SUPPORTING INFORMATION

Astemizole-based Turn-On Fluorescent Probes for Imaging hERG Potassium Channel

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1. Materials and instruments

All solvents and reagents available from commercial sources were used as received unless otherwise noted. Water used for the fluorescence studies was doubly distilled and further purified with a Mill-Q filtration system. Melting points were determined on an electrothermal melting point apparatus and were uncorrected. $^1$H NMR and $^{13}$C NMR were recorded on a Bruker 400 MHz NMR spectrometer. Mass spectra were performed by the analytical and the mass spectrometry facilities at Shandong University and Shandong Provincial Academy of Sciences. Absorption spectra and fluorescence spectra were obtained with a Thermo Varioskan microplate reader. Fluorescence imaging was performed using Zeiss Axio Observer A1 fluorescence microscopy.

2. Synthesis

2.1 Synthesis of intermediates

Scheme S1. Synthesis route of the intermediates

2-chloro-1-(4-fluorobenzyl)-1H-benzo[d]imidazole (1)

A mixture of 2-chlorobenzimidazole (2.5 g, 16.35 mmol) and potassium carbonate (1.31 g, 23.3 mmol) in acetonitrile (15 mL) were refluxed for 30 min. The solution was cooled to room temperature and then 4-fluorobenzyl bromide (4.65 g, 24.05 mmol) was
added. The mixture was refluxed for another 5 h. Water was added and the mixture was extracted with dichlormethane. The combined organic layers were dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated in vacuo. The crude product was recrystallized in petroleum ether to afford \textbf{1} as white solid in 47% yield. M.p.: 66-69 °C. \textsuperscript{1}H-NMR (400 MHz, DMSO-\textit{d6}): \(\delta\) ppm: 7.65-7.62 (m, 2H), 7.32-7.24 (m, 4H), 7.22-7.16 (m, 2H), 5.53 (s, 2H); ESI-MS: ([M+H]\textsuperscript{+}): 261.2.

\textbf{Ethyl-4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidine-1-carboxylate (2)}

A mixture of compound \textbf{1} (1.5 g, 5.7 mmol) and ethyl 4-aminopiperidine-1-carboxylate (1.485 g, 8.55 mmol) were subjected to Schlenk tube (170 °C, 8 h). After completion of the reaction, the solid was dissolved in dichloromethane and concentrated in vacuo. The crude product was purified by column chromatography to afford \textbf{2} as white solid in 37% yield. M.p.: 178-180 °C. \textsuperscript{1}H-NMR (400 MHz, DMSO-\textit{d6}): \(\delta\) ppm: 7.23-7.18 (m, 1H), 7.17-7.13 (m, 2H), 7.08-7.06 (m, 1H), 6.96 (td, \(J_1 = 8.0\) Hz, \(J_2 = 4.0\) Hz, 1H), 6.86 (t, \(J = 8.0\) Hz, 1H), 6.66 (d, \(J = 8.0\) Hz, 1H), 5.26 (s, 1H), 4.07-4.02 (m, 2H), 3.99-3.94 (m, 2H), 2.96 (s, 2H), 2.52-2.50 (m, 2H), 2.01-1.97 (m, 2H), 1.47-1.37 (m, 2H), 1.24-1.15 (m, 3H); ESI-MS: ([M+H]\textsuperscript{+}) : 397.4.

\textbf{1-(4-fluorobenzyl)-N-(piperidin-4-yl)-1H-benzo[d]imidazol-2-amine hydrobromide (3)}

A mixture of compound \textbf{2} (1 g, 2.52 mmol) and 40% HBr aqueous solution (25 mL) were stirred at 100 °C for 16 h. Thereafter, solvent was evaporated in vacuo and the crude product was recrystallized in ethyl acetate to afford \textbf{3} as white solid in 93% yield. M.p.: 261-263 °C. \textsuperscript{1}H-NMR (400 MHz, DMSO-\textit{d6}): \(\delta\) ppm: 9.05 (s, 1H), 8.06 (s, 2H), 7.52-7.50 (m, 1H), 7.43-7.41 (m, 2H), 7.39-7.35 (m, 2H), 7.33-7.26 (m, 2H), 7.24-7.18 (m, 2H), 5.51 (s, 2H), 4.06 (q, \(J = 8.0\) Hz, 1H), 3.46 (d, \(J = 8.0\) Hz, 2H), 3.03-2.97 (m, 2H), 2.20-2.15 (m, 2H), 1.99-1.84 (m, 2H); ESI-MS: ([M+H]\textsuperscript{+}) : 325.4.

