

Development and Evaluation of High Throughput Functional Assay Methods for hERG Potassium Channel

WEIMIN TANG,¹ JIESHENG KANG,^{2*} XIAYING WU,¹ DAVID RAMPE,² LIN WANG,² HONG SHEN,³ ZHUYIN LI,¹ DAMIEN DUNNINGTON,⁴ and TINA GARYANTES¹

ABSTRACT

Three functional hERG channel assay methods have been developed and evaluated. The methods were tested against five known hERG channel inhibitors: dofetilide, terfenadine (Seldane), sertindole (Serdolect), astemizole (Hismanal), and cisapride (Propulsid). The DiBAC₄(3)-based assays were found to be the most economical but had high false-hit rates as a result of the interaction of dye with the test compounds. The membrane potential dye assay had fewer color-quenching problems but was expensive and still gave false hits. The nonradioactive Rb⁺ efflux assay was the most sensitive of all the assays evaluated and had the lowest false-hit rate.

INTRODUCTION

THE GENE KNOWN AS hERG, the human homolog of the *ether-a-go-go* (*eag*)-related gene (*erg*) of the fruitfly *Drosophila melanogaster*, encodes a potassium channel whose activity is critical for the regulation of membrane potential in cardiac myocytes. Loss-of-function mutations in this channel are associated with the potentially lethal inherited long QT syndrome (LQT).^{1,2} Pharmacological blockage of the hERG channel leads to the development of acquired LQT.³ Acquired LQT can be induced by different classes of therapeutic agents, including antiarrhythmics,⁴ antibiotics,^{5,6} antihistamines,^{7,8} antipsychotics,^{9,10} and prokinetic agents.^{11,12} Since 1997, 5 of the 11 prescription drugs that were withdrawn from the U.S. market were withdrawn as a result of their potential to inhibit the hERG channel and thus causing LQT syndrome.¹³ Considering the expense associated with clinical trials, it is financially beneficial for pharmaceutical companies to conduct early compound safety assessments to eliminate chemical agents that have the potential to induce QT prolongation. This concern is also shared by many regulatory agencies in different countries, highlighted by the publication of *Points to Consider* by the Committee for Proprietary Medicinal Products (CPMP) of the European Agency for Evaluation of Medicinal Products (EMA) in 1997. In this document, the CPMP listed a series

of *in vivo* and *in vitro* assays for pharmaceutical companies to assess the LQT liability of noncardiovascular therapeutic agents.¹⁴ The ability of a compound to inhibit the hERG channel was regarded as an important indication of its possible LQT liability.^{15,16}

However, the number of methods available for functional hERG channel measurement is limited. Patch-clamp technology is sensitive and reliable but labor intensive. The ability to screen large amount of compounds using this technology is not presently available. Another relatively reliable and medium throughput screening method is the Rb-86-based radioactive assay. However, many high throughput screening (HTS) labs are reluctant to use this assay format because of the highly radioactive nature of Rb-86. The recent development of a nonradioactive Rb⁺ assay has greatly enhanced the ease of using this system.¹⁷ Other potential functional potassium channel assays use membrane-sensitive fluorescent dyes. One of the most widely used oxonol dyes is bis-(1,3-dibutylbarbituric acid)trimethine oxonol (DiBAC₄(3)). Recently, Molecular Devices (Sunnyvale, CA) released a new fluorometric imaging plate reader (FLIPR[®]) Membrane Potential Assay Kit (FMP) that has been widely accepted for Na⁺ and K⁺ channel screening. However, both dyes measure the change of membrane potential instead of channel activity. Thus they have the potential of selecting compounds that change membrane potential but that do not affect channel activity.

¹Department of Profiling and High Throughput Screening, ²Department of Safety Pharmacology, and ³Department of Analytical Chemistry, Aventis Pharmaceuticals, Bridgewater, NJ.

⁴Current address: Axiom Biotechnology, San Diego, CA.

*Equal contributor as first author.

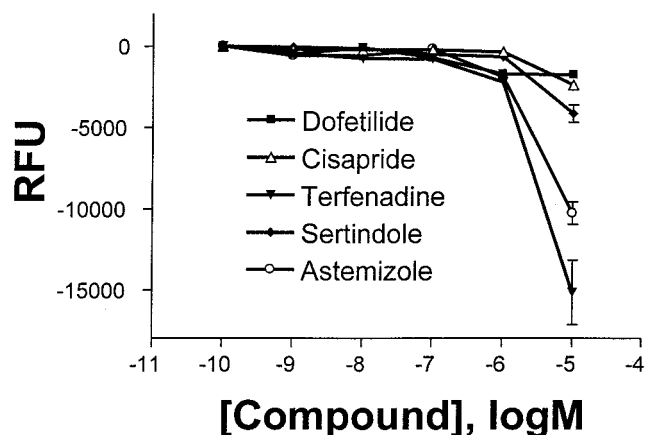


FIG. 1. hERG channel functional assay using the DiBAC₄(3) method. The cells were preloaded with 5 μ M DiBAC₄(3) and incubated with compound before the hERG channels were activated by addition of 50 mM KCl ($n = 3$). URF, relative fluorescence unit.

