Astemizole Derivatives as Fluorescent Probes for hERG Potassium Channel Imaging

Beilei Wang, † Zhenzhen Liu, Zhao Ma, Minyong Li, and Lupei Du [a] *

Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (MOE), School of Pharmacy, Shandong University, Jinan, Shandong 250012, China

*Tel./fax: +86-531-8838-2006, E-mail: dulupei@sdu.edu.cn.

Table of Contents

1. Materials and instruments

All reagents and solvents were purchased from commercial sources and used as received unless otherwise noted. Twice-distilled water was used throughout all experiments. Mass spectra were performed by the analytical and the mass spectrometry facilities at Shandong University. ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker 300 MHz NMR and 400M NMR spectrometer. Analytical HPLC was performed on Agilent Technologies 1260 Series using a C8 reversed-phase column (250 x 4.60mm, 5 μm, Phenomenex). Absorption spectra and fluorescence spectra were obtained with a Thermo Varioskan microplate reader. Fluorescence imaging was performed using Zeiss Axio Observer A1 fluorescence microscope.

2. Homology Modeling Study

The amino acid sequence of the human hERG potassium channel was retrieved from Swiss-Prot database (accession number **Q12809**, entry name KCNH2_HUMAN) and aligned to KcsA open-inactivated potassium channel (PDB accession number: **3F5W**) by ClustalX multiple alignment program. Using MODELLER 7v7 program [1] with default parameters, a monomer model of the S5/H5/S6 domains of hERG was then constructed based on KcsA cyrstal structure. The monomer coordinates were then aligned with template tetramer respectively to obtain the tetramer homology model.

3. Molecular Modeling Study

The 3D structures for all ligands were refined using the PM3 method in the MOPAC 7 program [2] and assigned with AM1-BCC partial charges [3-5] by the QuACPAC program. All partial charges on the atoms of the homology model were derived from AMBER 8 parameters. Docking of the molecules into the binding pocket around residues Tyr 652 and Phe 656 of the hERG homology model was performed by using DOCK 5.4 [6]. After docking, MD simulations were conducted with the ligand-receptor complexes by using the CHARMM c33b1 program [7] and an implicit membrane model, GBSW (Generalized Born model with a simple Switching function) [8]. Minimizations were carried out using 1500 steps of steepest descent, followed by Adopted Basis Newton-Raphson (ABNR) minimization until the root mean square gradient was less than 0.001 kcal/mol Å. The whole system was then equilibrated for 50 ps, followed by another 2 ns of the canonical ensemble (NVT)-MD simulation run. Then, the molecule-channel complexes were analyzed by Pymol 0.99 [9] and PoseViewWeb 1.97.0[10].

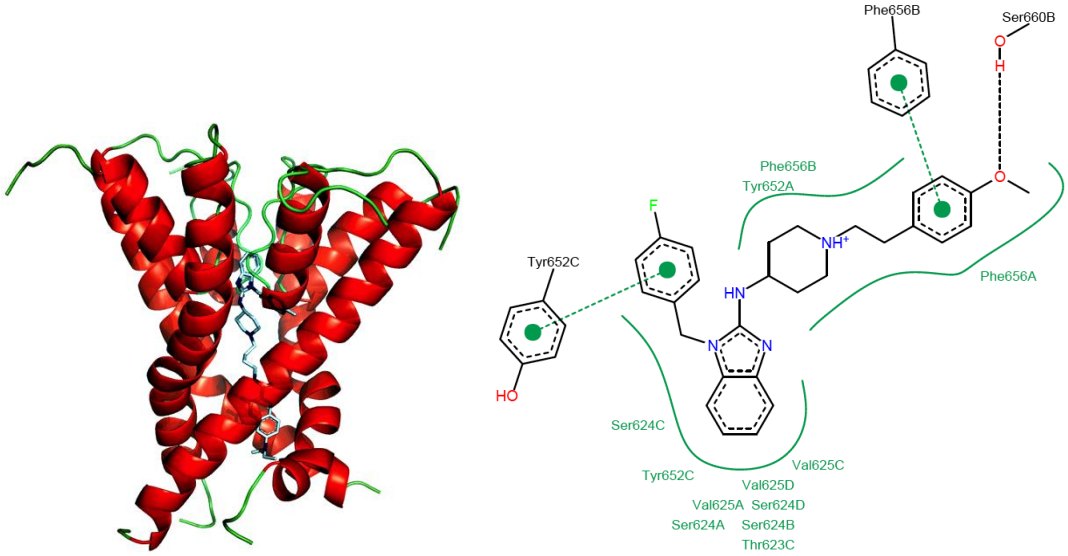


Figure S1. Proposed docking conformation of astemizole (white sticks) in the hERG binding pocket (left) and the proposed schematic interactions of astemizole with hERG (right)