\textbf{6-(dimethylamino)-1H,3H-benzo[de]isochromene-1,3-dione(a1)}

6-bromo-1H,3H-benzo[de]isochromene-1,3-dione (1.66 g, 5.91 mmol) and 3-(dimethylamino)propanenitrile (2.4 g, 23.96 mmol) in isopropyl alcohol (45 mL) were refluxed for 1 h. Then the reaction solution was cooled at room temperature, filtered to afford \textbf{a1} as yellow solid in 93% yield. M.p.: 201-205 °C. \textsuperscript{1}H-NMR (400 MHz, DMSO-\textit{d6}): \(\delta\) ppm: 8.61-8.58 (m, 1H), 8.48 (d, \(J = 8\) Hz, 1H), 8.34 (d, \(J = 8\) Hz, 1H), 7.79-7.75 (m, 1H), 7.22 (d, \(J = 12\) Hz, 1H), 3.17 (s, 6H); ESI-MS: ([M+H]\textsuperscript{+}) : 242.3.
2-(3-bromopropyl)-6-(dimethylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione (a)

A mixture of compound b1 (0.6 g, 3.03 mmol) and 3-aminopropan-1-ol (272.89 mg, 3.63 mmol) in ethyl alcohol (15 mL) were refluxed for 6 h. Thereafter, solvent was evaporated in vacuo and the crude product was dissolve in ethyl acetate (6 mL). Tribromophosphane (5 mL) was dissolve in ethyl acetate (5 mL) and was dropwise added to the reaction solution in ice bath condition. After 0.5 h, the reaction solution was moved to 80 °C for 8 h. Yellow solid appeared, then the reaction solution was filtered to afford a as yellow solid in 98% yield. M.p.: 120-123 °C. ¹H-NMR (400 MHz, DMSO-d6): δ ppm: 8.53 (d, J = 8.0 Hz, 1H), 8.48-8.46 (m, 1H), 8.36 (d, J = 8.0 Hz, 1H), 7.78-7.74 (m, 1H), 7.24 (d, J = 12.0 Hz, 1H), 4.17-4.14 (m, 2H), 3.61-3.58 (m, 2H), 3.10 (s, 6H), 2.22-2.15 (m, 2H); ESI-MS: ([M+H]⁺) : 361.2.

2-(4-bromobutyl)-6-(dimethylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione (b)

A mixture of compound b1 (350 mg, 1.45 mmol) and 4-aminobutan-1-ol (155.19 mg, 1.74 mmol) in ethyl alcohol (15 mL) were refluxed for 6 h. Thereafter, solvent was evaporated in vacuo and the crude product was dissolve in ethyl acetate (6 mL). Tribromophosphane (3.5 mL) was dissolve in ethyl acetate (5 mL) and was dropwise added to the solution of the crude product in ice bath condition. After 0.5 h, the reaction liquid moved to 80 °C for 8 h. Yellow solid appeared, then the reaction solution was filtered to afford b as yellow solid in 96% yield. M.p.: 176-179 °C. ¹H-NMR (400 MHz, DMSO-d6): δ ppm: 8.52-8.49 (m, 1H), 8.47-8.45 (m, 1H), 8.35 (d, J=8.0 Hz, 1H), 7.78-7.73 (m, 1H), 7.23-7.20 (m, 1H), 4.08 (t, J = 8.0 Hz, 2H), 3.59-3.56 (m, 2H), 3.10 (s, 6H), 1.93-1.80 (m, 2H), 1.78-1.73 (m, 2H) ; ESI-MS: ([M+H]⁺) : 375.3.

5-(dimethylamino)-N-(3-hydroxypropyl)naphthalene-1-sulfonamide (c1)

5-(dimethylamino)naphthalene-1-sulfonyl chloride (1 g, 2.52 mmol) was dissolved in dichlormethane (10 mL), and 3-hydroxypropan-1-aminium (0.22 g, 2.00 mmol) was dissolved in sodium carbonate solution (2M). The sodium carbonate solution of 3-hydroxypropan-1-aminium was added to the solution of 5-(dimethylamino)naphthalene-1-sulfonyl chloride by constant pressure titration at room temperature for 16 h. The reaction solution was slightly acidic with citric acid, extracted with dichloromethane, concentrated in vacuo, and recrystallized with petroleum ether, filtered to afford c1 as light yellow powder in 57% yield. M.p.: 122-125 °C. ¹H-NMR (400 MHz, DMSO-d6): δ ppm: 8.46 (d, J = 8.0 Hz, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.10-
8.08 (m, 1H), 7.64-7.56 (m, 2H), 7.26 (d, \( J = 8.0 \) Hz, 1H), 3.31-3.28 (m, 2H), 2.83 (s, 6H), 2.82-2.79 (m, 2H), 1.50-1.44 (m, 2H); ESI-MS: ([M+H]+) : 309.4.

