Astemizole Derivatives as Fluorescent Probes for hERG Potassium Channel Imaging

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S Supporting Information

ABSTRACT: The detection and imaging of hERG potassium channels in living cells can provide useful information for hERG-correlation studies. Herein, three small-molecule fluorescent probes, based on the potent hERG channel inhibitor astemizole, for the imaging of hERG channels in hERG-transfected HEK293 cells (hERG-HEK293) and human colorectal cancer cells (HT-29), are described. These probes are expected to be applied in the physiological and pathological studies of hERG channels.

KEYWORDS: hERG potassium channel, fluorescent probes, astemizole, cell imaging

hERG (human ether-a-go-go-related gene) encodes a potassium channel that is expressed in several tissues and cell types, such as cardiac and smooth muscle; liver, pancreas, and neural tissue; and certain tumor cells.^{1,2} In the heart, hERG is highly expressed and is responsible for the primary component of the delayed rectifier K⁺ current (IKr), which plays a critical role in cardiac action potential repolarization.^{3,4} Functional abnormality of hERG potassium channels can cause inherited or acquired long QT syndrome (LQTS).^{5,6} Long QT intervals can be caused by mutations in the hERG gene and by drug interactions. Because various drugs have been found to be related to LQTS, drug interactions have become an important safety concern in both drug discovery and clinical practice.^{6,7} Therefore, hERG potassium channels have been recognized as a primary target to evaluate cardiotoxicity in the screening of drug candidates.⁸

In recent years, many studies have shown that hERG-channel expression is upregulated in tumor cells such as breast cancer (MCF-7), neuroblastoma (SH-SY5Y), and colon cancer cells (HT-29), whereas expression is low or absent in the corresponding normal cells.^{9–11} In addition, it was also found that hERG channels influence cell proliferation, survival and apoptosis, and sensitivity to chemotherapy in tumor cells.^{12,13} However, the expression and the role of the hERG channels in human tumor cells are still poorly understood and unexplored. It is possible that hERG potassium channels could become a new target and biomarker for tumor research. Due to the importance of understanding the physiological and pathological studies of hERG channels, there is an urgent demand for a simple, safe, and cost-effective method to label hERG channels for their study. Small-molecule fluorescent probes are a highly sensitive and convenient option, which can provide advantageous information in real-time and with high spatial-temporal resolution.¹⁴ Currently, small-molecule fluorescent probes have been widely used in biology and pharmacology for the

detection of proteins, nucleic acids, and other vital biological molecules.^{15–17}

hERG channels are blocked by structurally diverse drugs, including antiarrhythmic agents (dofetilide), antihistamines (astemizole), and antipsychotics (chlorpromazine).^{18,19} So far, among the drugs that have an inhibitory effect on hERG channels, astemizole is the most effective blocker.²⁰ Studies of the molecular mechanisms between the hERG channel and its inhibitors suggest that an aromatic group and a basic nitrogen of the blocker are required for high-affinity binding of the hERG channel.^{21,22} Based on these results, the major interaction sites for astemizole binding were chosen as the recognition moiety of the hERG channel for the development of the fluorescent probes. In addition, the fluorophores coumarin, 4-chloro-7-nitrobenzoxadiazole (NBD), and naphthalimide, which could provide good fluorescent properties, were conjugated to the recognition moiety using an aliphatic spacer. Subsequently, several small-molecule fluorescent probes (**1a–1c**) for the hERG channel were designed (Scheme 1).

To establish a preliminary understanding of the interaction between these fluorescent derivatives of astemizole and the hERG channels, molecules **1a–1c** were docked into an open-inactivated hERG homology model following our method published in 2007.¹⁹ The optimized docking conformations and orientations of **1a–1c** at the binding pocket of the hERG channel were highly consistent with those of astemizole (Figures 1 and S1–S4). These computational results suggest that compounds **1a–1c** may be recognized by the hERG channel. More modeling details can be found in the Supporting Information.

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Scheme 1. Strategy of Small-Molecule Fluorescent Probes for the hERG Channel

Figure 1. Proposed docking conformation of astemizole (white sticks), 1a (cyan sticks), 1b (purple sticks), and 1c (yellow sticks) in the hERG homology model.

Scheme 2. Synthetic Routes of the Fluorescent Probes

Table 1. Photophysical Properties of Synthesized Probes

compd	$\lambda_{\max}$ (nm)	λ_{ex} (nm)	λ_{em} (nm)	Φ (%)
1a	430	416	547	3.5
1b	478	476	547	2.8
1c	355	354	469	79.1

Table 2. Inhibitory Activity of the Synthesized Probes against the hERG Potassium Channel^a

compd	IC ₅₀ (μM)	K _i ^b (μM)
1a	0.434 $\pm$ 0.029	0.217
1b	0.025 $\pm$ 0.002	0.012
1c	0.182 $\pm$ 0.016	0.091
astemizole	0.023 $\pm$ 0.011	0.011

^aSee Supporting Information. ^bThe inhibition constant (K_i) was calculated from each IC₅₀ value using the Cheng–Prusoff equation.