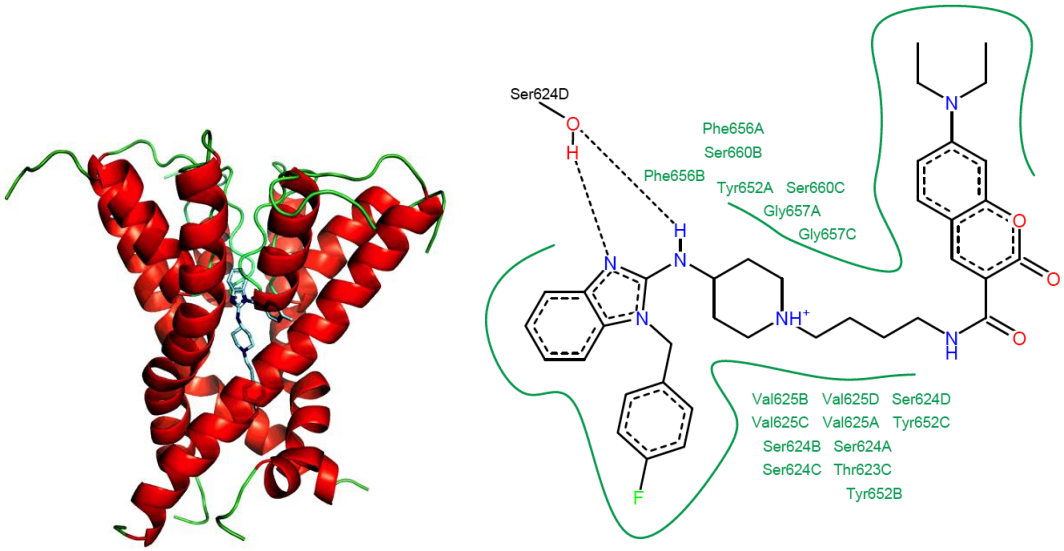


Figure S2. Proposed docking conformation of **1a** (white sticks) in the hERG binding pocket (left) and the proposed schematic interactions of **1a** with hERG (right)

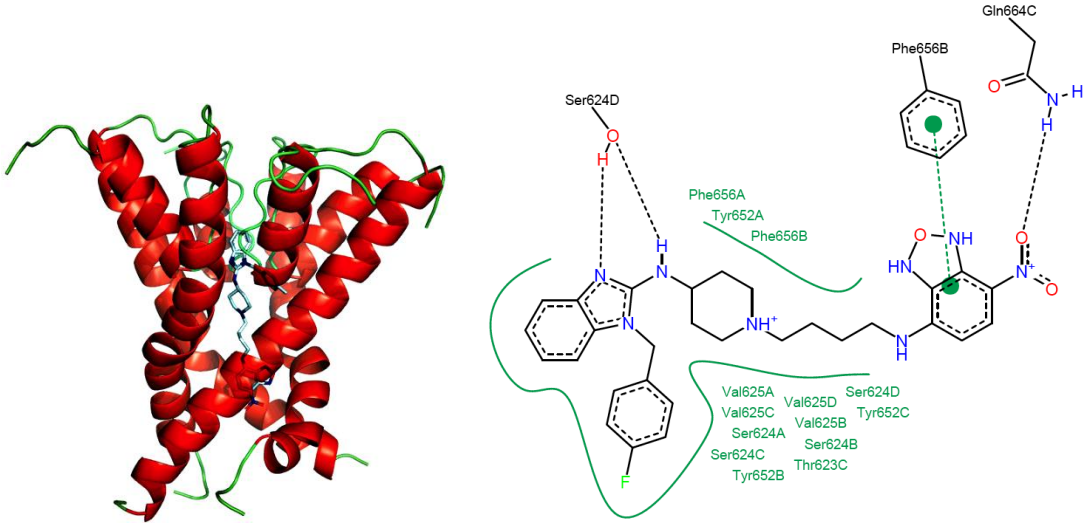


Figure S3. Proposed docking conformation of **1b** (white sticks) in the hERG binding pocket (left) and the proposed schematic interactions of **1b** with hERG (right)