3-((5-(dimethylamino)naphthalene)-1-sulfonamido)propyl 4 methylenesulphonate (c)

Compound c1 (0.2 g, 648.52 \( \mu \)mol) was dissolved in dichlormethane (6 mL), and triethylamine was added. 4-methylbenzenesulfonyl chloride (185.46 mg, 972.78 \( \mu \)mol) was dissolve in dichlormethane (10 mL) and the solution was dropwise added to the solution of compound c1 in ice bath condition. After the ice bath reaction for 2 h, the reaction solution was moved to the room temperature for 48 h. The combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo. Thereafter, solvent was evaporated in vacuo and the crude product was recrystallized in petroleum ether to afford c as white solid in 47% yield. \(^1\)H-NMR (400 MHz, DMSO-\( d_6\)): \( \delta \) ppm: 8.49-8.46 (m, 1H), 8.25 (d, \( J = 8.0 \) Hz, 1H), 8.05-8.06 (m, 1H), 7.95-7.92 (m, 1H), 7.70-7.68 (m, 2H), 7.65-7.56 (m, 2H), 7.46-7.43 (m, 2H), 7.28-7.26 (m, 1H), 3.96-3.93 (m, 2H), 2.84 (s, 6H), 2.78-2.84 (m, 2H), 2.41 (s, 3H), 1.66-1.63 (m, 2H).

2.2 Synthesis of probes

2.2.1 Synthesis of probe N1

In a 100 mL round bottom flask, compound 3 (150.00 mg, 415.24 \( \mu \)mol), compound a (168.30 mg, 415.24 \( \mu \)mol) and anhydrous K\(_2\)CO\(_3\) (143.47 mg, 1.04 mmol) were added in dry MeCN (10 mL) and dry DMF (5 mL). After stirred at 80 °C for 18 h, the reaction solution was cooled to room temperature and concentrated in vacuo. The crude product was purified by silica gel chromatography, then formed to be salt by ethyl acetate saturated with HCl to afford compound N1 as yellow solid in 23% yield. mp: 189-191 °C. \(^1\)H-NMR (400 MHz, DMSO-\( d_6\)): \( \delta \) ppm: 14.48 (s,1H), 10.69 (s,1H), 9.48 (s,1H), 8.55 (d, \( J = 8 \) Hz, 1H), 8.50 (d, 1H, \( J = 8 \) Hz, 1H), 8.39 (d, \( J = 8 \) Hz, 1H), 7.80 (t, \( J = 8 \) Hz, 1H), 7.51-7.48 (m, 1H), 7.41-7.36 (m, 3H), 7.28-7.17 (m, 5H), 5.56 (s, 2H), 4.16-4.14 (m, 4H), 3.62 (d, \( J = 12 \) Hz, 2H), 3.23-3.17 (m, 2H), 3.11 (s, 6H), 3.04-2.95 (m, 2H), 2.23-2.12 (m, 5H). \(^{13}\)C-NMR (100 MHz, DMSO-\( d_6\)): \( \delta \) ppm: 164.63, 164.09, 156.93, 153.31, 142.44, 134.54, 132.59, 131.11, 130.97, 130.24, 128.26, 128.18, 125.32, 124.89, 123.15, 121.49, 119.68, 116.62, 116.34, 116.12, 115.09, 113.34, 107.01, 56.09, 51.92, 49.62, 45.05, 44.80, 38.68, 32.68, 25.56. ESI-HRMS: ([M+H]+) calcd for \( C_{36}H_{37}FN_6O_2\): 605.3035, found: 605.3032.
2.2.2 Synthesis of probe N2