Here, we compare three potential assay systems for functional hERG channel analysis. These three assays are the DiBAC₄(3), Molecular Devices' FMP dye, and the nonradioactive Rb⁺ assay. The throughput, sensitivity, and false-hit rate of the three methods are compared.

MATERIALS AND METHODS

DNA and cell line selection

The hERG channel expression vector and stable cell line (CHO-K1) construction were described previously.⁶ G418-resistant clones were transferred into 96-well plates and screened for activity using the DiBAC₄(3) assay as described below. The transfected cells were cultured in Ham's F-12 medium supplemented with 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD), 1 \times penicillin/streptomycin, and 500 μ g/ml of G418. The cell density was kept below 80% confluency prior to microplate culture. The hERG cells were dispensed into microplates with a Multidrop (Labsystems, Helsinki, Finland) at a density of 10,000 cells/well into a 384-well plate or a density of 50,000 cells/well into a 96-well plate (Costar[®], TC-treated plate; Corning Incorporated, Acton, MA). The plates were incubated at 37°C for a minimum of 12 h prior to the channel functional assays.

DiBAC₄(3) assay method

Cells were loaded with 5 μ M DiBAC₄(3) in Hank's/HEPES buffer (Hank's solution, 20 mM HEPES [pH 7.4], 0.2% glucose) and incubated at 37°C for 30 min before adding KCl to a final concentration of 50 mM with a FLIPR to activate the hERG channel. The temperature was kept constant at 37°C by using the FLIPR heated stage as well as preheated compounds and buffers. A panel of the five known hERG channel inhibitors were tested at concentrations of 10, 1, 0.1, 0.01, and 0.001 μ M. The response to potassium addition without compound was used as a control. Sample curves were plotted as the difference

of the observed response to that of the control. A plate of compounds from the Aventis collection was screened at a final concentration of 10 μ M. Compounds whose response was less than 3 SD from that of the control were presumed not to inhibit the hERG channel (FLIPR negative) and those more than 3 SD from the control were designated as hERG channel inhibitors (FLIPR positive). Selected compounds from this screening were further tested using the patch-clamp method as described below.

Patch-clamp analysis

hERG channel currents were recorded with an Axopatch 200B amplifier (Axon Instruments, Union City, CA) at room temperature using the whole-cell configuration of the patch-clamp technique.¹⁸ Thin-walled glass capillary tubes (World Precision Instruments, Sarasota, FL) were fabricated to a direct current resistance of 3–6 M Ω . Electrodes were filled with the following solutions (in mM): potassium aspartate, 120; KCl, 20; Na₂ATP, 4.0; HEPES, 5.0; MgCl₂, 1.0 (pH adjusted to 7.2 with KOH). The external solution contained (in mM): NaCl, 130; KCl, 5; sodium acetate, 2.8; MgCl₂, 1.0; HEPES, 10; glucose, 10; CaCl₂, 1.0 (pH adjusted to 7.4 with NaOH). Current readings were digitally stored and analyzed using the pCLAMP suite of software (Axon Instruments).

FMP dye

The cells were washed with Hank's/HEPES buffer 3 times, and then 25 μ l of the loading buffer was added (Hank's/20 mM HEPES buffer; pH 7.4) with a Multimek[™] (Beckman Coulter, Inc., Fullerton, CA). Next 25 μ l of the loading dye (loading buffer with 1 \times dye) was added and the cells were incubated at room temperature for 30 min. Compounds were added and the cells were incubated for another 10 min before the response to 50 mM KCl was measured with the FLIPR. A compound plate containing 88 Aventis compounds was screened at a final concentration of 10 μ M, and samples with a response more than 3 SD from the negative control were selected as hERG channel inhibitors (positive). The selected compounds were retested by patch clamp.