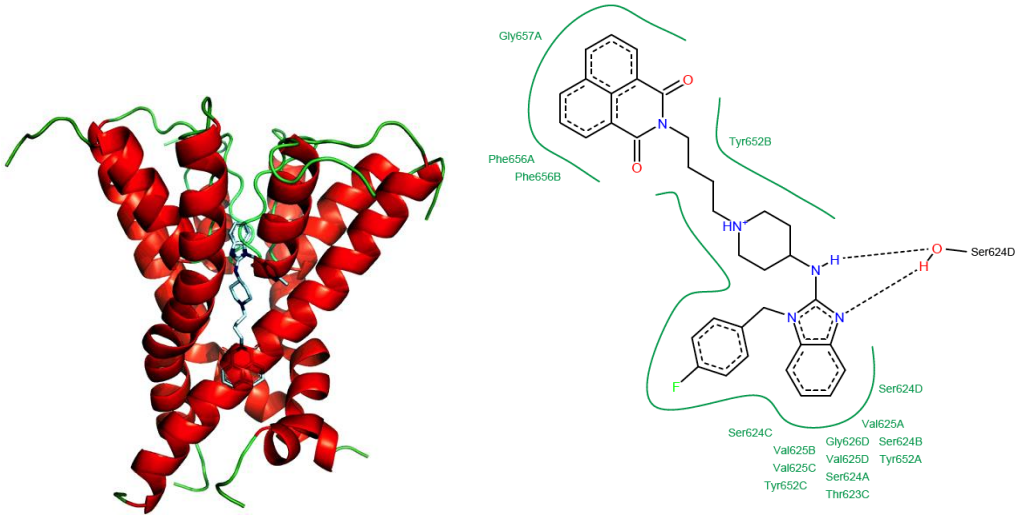
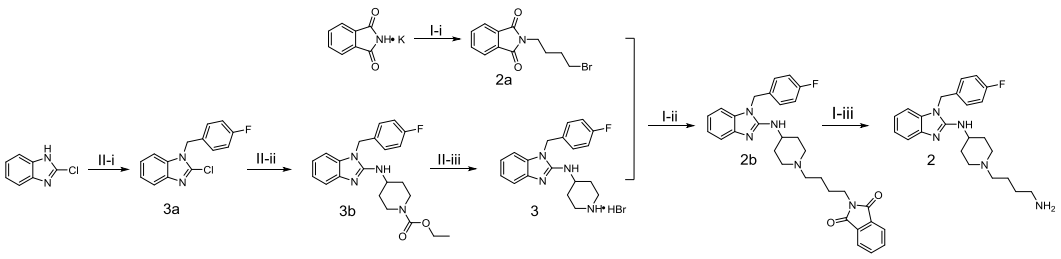


Figure S4. Proposed docking conformation of **1c** (white sticks) in the hERG binding pocket (left) and the proposed schematic interactions of **1c** with hERG (right)

4. Synthesis

4.1 Synthesis of recognition motif



Scheme S1. Synthesis of recognition motif (2 and 3)

2-(4-bromobutyl) isoindoline-1, 3-dione (2a)

A mixture of phthalimide potassium (2.0 g, 10.7 mmol) and 1, 4-dibromobutane (2.8 g, 12.9 mmol) in dry DMF (25mL) was stirred at room temperature for 26 h. After that, the reaction mixture was extracted with CH_2Cl_2 and washed with water. The combined organic layer was dried over anhydrous MgSO4, filtered and evaporated in vacuo. The resulting residue was recrystallized in distilled water to afford **2a** as a white solid in 92.4% yield. mp: 75-77 ℃. ^{1}H-NMR (400 MHz, CD_3Cl): δ ppm: 7.90(m, 4H), 3.65(t, *J* = 8.0 Hz, 2H), 3.60(t, *J* = 8.0 Hz, 2H), 1.87(m, 2H), 1.76(m, 2H). ESI-MS: ($[M+H]^+$): 283.4.

2-chloro-1-(4-fluorobenzyl)-1*H*-benzuo[*d*]imidazole (3a)

A mixture of 2-chloro-1*H*-benzo [*d*] imidazole (5 g, 32.7 mmol), KOH (2.62 g, 46.6 mmol), and acetonitrile (30 mL) was heated to 80℃ and stirred for 30 min. Then, the solution was cooled to room temperature, 1-(bromomethyl)-4-fluorobenzene (9.29 g，49.1 mmol) was added and the reaction mixture was refluxed with stirring for 5 h. After cooling, the resulting solution was washed with water and extracted with CH_2Cl_2. The organic layer was dried over Mg_2SO_4, filtered and evaporated under reduced pressure. Then, the crude product was recrystallized in acetone/petroleum ether to yield **3a** as a white solid in 91.1% yield. mp: 78-80 ℃. ^{1}H-NMR (300MHz, DMSO): δ ppm: 7.64(d, *J* = 6.6 Hz, 2H), 7.32-7.24(m, 4H), 7.19(t, *J* = 4.9Hz, 2H), 5.53(s, 2H). ESI-MS: ($[M+H]^+$): 261.6.

