buffer (1.92 µL ± 30 µM E-4031) were dispensed to the assay plate with a Cybi-Drop® 3D (CyBio).
Concentration response curves were performed in quadruplicate wells.

Conclusion

- **The Predictor™ hERG fluorescence polarization assay kit provides:**
  - **Accuracy:**
    Determines IC_{50} data that tightly correlate to radioligand and patch-clamp data
  - **Automation:**
    Can be easily automated using a variety of liquid handling instruments
  - **Screening:**
    Has been implemented in an HTS screening environment with robust and reproducible data generation
    Available in the SelectScreen™ screening service with the same high quality, fast turnaround, and intimate customer relationship experience as Invitrogen's other SelectScreen™ services
- **Product:** predictor@invitrogen.com
- **SelectScreen™ Service:** www.invitrogen.com/profilingpartner

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