Dye–compound interaction test

An aliquot of 45 μ l of 1 \times dye was added to a 384-well plate with or without cells. Five microliters of one of the selected compounds was added to each well and the signal was measured with the FLIPR 5 min thereafter. The color-quenching effect was cal-

TABLE 1. COMPOUND IC₅₀ VALUES AND THEIR MINIMUM DETECTION DOSE (MDD) IN DIFFERENT DETECTION FORMATS

	IC ₅₀ (nM)		MDD (nM)	
	Patch Clamp	Rb ⁺ efflux	DiBAC ₄ (3)	FMD Dye
Dofetilide	10	69	1,000	100
Sertindole	14	352	10,000	10,000
Cisapride	45	1,500	10,000	10,000
Terfenadine	56	1,800	1,000	1,000
Astemizole	6	59	1,000	100

TABLE 2. WHOLE-CELL PATCH-CLAMP TEST OF COMPOUNDS CLASSIFIED IN DiBAC₄(3) METHOD

Compound	FLIPR Response	Patch Clamp ^a
A	Negative	—
B	Negative	—
C	Negative	—
D	Negative	—
E	Negative	—
F	Negative	+
G	Negative	—
H	Negative	—
I	Negative	—
J	Negative	—
K	Positive	+
L	Positive	+
M	Positive	—
N	Positive	—
O	Positive	—
P	Positive	—
Q	Positive	—

^aCompounds that inhibit over 50% of the hERG channel current at a concentration of 1 μ M were designated “+” or strong hERG channel inhibitors; compounds that inhibit less than 50% of the hERG channel current at a concentration of 1 μ M were designated “—” or weak hERG channel inhibitors.

culated by determining the ratio of the reading of samples with compounds to the reading with buffer alone. Four different conditions were tested: buffer alone, buffer with CHO cells, buffer with 1% FBS, and buffer alone with 100 μ M compound.

Rb⁺ efflux assay

A total of 50,000 hERG transfected cells/well were plated into a clear black 96-well plate (Costar #3603). After overnight culture at 37°C, the hERG cells were washed with buffer I (150 mM NaCl, 2 mM CaCl₂, 0.8 mM NaH₂PO₄, 1 mM MgCl₂, 5 mM glucose, 25 mM HEPES; pH 7.4) three times. Then 200 μ l of Rb⁺ loading buffer (buffer I with 5.4 mM RbCl) was added and the cells were incubated at 37°C for 4 h. The compounds were added and the cells were incubated at 37°C for another 30 min. The Rb⁺ buffer was then removed by washing the cells 3 times with 200 μ l of 5 mM KCl washing buffer (buffer I with 5 mM KCl). The hERG channel was activated by adding 200 μ l of 50 mM KCl buffer (buffer I with 50 mM KCl). The supernatant (SN) was transferred to a new 96-well plate after 10 min of incubation at room temperature. Cells were lysed by adding 200 μ l lysis buffer (1% Triton X-100 in buffer I). Rb⁺ concentration in the cells and SN was measured using the Analyst™ 100 atomic adsorbance spectrometer (AAS) (PE Bioscience, Foster City, CA). Rb⁺ efflux rate (RE) was calculated using the formula $[Rb^+_{SN}]/[total Rb^+]$. Rb⁺ efflux efficiency was calculated as

$$Rb^+ \text{ efflux efficiency} = \frac{(RE_{\text{sample}} - RE_{\text{negative}})}{(RE_{\text{positive}} - RE_{\text{negative}})}$$

The five known hERG channel inhibitors were tested at final concentrations of 10, 1, 0.1, 0.01, and 0.001 μ M and their IC₅₀ values were calculated with ABASE XLFIT (IDBS, Emeryville, CA). All data were collected in triplicate.

For screening quality evaluation, four 96-well plates were tested. Twenty-one wells of each 96-well plate were treated with 10 μ M astemizole and the Rb⁺ efflux of the astemizole-treated samples was calculated and used as the low control. The other 72 wells were treated with buffer only and their Rb⁺ efflux efficiencies were calculated and used as high control. The other 3 wells were spotted with unknown compounds. One 96-well plate of vector control cells was also tested for Rb⁺ efflux rate. The Z factor was calculated as described by Zhang et al.¹⁹

Forty-two compounds with known IC₅₀ values and 36 unknown compounds were tested in this format at a final concentration of 10 μ M. All samples were tested in triplicate, and the compounds were tested with patch-clamp technology for validation.

RESULTS

Cell line selection

Five cell lines were selected out of 400 screened with the DiBAC₄(3) FLIPR assay. Four of the cell lines quickly lost their channel activity during cell line maintenance. However, one CHO cell line, named P15C, was found to have a detectable and stable signal. This cell line was later used in all of the assays in this report.