Ethyl 4-((1-(4-fluorobenzyl)-1H-benzo[*d*]imidazol-2-yl) amino) piperidine-1-carboxylate (3b)

Compound **3a** (0.5 g, 1.9 mmol) was added to the colorless oil liquid ethyl 4-aminopiperidine 1-carboxylate (0.495 g, 2.8 mmol) and stirred well. Then, under microwave reaction conditions (180℃, 30 W), the reaction was completed within one minute to produce brown solid and dissolved in MeOH/CH_2Cl_2. The crude product purified by column chromatography (silica gel, gradient CH_2Cl_2/MeOH) to obtain white solid in 34.0% yield. mp: 170- 172 ℃. ^{1}H-NMR (300 MHz, DMSO): δ ppm: 7.21(m，3H), 7.14(t, *J* = 6.6 Hz，2H)，7.06(d, *J* = 5.8 Hz, 1H), 6.93(t, *J* = 5.6 Hz, 1H) , 6.84(t, *J* = 5.6 Hz, 1H), 6.64(d, *J* = 5.6 Hz，1Hz), 5.26(s, 2H), 4.02(m, 5H), 2.96(s, 2H), 1.99(d, *J* = 7.4 Hz, 2H), 1.42(m, 2H), 1.19(t, *J* = 5.3 Hz, 3H). ESI-MS: ($[M+H]^+$) : 397.5.

1-(4-fluorobenzyl)-*N*-(piperidin-4-yl)-1H-benzo[*d*]imidazol-2-amine hydrochloride (3)

Compound **3b** (1.2 g, 3.02 mmol) in 40% hydrobromic acid (30 mL) was stirred at 100℃ for 15 h. Then, the reaction solution was removed in vacuo to give the crude product and recrystallized in ethyl acetate to afford **3** as a white solid in 95.0% yield. mp: > 280 ℃. ^{1}H-NMR (300 MHz, DMSO): δ ppm: 7.17(m, 5H), 7.05(d, *J* = 13.8 Hz, 1H), 6.91(td, *J* = 7.5 Hz, 1.2 Hz, 1H), 6.81(td, *J* = 7.5 Hz, 0.9 Hz, 1H), 6.61(d, *J* = 18.9 Hz, 1H)，3.81(brs, 1H), 2.97(d, *J* = 12.3 Hz, 2H), 2.55(t, *J* = 9.9Hz, 2H), 1.92(d, 9.6 Hz, 2H), 1.38(qd, *J* = 11.7 Hz, 3.6 Hz, 2H). ESI-MS: ($[M+H]^+$): 325.3.

2-(4-(4-((1-(4-fluorobenzyl)-1*H*-benzo[*d*]imidazol-2-yl) amino) piperidin-1-yl) butyl) isoindoline-1, 3-dione (2b)

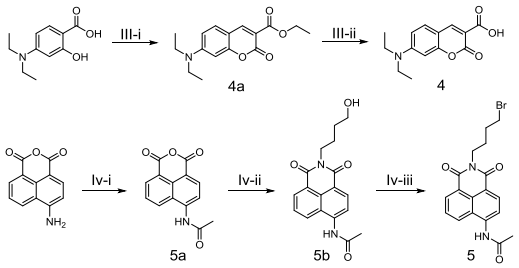
A mixture of compound **3** (1.20 g, 2.96 mmol), compound **2a** (1.67 g, 5.92 mmol) and K_2CO_3 (0.82 g, 5.92 mmol) in 30 mL MeCN was refluxed at 80 ℃ for 10 h. Then, the solution was cooled to room temperature, washed with water and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous MgSO4, filtered and evaporated in vacuo. The resulting residue was purified by column chromatography (silica gel, gradient CH_2Cl_2/MeOH) to obtain a white solid in 63.0% yield. mp: 96-99 ℃. ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 7.84(dd, *J* = 6.0 Hz, 3.0 Hz, 2H), 7.71(dd, *J* = 5.4 Hz, 3.0 Hz, 2H), 7.51(d, *J* = 7.8 Hz, 1H), 7.16-7.09(m, 3H), 7.07-6.99(m, 4H), 5.06(s, 2H), 3.98-3.90(m, 2H), 3.70(t, *J* = 6.9 Hz, 2H), 2.86(d, *J* = 10.2 Hz, 2H), 2.46(t, *J* = 7.5 Hz, 2H), 2.27(t, *J* = 10.8 Hz, 2H),

2.12-2.03(m, 3H), 1.72(q, *J* = 6.6 Hz, 2 H), 1.63-1.50 (m, 2H). ESI-MS: ([M+H]$^+$): 526.5.