Table 3. Cytotoxicity Results for the Synthesized Probes

compd	IC ₅₀ (μM)	
	hERG-HEK293 cells	HT-29 cells
1a	4.7 $\pm$ 0.2	9.9 $\pm$ 1.1
1b	12.5 $\pm$ 2.3	36.1 $\pm$ 3.3
1c	7.2 $\pm$ 1.6	18.6 $\pm$ 0.6
astemizole	5.1 $\pm$ 0.8	15.4 $\pm$ 2.7

Figure 2. Fluorescence microscopy imaging of hERG transfected HEK293 cells incubated with 1 μM probe 1a (A1, bright field; A2, GFP channel), 0.5 μM probe 1b (B1, bright field; B2, GFP channel), and 1 μM probe 1c (C1, bright field; C2, DAPI channel), respectively. The imaging of inhibition of the hERG channels was accomplished by incubating 100 μM astemizole with probe 1a (1 μM ; A3, bright field; A4, GFP channel), 1b (0.5 μM ; B3, bright field; B4, GFP channel), and 1c (1 μM ; C3, bright field; C4, DAPI channel). All cells were incubated with each probe at 37 $^{\circ}\text{C}$ for 10 min and washed immediately. The background was adjusted by ImageJ software. Imaging was performed using a Zeiss Axio Observer A1 microscope with a 63 $\times$ objective lens.

Figure 3. Fluorescence microscopic imaging of HT-29 cells incubated with 1 μM probe **1a** (A1, bright field; A2, GFP channel), 0.5 μM probe **1b** (B1, bright field; B2, GFP channel), and 1 μM probe **1c** (C1, bright field; C2, DAPI channel), respectively. The imaging of inhibition of hERG channels was accomplished by incubating 100 μM astemizole with probe **1a** (1 μM ; A3, bright field; A4, GFP channel), **1b** (0.5 μM ; B3, bright field; B4, GFP channel), and **1c** (1 μM ; C3, bright field; C4, DAPI channel). HT-29 cells were incubated with each probe at 37 $^{\circ}\text{C}$ for 10 min and washed immediately. The background was adjusted by ImageJ software. Imaging was performed using a Zeiss Axio Observer A1 microscope with a 63 $\times$ objective lens.

The convenient syntheses of the fluorescent derivatives of astemizole are shown in [Scheme 2](#). Coupling the coumarin acid with compound **2** under HOBt/EDCI conditions yielded the fluorescent product **1a**. NBD was alkylated with compound **2** in the presence of K_2CO_3 in 1,4-dioxane to produce fluorescent derivative **1b**. Compound **3** and naphthalimide in the presence of K_2CO_3 in acetonitrile yielded the fluorescent compound **1c**. Further details on the synthesis of the recognition moieties and fluorophores can be found in the [Supporting Information](#).

The spectroscopic properties of the target compounds **1a–1c** were measured in 10 μM solutions of the corresponding probe in PBS (pH = 7.4). The results showed that all probes possessed good fluorescent properties. In particular, probe **1c** has a maximum emission wavelength at 469 nm and a high fluorescence quantum yield of 79.1% ([Table 1](#)). Compared with probe **1c**, the emission wavelengths of probes **1a** and **1b** were significantly red-shifted. However, the fluorescence quantum yields of these compounds were relatively low.

Next, the inhibitory activities of these three probes against the hERG potassium channel were determined by radioligand binding assays using [^3H] dofetilide in membranes from hERG-transfected HEK293 cells. For these assays, astemizole was chosen as a positive control (see details in the [Supporting Information](#)).^{20,23,24} The results revealed that all three probes have a high affinity for the hERG channel. Probe **1b** has calculated IC_{50} and K_i values of 0.025 ± 0.002 and $0.012 \mu\text{M}$, respectively, which are equivalent to those of astemizole (0.023 ± 0.011 and $0.011 \mu\text{M}$, respectively; see [Table 2](#)). Compounds **1a** and **1c** also showed potent inhibitory activity against the hERG channel, although lower than that of probe **1b**, with IC_{50} values of 0.434 ± 0.029 and $0.182 \pm 0.016 \mu\text{M}$, respectively.