DiBAC₄(3) assay

The hERG channel cells were exposed to compound before being activated by 50 mM of KCl. A test of a panel of five known hERG channel inhibitors indicated that this assay has a minimal detection dose (MDD) of 1, 10, 1, 1, and 10 μ M for dofetilide, sertindole, terfenadine, astemizole, and cisapride, respectively (Fig. 1; Table 1). The MDD of each compound was 100-fold higher than its respective IC₅₀ value as measured by patch-clamp electrophysiology. The MDD rankings of these compounds did not match with the ranking of

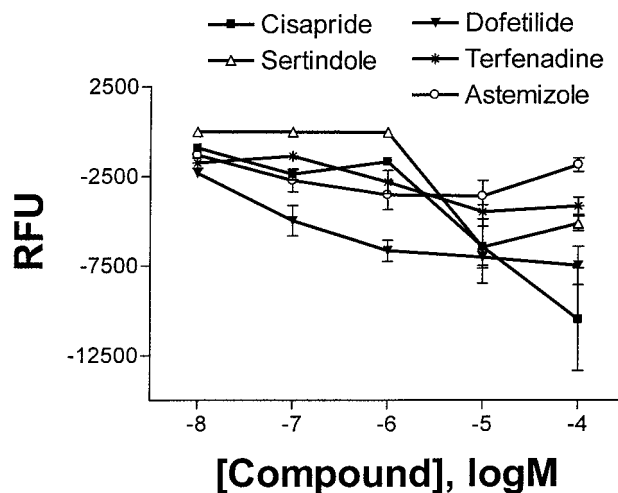


FIG. 2. Dose-response curve of the five known hERG channel inhibitors tested with FMP dye. Assay was performed in a 384-well format ($n = 3$).

TABLE 3. WHOLE-CELL PATCH-CLAMP TEST OF COMPOUNDS IDENTIFIED IN BOTH DiBAC₄(3) METHOD AND FMD DYE

Compounds	MD test ^a	DiBAC ₄ (3) ^a	Patch Clamp ^b
Dofetilide	+	+	+
Sertindole	+	+	+
Seldane	+	+	+
Hismanal	+	+	+
Propulsid	+	+	+
A1	+	+	—
B1	+	+	—
C1	+	+	—
D1	+	+	—
H1	—	+	—
A2	—	+	—

^aTesting positive (+) in either the FMD dye system or DiBAC₄(3) assay suggests the compound is a potent hERG channel inhibitor (IC₅₀ < 1 μM), while testing negative (—) means it is a weak hERG channel inhibitor (IC₅₀ > 1 μM).

^bCompounds that inhibit 50% of the hERG channel current at 1 μM were designated “+” and those with less than 50% inhibition were designated as “—”.

their IC₅₀ values either. Thus this method could only be used for identifying potential hERG channel inhibitors and could not be used for ranking compounds by their potency. Screening of a panel of 88 compounds selected 7 compounds as hERG channel inhibitors and 81 as noninhibitors. Ten of the noninhibitors were retested with patch-clamp electrophysiology and nine compounds were reconfirmed (Table 2). However, five of the seven positive compounds did not reconfirm with such retesting (Table 2).

FMP dye

Dofetilide, sertindole, cisapride, terfenadine, and astemizole were tested with this dye system and were all found to be positive, with MDDs of 0.1, 10, 10, 1, and 0.1 μM, respectively (Fig. 2). Like the DiBAC₄(3)-based assay, the detection limits were higher than the respective compounds' IC₅₀ values as measured by electrophysiology. The ranking of the MDDs of these compounds did not match with the ranking of their IC₅₀ values either. However, examination of a panel of 88 compounds indicated that screening hERG channel modulators with this dye may have a lower false-hit rate than that of the DiBAC₄(3) method (Table 3).

Color-quenching effect on a fluorescence-based detection system

The hERG channel inhibitors terfenadine and astemizole quenched the DiBAC₄(3) signal by as much as 50% at 10 μM in the presence of CHO cells (Fig. 3A). However, these compounds did not appear to quench the signal from the new FMP dye. This behavior was the same whether cells were present or not (Fig. 3B). The addition of 1% FBS, which increased the DiBAC₄(3) signal by 100%, did not affect the signal of the FMP dye (Fig. 3C). Thus, the FMP dye had less compound–dye interaction than did DiBAC₄(3). However, there was still a detectable color-quenching effect when 100 μM astemizole or terfenadine was used (Fig. 3D). Thus this new dye is not free of compound–dye interaction, at least at high drug concentrations.