N- (1-(4-aminobutyl) piperidin-4-yl)-1- (4-fluorobenzyl) -1*H*-benzo [*d*] imidazol-2-amine (2)

To a solution of compound **2b** (0.50 g, 0.95 mmol) in anhydrous EtOH (30 mL), 80% hydrazine hydrate (5 mL) was added. After stirring at 80 ℃ for 5 h, the reaction solution was concentrated under reduced pressure. Then, CH_2Cl_2 (30 mL) was added with precipitating white floc, filtered and collected the filtrate. Thereafter, the filtrate was concentrated under reduced pressure and recrystallized in CH_2Cl_2/PE to afford compound **2** as a white solid in 90.0% yield. mp: 139-142 ℃. ^{1}H-NMR (400 MHz，DMSO)：δ ppm: 7.23-7.18(m, 3H)，7.14(t, *J* = 8.0 Hz, 2H), 7.05(d，*J* = 8.0 Hz, 1H), 6.92(t, *J* = 8.0 Hz, 1H), 6.82(t, *J* = 8.0 Hz, 1H), 6.57(d, *J* = 8.0 Hz, 1H), 5.26(s, 2H), 3.73(brs, 1H), 2.84(d, *J* = 12.0 Hz, 2H), 2.55(s, 2H), 2.25(t, *J* = 8 Hz, 2H), 1.96(m, 4H), 1.57-1.35(m, 7H). ESI-MS: ([M+H]$^+$): 396.5.

4.2 Synthesis of fluorophores



Scheme S2. The synthesis of fluorophore motif (4 and 5)

Ethyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate (4a)

To a solution of 4-(diethylamino)-2-hydroxybenzoic acid (0.50 g, 2.59 mmol) in anhydrous EtOH (20 mL), diethyl malonate (0.42 g, 2.59 mmol), a mixture of morpholine (22.56 mg，0.26 mmol) and acetic acid (10 μL) in 4 mL anhydrous EtOH were added. After stirring at 80 ℃ for 8 h, the reaction solution was concentrated under reduced pressure. The resulting residue was purified by column chromatography (silica gel, gradient CH_2Cl_2/MeOH) to obtain a red-brown oil in 81.6% yield. ^{1}H-NMR (400 MHz, DMSO): δ ppm: 8.55(s, 1H), 7.63(d, *J* = 8 Hz, 1H), 6.77(dd, *J* = 8 Hz, 8 Hz, 1H), 6.54(d, *J* = 2.4 Hz, 1H), 4.23(q, *J* = 8 Hz, 2H), 3.48(q, *J* = 8 Hz, 4H), 1.28(t, *J* = 8 Hz, 3H), 1.14(t, *J* = 8 Hz, 6H). ESI-MS: ([M+H]$^+$): 290.5.

7- (diethylamino)-2-oxo-2*H*-chromene-3-carboxylicm acid (4)

Compound **4a** was added in the 2 N NaOH (20 mL) to stir at room temperature for 18 h. Thereafter, the reaction solution was acidified with aqueous 2 N HCl with some orange precipitations was appearing. Then, the solution was filtered to obtain an orange solid in 96.0% yiled. mp: 222-225 ℃. ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 12.33(s, 1H), 8.66(s, 1H), 7.45(d, *J* = 9.0 Hz, 1H), 6.71(dd, *J* = 9.0 Hz，2.4 Hz, 1H), 6.53(d, *J* = 2.4 Hz, 1H), 3.49(q, *J* = 7.2 Hz, 4H), 1.26(t, *J* = 7.2 Hz, 6H). ESI-MS: ([M+H]+): 262.3.