In a 100 mL round bottom flask, compound 3 (324.02 mg, 799.43 μmol), compound b (300.00 mg, 799.43 μmol) and anhydrous K2CO3 (276.21 mg, 2.00 mmol) were added in dry MeCN (10 mL) and dry DMF (5 mL). After stirring at 80 °C for 18 h, the reaction solution was cooled to room temperature and concentrated in vacuo. The crude product was purified by silica gel chromatography, then formed to be salt by ethyl acetate saturated with HCl to afford compound N2 as yellow solid in 24% yield. mp: 196-198 °C. 1H-NMR (400 MHz, DMSO-d6): δ ppm: 14.33 (s, 1H), 10.89 (s, 1H), 9.54 (s, 1H), 8.54 (d, J = 8 Hz, 1H), 8.49 (d, 1H, J = 8 Hz, 1H), 8.38-8.35 (m, 1H), 7.79-7.75 (m, 1H), 7.51-7.49 (m, 1H), 7.42-7.37 (m, 3H), 7.31-7.26 (m, 1H), 7.24-7.17 (m, 4H), 5.58 (s, 2H), 4.15 (s, 1H), 4.10 (d, J = 8 Hz, 2H), 3.62 (d, J = 12 Hz, 2H), 3.15-3.14 (m, 2H), 3.10 (s, 6H), 2.98-2.96 (m, 2H), 2.22-2.17 (m, 4H), 1.85-1.81 (m, 2H), 1.72-1.67 (m, 2H). 13C-NMR (100 MHz, DMSO-d6): δ ppm: 164.19, 163.53, 157.02, 149.36, 132.81, 132.06, 131.10, 130.81, 129.88, 129.80, 129.71, 125.51, 124.68, 124.16, 122.80, 116.21, 116.00, 113.79, 113.51, 112.10, 111.01, 55.92, 51.29, 49.99, 45.30, 44.88, 39.24, 28.85, 25.45, 21.45. 


2.2.3 Synthesis of probe N3

In a 100 mL round bottom flask, compound 3 (157.72 mg, 389.12 μmol), compound c (150.00 mg, 389.12 μmol) and anhydrous K2CO3 (134.45 mg, 972.81 μmol) were added in dry MeCN (10 mL) and dry DMF (5 mL). After stirring at 80 °C for 18 h, the reaction solution was cooled to room temperature and concentrated in vacuo. The crude product was purified by silica gel chromatography, then formed to be salt by ethyl acetate saturated with HCl to afford compound N3 as yellow solid in 49% yield. mp: 189-191 °C. 1H-NMR (400 MHz, DMSO-d6): δ ppm: 14.33 (s, 1H), 10.95 (s, 1H), 9.48 (d, J = 8 Hz, 1H), 8.66-8.61 (m, 1H), 8.46-8.42 (m, 1H), 8.21-8.16 (m, 2H), 7.74-7.70 (m, 2H), 7.51-7.49 (m, 1H), 7.41-7.36 (m, 3H), 7.31-7.26 (m, 2H), 7.24-7.18 (m, 2H), 5.56 (s, 2H), 4.11 (s, 3H), 3.48-3.45 (m, 2H), 2.98 (s, 6H), 2.88-2.87 (m, 4H), 2.19-2.13 (m, 4H), 1.87-1.83 (m, 2H). 13C-NMR (100 MHz, DMSO-d6): δ ppm: 149.36, 136.39, 131.54, 130.81, 129.85, 129.77, 129.70, 129.36, 128.37, 124.18, 116.22, 112.11, 111.01, 60.22, 53.89, 51.33, 49.89, 45.92, 45.28, 28.79, 24.37.

HPLC, t_R = 5.838 min, mobile phase: methanol-water (85:15, v/v), λ = 270 nm.

3. Fluorescent excitation and emission spectra of free probe N1-N3

Figure S1. Fluorescent excitation (the emission wavelength was 535 nm) and emission spectra (the excitation wavelength was 440 nm) of probe N1 in DMSO, dichlormethane, MeCN, MeOH and H₂O.

Figure S2. Fluorescent excitation (the emission wavelength was 545 nm) and emission spectra (the excitation wavelength was 430 nm) of probe N2 in DMSO, dichlormethane, MeCN, MeOH and H₂O.
Figure S3. Fluorescent excitation (the emission wavelength was 495 nm) and emission spectra (the excitation wavelength was 335 nm) of probe N3 in DMSO, dichlormethane, MeCN, MeOH and H2O.

4. hERG potassium channel inhibition assay

Figure S4. Competitive binding curve of probe N1-N3 and astemizole to hERG potassium channel was determined by radio-ligand binding assay.