Rb⁺ efflux assay

The loading of mammalian cells with Rb⁺ was achieved by the use of Rb⁺ loading buffer containing 5.4 mM RbCl. hERG

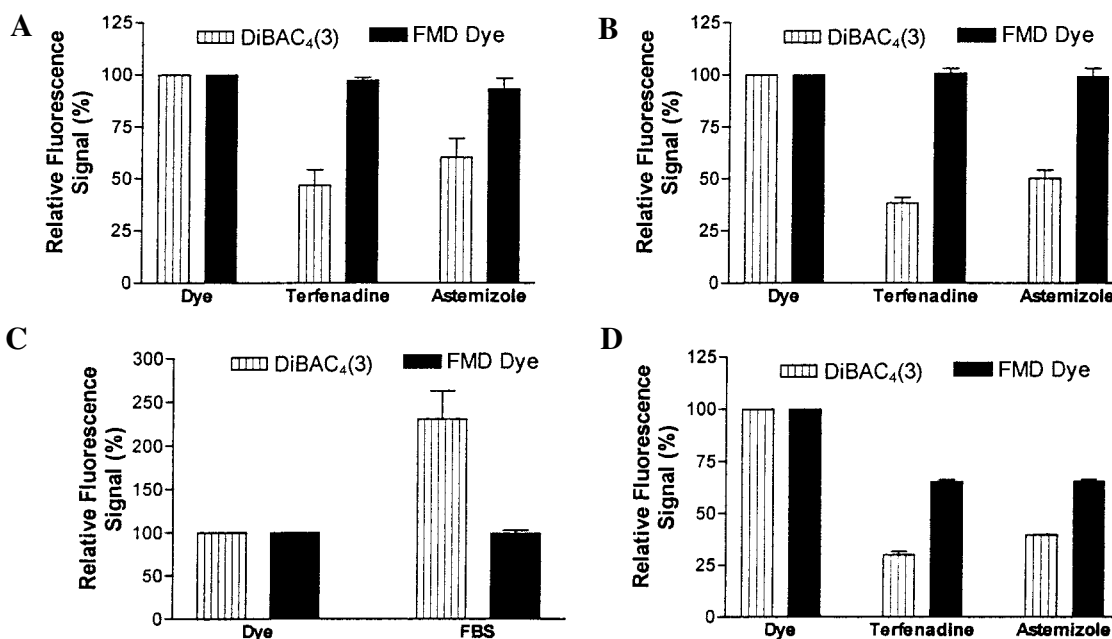


FIG. 3. Dye–compound interaction. Relative fluorescence signal was calculated as the fluorescence signal average of samples/dye alone × 100. (A) Interaction of 5 μM dye–10 μM compound in the presence of CHO cells. (B) Dye–compound interaction in the absence of cells. (C) Dye–FBS interaction in the absence of cells. (D) Dye–100 μM compound interaction in the absence of cells.

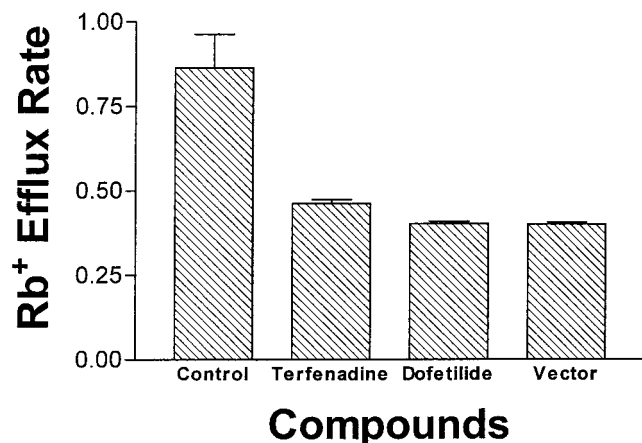


FIG. 4. Rubidium efflux assay for hERG potassium channel inhibitors. Each compound was tested at a final concentration of 30 μM . The Rb^+ efflux rate was calculated with the formula described in *Materials and Methods* ($n = 3$).

channels were activated by adding 50 mM KCl. After the hERG channel was activated, Rb^+ was released into the medium that contained no Rb^+ . The Rb^+ efflux rate was 85% in the absence of inhibitors and 40% in a pcDNA3 vector-transfected cell line (Fig. 4). The hERG-expressing cell line Rb^+ efflux rate was reduced to 40.2% and 46%, respectively, in the presence of 30 μM dofetilide and terfenadine (Fig. 4). IC_{50} values were 69, 1,800, 1,500, 352, and 59 nM for the five known hERG inhibitors dofetilide, terfenadine, cisapride, sertindole, and astemizole, respectively (Fig. 5), when they were tested in this system. These values are 5- to 20-fold higher than the reported IC_{50} values obtained from patch-clamp electrophysiology. However, the rank order of the compounds according to the IC_{50} values obtained