N- (1, 3-dihydrobenzo [*de*]isochromen-6-yl) acetamide (5a)

A mixture of 6-amino-1H, 3H-benzo [*de*] isochromene-1, 3-dione (2 g, 9.3 mmol), acetic acid (4 mL), and pyridine (20 mL) was stirred at 115 ℃ for 1 h. After that, 20 mL acetic oxide was added to the reaction solution to reflux at 119 ℃ for 5 h. The resulting solution was cooled to room temperature and was poured into crushed ice, filtered and washed with water to obtain a brown solid in 71.3% yield. mp: 259-261 ℃. ^{1}H-NMR (400 MHz, DMSO：δ ppm: 10.53(s, 1H), 8.82(d, *J* = 8 Hz, 1H), 8.55(d, *J* = 8 Hz, 1H), 8.52(d, *J* = 8 Hz, 1H), 8.39(d, *J* = 8 Hz, 1H), 7.93(t, *J* = 8 Hz, 1H), 2.31(s, 3H). ESI-MS: ([M+H]$^+$): 256.3.

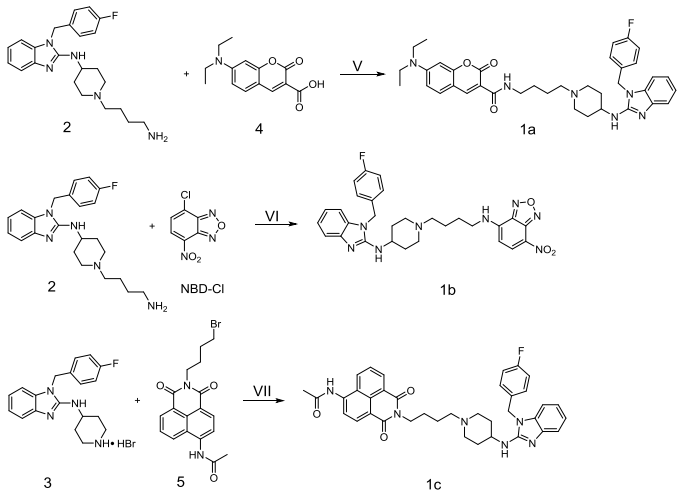
N-(2-(4-hydroxybutyl)-1, 3-dioxo-2, 3-dihydro-1H-benzo [de]isoquinolin-6-yl) acetamide (5b)

To a solution of compound **5a** (1.7 g, 6.66 mmol) in anhydrous EtOH (30 mL), 4-amino-1-butanol (0.71 g, 7.96 mmol) was added. After stirring at 80 ℃ for 5 h, the reaction solution was concentrated under reduced pressure. Then, the resulting solution was recrystallized in EA to afford **5b** as a brown solid in 73.7% yield. mp: 209-212 ℃. ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 8.63(dd, *J* = 7.2, 0.9 Hz, 1H), 8.59(d, *J* = 8.4 Hz, 1H), 8.37(brs, 1H), 8.19(d, *J* = 8.1 Hz, 1H), 7.83-7.75(m, 2H), 4.24(t, *J* = 7.2 Hz, 2H), 3.76(t, *J* = 6.3 Hz, 2H), 1.89 - 1.79(m, 2H), 1.74 - 1.57(m, 2H). ESI-MS: ($[M+H]^+$): 327.5.

N-(2-(4-bromobutyl)-1, 3-dioxo-2, 3-dihydro-1*H*-benzo [de]isoquinolin-6-yl) acetamide (5)

To a solution of compound **5b** (0.60 g，1.84 mmol) in dry EA, PBr_3 (0.5 mL) in dry EA (5 mL) were dropped into at 0 ℃. After that, the solution was heated to 80 ℃ and stirred for 6 h. Then, the reaction solution was cooled to room temperature and was poured into crushed ice, filtered and washed with water to obtain a brown crude solid in 95.2% yield. mp: 216-222 ℃. ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 8.63(d, *J* = 7.2 Hz, 1H), 8.60(d, *J* = 8.1 Hz, 1H), 8.39(brs, 1H), 8.20(d, *J* = 8.4 Hz, 1H), 7.81-7.75(m, 2H), 4.24(t, *J* = 6.9 Hz, 2H), 3.49(t, *J* = 6.3 Hz, 2H), 2.38(s, 3H), 2.04-1.85(m, 4H). ESI-MS: ($[M+H]^+$): 389.4.