Additionally, the cytotoxicity of these probes in living cells was evaluated by MTT assays using hERG-transfected HEK293 and HT-29 cells. The results revealed that the half maximal inhibitory concentration (IC_{50}) values of probes **1a–1c** were 4.7 ± 0.2 , 12.5 ± 2.3 , and $7.2 \pm 1.6 \mu\text{M}$ in hERG-HEK293 cells (see [Table 3](#)). Furthermore, the IC_{50} values of probes **1a–1c** for HT-29 cells were 9.9 ± 1.1 , 36.1 ± 3.3 , and $18.6 \pm 0.6 \mu\text{M}$. The results indicated that these probes have acceptable cell toxicity for use in detection and imaging of hERG channels in living cells.

Because of their potent inhibitory activity, good fluorescent properties, and acceptable cell toxicity, compounds **1a–1c** were chosen as suitable candidates for labeling hERG channels. Thus, the specific selectivity of probes **1a–1c** for hERG channels in living cells was evaluated using hERG-transfected HEK293 cells. The imaging results showed that these probes exhibit strong fluorescence and rapid responses to hERG-HEK293 cells ([Figure 2](#)). As a negative control, the inhibition of the hERG channels was imaged by incubating the cells with 100 μM astemizole together with each probe. Inhibition of hERG by astemizole resulted in a decrease of fluorescence intensity. The results showed that these probes display favorable selectivity for hERG potassium channels and could be used in hERG channel detection. In addition, cell autofluorescence and the effect of astemizole on cell autofluorescence were also investigated (see [Supporting Information](#)). The results showed that the autofluorescence of the cells used is negligible in both the absence and presence of astemizole ([Figure S8](#)) and would not interfere with the imaging of cells using probes **1a–1c**.

To extend the application of probes **1a–1c**, we also evaluated whether they can be used in the imaging of tumor cells with high levels of hERG channel expression. Previous studies have demonstrated that the hERG potassium channels are indeed highly expressed in some cancer cell lines.¹² Cherubini et al. have shown that the hERG gene and hERG proteins are highly expressed in endometrial cancer tissue, compared with the normal or hyperplastic endometrium.⁹ Elena Lastraioli et al. studied the expression of hERG channels in human colorectal cancer cells and showed that hERG channels are highly expressed in a high percentage of colon

cancer cell lines.²⁵ Furthermore, significant levels of hERG channel expression have been found in a variety of other tumor cell lines including epithelial, neuronal, and leukemic cells.¹² Because hERG is expressed in tumor tissue and is absent in normal tissue, hERG may be regarded as a potential biomarker for tumors. Accordingly, the imaging of hERG channels could provide useful information for tumor detection and labeling. Because of the high levels of hERG expression in colorectal cancer cells (HT-29 cells), the potential of the synthesized probes **1a–1c** in tumor cell imaging was evaluated using HT-29 cells (see [Supporting Information](#)). The imaging results demonstrated that these probes are able to label hERG channels for visualization. When 100 μ M of astemizole, a high-affinity inhibitor, was delivered into the HT-29 cells, the fluorescence intensity observed was quenched ([Figure 3](#)), indicating that these probes can be used as a labeling toolkit for hERG highly expressing tumor cells. In addition, hERG channel blockers were found to induce apoptosis and produce antiproliferative effects in tumor cells by directly blocking the hERG channels, and thus play a role in anticancer therapy.¹² Cisapride has been confirmed to prevent gastric cancer cell proliferation by inhibition of hERG channels.²⁶ Thus, the hERG channels could be used not only as a tumor biomarker but also as a future drug target. Small-molecule fluorescent probes have been used for drug screening in recent years. Therefore, we expect that these fluorescent probes for hERG channels could be employed as competitive substrates for hERG inhibitor activity screening and applied in the detection of tumors. Based on these preliminary results, our laboratory will pursue the further development of fluorescent probes for hERG channels.

In conclusion, three small-molecule fluorescent probes, with excellent fluorescent properties for hERG channel localization and visualization, have been designed and synthesized. The probes have been successfully used to label the hERG channels in hERG-transfected HEK293 (hERG-HEK293) cells and human colorectal cancer (HT-29) cells at the micromolar level. Moreover, these probes exhibited high inhibitory activity on the hERG channels and acceptable toxicity in cells. The preparation of these small fluorescent probes is also convenient. These features make the probes favorable for drug screening and cell staining. Therefore, these probes are anticipated to be applied in the detection of hERG channels, as well as in physiological and pathological studies of hERG channels.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmchemlett.5b00360](https://doi.org/10.1021/acsmchemlett.5b00360).

Full experimental procedures; analytical and spectral characterization data of all compounds ([PDF](#))

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Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

hERG, human ether-a-go-go-related gene; LQTS, long QT syndrome; hERG-HEK293 cells, hERG transfected HEK293 cells

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