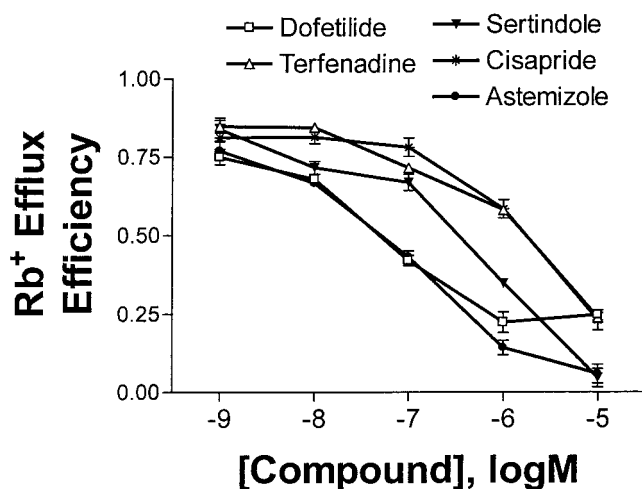


FIG. 5. IC_{50} values of five known hERG channel inhibitors tested in Rb^+ efflux assay. Rb^+ efflux efficiency was calculated by the efflux rate of (sample - negative control)/(positive control - negative control). The IC_{50} values for dofetilide, terfenadine, cisapride, sertindole, and astemizole are 69, 1,885, 1,518, 353, and 59 nM, respectively.

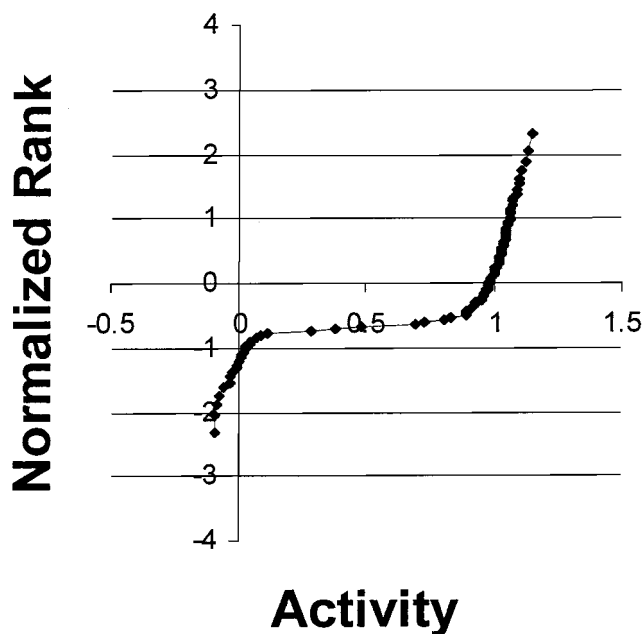


FIG. 6. Normal probability plot of sample hERG channel activity test in Rb^+ efflux assay format. Negative hERG channel population represents 21 samples inhibited by 10 μM astemizole and active population represents average of 72 samples without any inhibitor.

with this method was identical to that of their reported IC_{50} values with patch-clamp technique. Thus, the Rb^+ AAS assay can be used for ranking unknown compounds' hERG channel potency and meets the requirement for compound profiling.

This assay was further developed for the HTS standard evaluation. The average hERG activity for cells treated with 10 μM astemizole was 0, with a standard deviation of 5.8%. Cells without any channel blockers had an average hERG channel activity of 92% and standard deviation of 8.5%. The Z factor calculated based on those 384 samples was 0.53. Thus this assay was validated to meet HTS standards.¹⁹ A data normalization plot indicated that this assay has a good window to separate negative compounds from positive ones (Fig. 6).²⁰[FIG6] A 78-compound plate was screened at a final concentration of 10 μM with this method, and 33 compounds were found to have low potency for the hERG channel. Thirty compounds were reconfirmed by the patch-clamp technique to have IC_{50} values over 1.5 μM . Forty-five compounds were determined to be positive (potent hERG channel blocker) with this assay. Thirty-six of the positive compounds were tested with the patch-clamp technique and all of those were found to have IC_{50} values less than 1 μM .

DISCUSSION

The development of a functional hERG channel assay, as for other voltage-gated potassium channels, is hindered by the lack of reliable detection methods. Two traditional methods are widely accepted as standard methods for a potassium channel functional assay. One is patch-clamp electrophysiology, which

measures channel currents directly and is regarded as the most reliable functional potassium channel assay available. However, this is a labor-intensive and low throughput assay and thus does not meet today's need for quick profiling of primary hits from HTS. The throughputs of this assay are at best on the order of 10–20 compounds per day, depending on the researcher.²¹ Conversion of this technology to HTS is still not a possibility and not seriously considered by most HTS laboratories. The other method for a functional potassium channel assay is the Rb-86 efflux assay. This assay is based on the idea that potassium channels can transport not only K⁺ ions but also Rb⁺ ions. Since mammalian cells do not have intrinsic Rb⁺ ions, any concentration change can easily be detected by measuring the radioactivity change of the Rb-86. However, HTS assay development based on this readout is not a favorite among HTS laboratories because Rb-86 is highly radioactive.