The inhibitory activity on hERG potassium channel was determined by a radio-ligand binding assay.\textsuperscript{1, 2} Astemizole (Cat. No.#A2861-10MG, Sigma-Aldrich) was chosen as positive control.\textsuperscript{3} The affinity with hERG potassium channel was assessed in the presence of 7.5 nM \textsuperscript{3}H-dofetilide (Perkin Elmer# NET1144100UC). The probe’s binding abilities with the hERG potassium channel were displayed with displacement curves and compared to the positive controls. In brief, probe N1-N3 and astemizole were dissolved in DMSO as stock solution (10 mM), which was further diluted with
binding assay buffers when applied to the binding assays. Cell membranes were prepared in HDB. First, each well of Uni-filter 96 GF/B microplate was incubated with 80 μL hERG membrane (10 μg protein/well), 10 μL of 3H-dofetilide (7.5 nM final) and 10 μL of compounds (10 points, 4 fold dilution from 10 μM) in binding assay buffer at 37 °C for 1.5 h. Then stop the binding reaction by rapid filtration through GF/B plates using cell harvester. The wells was washed with cold wash buffer for three times and dried at 37 °C for 30 minutes. 50 μL scintillation cocktail was added to each well. Radioactivity was determine by MicroBeta Trilux (Perkin Elmer 1450). Data were recorded by Topcount NXT and stored on the GenScript computer network for off-line analysis. Data acquisition was performed by Microsoft Excel (version 2010) program; IC₅₀ values were obtained by GraphPad Prism 6 using the Cheng-Prusoff equation. The binding data was converted to %displacement according to the below equation: %displacement=100 × (1-(sample CPM/Total binding CPM)) (in which total binding CPM values were obtained by testing binding of 3H-dofetilide to the targets without competitors).

5. Cell membrane preparation

HEK 293 cell line stably transfected with hERG gene was purchased from Shanhai Genechem Company and cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Before collecting the membrane, hERG-transfecting HEK 293 cells were cultured in 10-cm dishes. When they reached 90% confluency, cells were collected, and washed with PBS buffer (pH 7.4) three times by centrifugation for 6 min at 1200 RMP. The cell pellets were resuspended in assay buffer (50 mM Tris-HCl, 1 mM MgCl₂, 10 mM KCl, pH 7.4, at 4 °C) and stored in -80 °C. Until use, the cell suspension was thawed at 4 °C and was lysed by passing 20-30 times through 27G ½ needle on ice bath. The cell lysates were centrifuged at 12,000 g for 20 min (4 °C). The supernatant solution was discarded and the pellet was suspended in assay buffer and homogenized using 27G ½ needle and centrifuged at 12,000 g for 20 min (4 °C). The supernatant solution was discarded and the obtained membrane pellets was resuspended in assay buffer and aliquoted in 1.5 mL tube and stored in -80 °C before use. Protein concentration was determined using a BCA Protein Assay kit as manufacturer’s instructions (47T00150, Dingguo Changsheng Biotechnology Co., LTD).

6. The quantum yields measurement
The probes N1-N3 (10 mM) was diluted with PBS (pH = 7.4) into dilute solutions of different concentrations (1-5 μM). The standard (10 mm x 10 mm) quartz sample pool with white PTFE plugs was used in the determination, one for PBS (pH = 7.4) and one for the solution of probe. Then, the quantum yields were determined by combined steady state/transient fluorescence spectrometer (FLS920).

7. Fluorescent properties of the probes N2-N3 after incubating with hERG transfected HEK293 membranes

Figure S5. Fluorescent emission spectra of 5 μM probe N2 incubated with different concentrations of hERG transfected HEK293 membrane (0.8, 0.6, 0.4, 0.2, 0.1, and 0 mg/mL) for 20–30 min in the assay buffer (50 mM Tris-HCl, 1 mM MgCl2, 10 mM KCl) at room temperature (λex = 430 nm).