4.3 Synthesis of probes



Scheme S3. The synthesis of fluorescent probes (1a, 1b, and 1c) for hERG potassium channel

4.3.1 Synthesis of probe 1a

A mixture of compound **4** (66 mg, 0.25 mmol), EDCI (47 mg, 0.30 mmol), HOBT (47 mg, 0.30 mmol) in dry MeCN was stirred at 0 ℃ for 30 min. Then, compound **2** (100 mg，0.25 mmol) , Et_3N (40 μL，0.50 mmol) were added and stirred at room temperature for 14 h. Thereafter, the reaction solution was extracted with CH_2Cl_2 and washed with water. The combined organic layer was dried over anhydrous MgSO4, filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography (silica gel, gradient CH_2Cl_2/MeOH) to obtain a bright yellow solid in 40.0 % yield. mp: 201-204 ℃. ^{1}H-NMR (400 MHz, CD_3OD): δ ppm: 8.65(s, 1H), 7.57(d, *J* = 8 Hz, 1H), 7.34(m, 1H), 7.16(dd, *J* = 12 Hz, 8 Hz, 2H), 7.07(m, 4H), 6.99(m, 1H), 6.84(dd, *J* = 8 Hz, 8 Hz, 1H), 6.60(d, *J* = 4 Hz，1H)，5.29(s, 2H), 3.95(brs, 1H), 3.55(q, *J* = 8 Hz, 4H), 3.49(t, *J* = 8 Hz, 2H)，2.86(m, 2H), 2,75(t, *J* = 12 Hz, 2H), 2.23(d, *J* = 12 Hz, 2H), 1.74(m, 7H), 1.25(t, *J* = 8 Hz, 6H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm: 163.62, 163.36, 162.80, 161.17, 157.65, 153.20, 152.61, 148.12, 141.93, 134.41, 131.28, 131.25, 131.18, 128.41, 128.32, 121.58, 119.88, 116.32, 116.15, 115.94,

110.15, 110.01, 108.41, 107.40, 96.58, 57.55, 52.08, 48.93, 45.08, 45.04, 38.88, 31.12, 27.40, 23.12, 12.43. ESI-HRMS: ($[M+H]^+$) calcd for $C_{37}H_{43}FN_5O_3$: 639.3453, found: 639.3452. HPLC, t_R = 12.02 min, mobile phase: methanol-water (77:23, v/v), λ = 430 nm.

4.3.2 Synthesis of probe 1b

To a solution of compound **2** (60 mg, 0.15 mmol) in dry 1,4-dioxane, then, 4-chloro-7-nitrobenzofurazan (NBD-Cl; 30.27 mg, 0.15 mmol) and K_2CO_3 (41.93 mg, 0.30 mmol) were added. After stirring at room temperature for 14 h, the reaction solution was filtered and collected the filtrate. Then, the filtrate was concentrated under reduced pressure and purified by column chromatography (silica gel, gradient CH_2Cl_2/MeOH) to obtain a bright yellow solid in 47.2% yield. mp: 156-159 ℃. ^{1}H-NMR (400 MHz, DMSO): δ ppm: 9.59(s, 1H), 8.52(d, *J* = 8 Hz, 1H), 7.19(t, *J* = 8 Hz, 3H)，7.14(t, *J* = 8 Hz, 2H), 7.05(d, *J* = 8 Hz, 1H), 6.92(t, *J* = 8 Hz, 1H), 6.83(t, *J* = 8 Hz，1H), 6.60(d, *J* = 8 Hz, 1H), 6.44(d, *J* = 8 Hz, 1H), 5.27(s, 2H), 3,75(s, 1H), 3.50(s, 2H), 2.89(d, *J* = 8 Hz, 2H), 2.36(s, 2H), 1.99(m, 4H), 1.71(m, 2H), 1.56(t, *J* = 8 Hz, 4H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm: 161.96, 159.54, 153.35, 144.60, 142.24, 137.29, 133.67, 132.81, 132.78, 128.40, 128.32, 119.87, 117.75, 114.82, 114.61, 114.41, 107.19, 98.54, 56.55, 51.70, 49.41, 43.06, 42.71, 31.19, 24.97, 23.30. ESI-HRMS: ($[M+H]^+$) calcd for $C_{29}H_{31}FN_8O_3$: 559.2576, found: 559.2537. HPLC, t_R = 9.76 min, mobile phase: methanol-water (70:30, v/v), λ = 475 nm.