Fluorescence-based assays have been widely pursued as an alternative potential HTS readout for potassium channel targets. Many of the successful high throughput functional potassium channel assays developed thus far have been focused on the synthesis of membrane potential-sensitive dyes.²² DiBAC₄(3) is one of the most widely used membrane potential dyes. Its distribution across biological membranes is dependent on membrane potential. Thus the fluorescence signal change can be used as a surrogate marker of potassium channel function. However, less than 20% of the dye signal change detected is associated with a 100-mV membrane potential change. Thus the development of a direct fluorescence signal change is dependent on the channel activity. Cells expressing hERG channels may have small currents, so the membrane potential changes associated with the blockage of this potassium channel may be limited. This is translated into a small fluorescence signal for this assay. One method to increase the signal is to increase cell number, as described in the DiBAC₄(3) section of *Materials and Methods*. A high concentration of KCl (50 mM) was applied in order to activate the hERG channels. High cell number combined with the high concentration of potassium ion in the medium may contribute to the low sensitivity of the DiBAC₄(3) assay. As shown in the *Results* section, the MDDs of the five hERG channel inhibitors are 100 times higher than the reported IC₅₀ values obtained with the patch-clamp technique. The screening of hERG channel inhibitors with this format was further complicated by the finding that three of the five compounds tested showed a strong quenching effect on the dye signal. Compounds that quench the dye signal could be misidentified as hERG channel inhibitors. This could contribute to the fact that the MDD sequence of the compounds tested did not match with their respective IC₅₀ values as determined with the patch-clamp technique.

A second assay format was reported by Gonzalez and co-workers using the fluorescence resonance energy transfer mechanism.^{23,24} The combination of the dyes with voltage/ion probe reader equipment has greatly increased the assay sensitivity and reduced the data variation.²⁵ However, the expense of this system may keep many research laboratories and companies from using this technology.

Molecular Devices' FMP dye provided a third tool for the potassium channel functional assay. The dye was reported to be temperature insensitive and quickly responsive to membrane potential changes.²⁶ Application of this FMP dye in our hERG

channel screen suggested that it displayed less dye–compound interaction. Our known inhibitor screening confirmed the advantage of using this dye. For instance, two known hERG channel inhibitors that quenched the DiBAC₄(3) signal did not interfere with the new FMP dye signal. A comparison of the results for the 88 Aventis compounds screened with DiBAC₄(3) or FMP dye identified additional compounds that quenched the DiBAC₄(3) signal, but not the signal from the new FMP dye (Table 3). Thus this dye could provide an alternative system for membrane potential-based screening. However, this system measures the membrane potential but not channel activity. Thus compounds affecting other determinants of membrane potential could be falsely identified as hERG channel inhibitors. Our results also indicated that 100 μM cisapride and terfenadine quenched the dye signal through compound–dye interaction.

The development of the nonradioactive, AAS-based functional hERG channel assay has opened up yet another avenue of screening. It has previously been reported to work for both Kv1.1 and Kv1.4 K⁺ channels with reasonable throughput.¹⁷ Our data suggested that this assay had a Z factor of 0.53 in the 96-well format for the hERG channel assay. Although the IC₅₀ values of the known hERG channel inhibitors are 5- to 20-fold higher than their respective IC₅₀ values as measured with the patch-clamp technique in mammalian cells, the IC₅₀ values are similar to those reported with hERG channels expressed in *Xenopus* oocytes.^{27,28} Most important of all, this assay generated IC₅₀ values with a ranking order similar to that determined with the patch-clamp technique.

CONCLUSIONS

We have evaluated different assay formats for functional hERG channel inhibition. The nonradioactive Rb⁺ efflux assay was proven to have the lowest false-hit rate. The assay was also optimized as a 96-well-based medium throughput assay. Current throughput of our system is limited by the AAS detection speed. Our system can process up to 200 samples per hour.

REFERENCES

1. Curren M, Splawski I, Timothy K, et al: A molecular basis for cardiac arrhythmia: hERG mutations cause long QT syndrome. *Cell* 1995;80:795–803.
2. Sanguinetti M, Jiang C, Curran M, et al: A mechanistic link between an inherited and an acquired cardiac arrhythmia hERG encodes the I_{Kr} potassium channel. *Cell* 1995;81:299–307.
3. Rampe D, Roy ML, Dennis A, et al: A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockage of the human cardiac potassium channel hERG. *FEBS Lett* 1997;417:28–32.
4. Ficker E, Jarolimek W, Kiehn J, et al: Molecular determinants of dofetilide block of hERG K⁺ channels. *Circ Res* 1998;82:386–395.
5. Bischoff U, Schmidt C, Netzer R, et al: Effects of fluoroquinolone on hERG currents. *Eur J Pharmacol* 2000;406:341–343.
6. Kang J, Wang L, Chen X-L, et al: Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K⁺ channel hERG. *Mol Pharmacol* 2001;59:122–126.