Figure S6. (A) Fluorescent emission spectra of 5 μM probe N2 incubated with 1 mg/mL trypsin, 1 mg/mL BSA, 1 mg/mL hERG transfected HEK293 membrane, and 1 mg/mL cell membrane combined with hERG channel inhibitor astemizole (10 μM) for 20–30 min in the assay buffer (50 mM Tris-HCl, 1 mM MgCl2, 10 mM KCl) at room temperature (λex = 430 nm). (B) The corresponding fluorescent intensity changes (normalized based on the last point that is seen as 1) at 545 nm (λex = 430 nm).
Figure S7. Fluorescent emission spectra of 5 μM probe N3 incubated with different concentrations of hERG and transfected HEK293 membrane (0.8, 0.6, 0.4, 0.2, 0.1, and 0 mg/mL) for 20–30 min in the assay buffer (50 mM Tris-HCl, 1 mM MgCl2, 10 mM KCl) at room temperature (λex = 335 nm).

Figure S8. (A) Fluorescent emission spectra of 5 μM probe N3 incubated with 1 mg/mL trypsin, 1 mg/mL BSA, 1 mg/mL hERG transfected HEK293 membrane, and 1 mg/mL cell membrane combined with hERG channel inhibitor Astemizole (10 μM) for 20–30 min in the assay buffer (50 mM Tris-HCl, 1 mM MgCl2, 10 mM KCl) at room temperature (λex = 335 nm). (B) The corresponding fluorescent intensity changes (normalized based on the last point that is seen as 1) at 495 nm (λex = 335 nm).

8. Cytotoxicity

The cytotoxicity effects of probes were determined by Cell Counting Kit-8 assays, using hERG transfected HEK293 cells. An amount of 5×10³ cells per well were seeded in 96-well plates in 100 μL culture medium and cultured in 5% CO₂ atmosphere at 37 °C for 24 h. Then, the cells were treated with 100 μL of different concentrations solutions of each probe (N1, N2, or N3) for 24 h, respectively. Subsequently, 20 μL of CCK-8 solution was added to each well, and then the plates were incubated for 2 h at 37 °C. After 2 h, the absorbance values of the wells were recorded using a microplate reader at 450 nm. Wells containing no probes were chosen as blanks. Then, the half
maximal inhibitory concentration (IC$_{50}$) of each probe was calculated by GraphPad Prism 6.

**9. Cell culture and fluorescence microscopy imaging**

hERG transfected HEK293 were grown in DMEM medium supplemented with 10% (v/v) fetal bovine serum in an atmosphere of 5% CO$_2$, 95% air at 37 °C. Cells were plated on confocal dish and allowed to adhere for 12 h~24 h. After the medium was removed, the cells were carefully washed with DMEM medium without fetal bovine serum, and then incubated at r.t. in the presence of the probe (prepared in DMEM medium without fetal bovine serum) or co-incubated with N1-N3 and astemizole (a potent hERG channel blocker) for 10 min. Fluorescence imaging was performed using Zeiss Axio Observer A1 fluorescence microscope.

![Figure S9. Fluorescence microscopic imaging of hERG transfected HEK293 cells in absence or presence of 10 µM Astemizole (A1,A3: bright field, A2, A4: GFP channel; B1, B3: bright field, B2, B4: DIPA channel). Performed in Zeiss Axio Observer A1; Objective lens: 63×](image)

In addition, further investigation has been done to detect whether the cells used in the current study have an autofluorescence, or astemizole can affect the autofluorescence of cells. In this assay, we incubated the cells with astemizole (10 µM, prepared in medium without fetal bovine serum) at 37 °C in an atmosphere of 5% CO$_2$, 95% air for 10 min. Then, fluorescence imaging of hERG-HEK293 cells was obtained in presence or absence of astemizole. The results displayed that the autofluorescence of cells is so weak in absence and presence of astemizole (Figure S9), which would not influence the imaging of cells using the obtained probes N1-N3.
10. $^1$H-NMR, $^{13}$C-NMR, ESI-HRMS, HPLC.

Figure S10. $^1$H-NMR spectrum of compound N1.

Figure S11. $^{13}$C-NMR spectrum of compound N1.
Figure S12. ESI- HRMS spectrum of compound N1.

Figure S13. $^1$H-NMR spectrum of compound N2.
Figure S14. $^{13}$C-NMR spectrum of compound N2.

Figure S15. ESI-HRMS spectrum of compound N2.
Figure S16. $^1$H-NMR spectrum of compound N3.

Figure S17. $^{13}$C-NMR spectrum of compound N3.
Figure S18. ESI-HRMS spectrum of compound N3.

HPLC assessment of compound purity.

All tested compounds (1a, 1b, 1c) with a purity of >95% were used for subsequent biological assays. We provided the spectra of HPLC assays as below.

N1, 99.7%

N2, 99.2%

N3, 98.2%

11. References