7. Roy M, Dumaine R, Brown A: hERG, a primary human ventricular target of the non-sedating antihistamine terfenadine. *Circulation* 1996;94:817–823.
8. Zhou Z, Vorperian VR, Gong Q, et al: Block of hERG potassium channels by the antihistamine astemizole and its metabolites desmethylastemizole and norastemizole. *J Cardiovasc Electrophysiol* 1999;10:836–843.
9. Rampe D, Murawsky MK, Grau J, et al: The antipsychotic agent sertindole is a high affinity antagonist of the human cardiac potassium channel hERG. *J Pharmacol Exp Ther* 1998;286:788–793.
10. Kang J, Wang L, Cai F, et al: High affinity blockage of the hERG cardiac K⁺ channel by the neuroleptic Pimozide. *Eur J Pharmacol* 2000;392:137–140.
11. Rampe D: Voltage-dependent potassium channels cloned from human heart: new tools to predict the pro-arrhythmic potential of drugs. *Ion Channel Modulators* 1997;2:331–337.
12. Mohammad S, Zhou Z, Gong Q, et al: Blockage of the hERG human cardiac K⁺ channel by the gastrointestinal prokinetic agent cisapride. *Am J Physiol* 1997;273:H2534–H2538.
13. Silverman E: Recall put FDA on the hot seat. *Star-Ledger* 2000;April 2:Section 3, p. 1.
14. European Agency for the Evaluation of Medicinal Products (EMA), Committee for Proprietary Medicinal Products: Points to consider in the assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products. London: EMA, 1997:986–996.
15. McDonald T, Yu Z, Ming Z, et al: A minK-hERG complex regulates the cardiac potassium current I_{Kr}. *Nature* 1997;388:289–292.
16. Trudeau M, Warmke J, Ganerzky B, et al: hERG, a human inward rectifier in the voltage gated potassium channel family. *Science* 1995;269:92–95.
17. Terstapen CG: Functional analysis of native and recombinant ion channels using a high capacity nonradioactive rubidium efflux assay. *Anal Biochem* 1999;272:149–155.
18. Hamill OP, Marty A, Neher E, et al: Improved patch clamp techniques for high resolution current recording from cells and cell free membrane patches. *Pflugers Arch Eur J Physiol* 1983;391:85–100.
19. Zhang J-H, Chung TDY, Oldenburg KR: A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen* 1999;4:67–73.
20. Li Z, Mehdi S, Patel I, et al: An ultra-high throughput screening approach for an adenine transferase using fluorescence polarization. *J Biomol Screen* 2000;5:47–51.
21. Denyer J, Worley J, Cox B, et al: HTS approaches to voltage-gated ion channel drug discovery. *Drug Discov Technol* 1999;3:323–332.
22. Fromherz P, Muller C: Voltage-sensitive fluorescence of amphiphilic hemicyanine dyes in neuron membrane. *Biochim Biophys Acta* 1993;1150:111–122.
23. Gonzalez J, Tsien R: Improved indicators of cell membrane potential that use fluorescence resonance energy transfer. *Chem Biol* 1997;4:269–277.
24. Gonzalez J, Tsien R: Voltage sensing by fluorescence resonance energy transfer in single cells. *Biophys J* 1995;69:1272–1280.
25. Gonzalez J, Oades K, Leychikis Y, et al: Cell-based assays and instrumentation for screening ion channel targets. *Drug Discov Today* 1999;4:431–439.
26. Xie M: Characterization of a novel membrane potential dye for voltage- and ligand-gated ion channels using FLIPR. Presented at the 4th International Cell Analysis Products User Meeting, Napa Valley, CA, 2000.
27. Lees-Miller JP, Duan Y, Duff HJ: Molecular determinant of high-affinity dofetilide binding to HERG1 expressed in *Xenopus* oocytes: involvement of S6 sites. *Circulation* 1999;100(Suppl 1):I424.
28. Suessbrich H, Waldegger S, Lang F, et al: Blockade of hERG channels expressed in *Xenopus* oocytes by the histamine-receptor antagonists terfenadine and astemizole. *FEBS Lett* 1996;385:77–80.

Address reprint requests to:

Weimin Tang
Department of Profiling and Screening
Aventis Pharmaceuticals
Route 206 North
Bridgewater, NJ 08807-2854

E-mail: weimin.tang@aventis.com