4.3.3 Synthesis of probe 1c

To a solution of compound **3** (90 mg，0.22 mmol) in dry MeCN (20 mL) and dry DMF (4 mL), compound **5** (110 mg, 0.28 mmol) and anhydrous K_2CO_3 (70 mg, 0.50 mmol) were added. After stirring at 80 ℃ for 6 h, the reaction solution was cooled to room temperature and concentrated under reduced pressure. The crude product was then purified by purified by silica gel chromatography to afford compound **1c** as a yellow solid in 50.5% yield. mp: 195-198 ℃. ^{1}H-NMR (400 MHz, DMSO): δ ppm: 10.45(s, 1H), 9.26(brs, 1H), 8.74(d, *J* = 8 Hz, 1H) , 8.55(d, *J* = 8 Hz, 1H), 8.50(d, *J* = 8 Hz, 1H), 8.33(d, *J* = 8 Hz, 1H), 7.91(t, *J* = 8 Hz, 1H), 7.23(s, 3H), 7.16(t, *J* = 8 Hz, 3H), 6.96(d, *J* = 24 Hz, 2H), 5.34(s, 2H), 4.11(t, *J* = 8 Hz, 2H), 4.00(brs, 1H), 3.56(d, *J* = 8 Hz, 2H), 3.10(s, 4H), 2.29(s, 3H), 2.23(d, *J* = 12 Hz, 2H), 2.03(s, 1H), 1.74(m, 5H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm: 171.26, 164.37, 163.88, 140.06, 133.22, 131.80, 131.48, 131.02, 128.78, 128.73, 128.34, 128.25, 126.43, 125.08, 122.42, 121.94, 120.75, 120.65, 118.45, 115.31, 115.09, 114.21, 108.54, 44.31, 38.78, 24.86, 22.51, 21.43. ESI-HRMS: ($[M+H]^+$) calcd for $C_{37}H_{37}FN_5O_3$: 633.2945, found: 633.3081. HPLC, t_R = 9.01 min, mobile phase: methanol-water (75:25, v/v), λ = 350 nm.

5. Fluorescence Spectroscopy Test

Each probe was dissolved in DMSO to obtain 10 mM stock solution, respectively. The stock solution was diluted in PBS (PH = 7.4) to acquired 10 μM solutions. The fluorescent properties of probes **1a**, **1b**, and **1c** were obtained on Thermo-Fisher Varioskan microplate reader.

The quantum yields of these probes under PBS solution (pH = 7.4) were calculated by comparison with fluorescein in 0.1 M NaOH (Φ_{ST} = 0.92) as a reference using the following equation:[11]

$$\Phi_X = \Phi_{ST} (A_{ST}/A_X) (F_X/F_{ST}) (\eta_X/\eta_{ST})^2$$

Where the subscripts ST and X denote standard and test respectively, Φ is the quantum yield, F is the integrated area under the fluorescence spectra, A is the absorbance, η is the refractive index of the solvent.

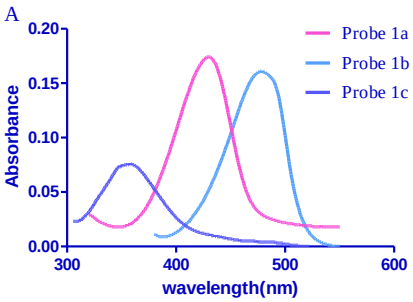


Figure S5. Absorption spectra of probes**1a, 1b,** and **1c**.

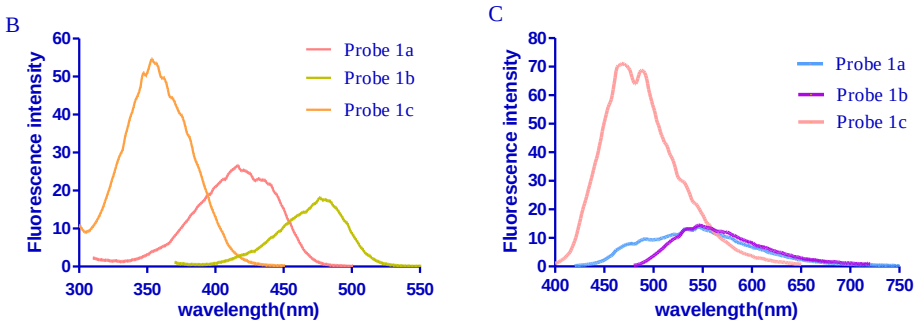


Figure S6. Fluorescence spectroscopy of probes **1a, 1b,** and **1c**：(B) fluorescence excitation spectrum; (C) fluorescence emission spectrum.

6. hERG Potassium Channel Inhibition Assay.[12-14]

The inhibitory activities against the hERG potassium channel of these three probes were determined by a radio-ligand binding test, in which the Astemizole was chosen as a positive control. The assay was measured in the presence of 10 nM [^{3}H] dofetilide. First, stock solutions (1 mM) of probes were prepared in DMSO, and then serial probe dilutions were prepared in binding buffers. The binding buffer contained 10 mM HEPES, 10 mM glucose, 60 mM KCl, 0.8 mM $MgCl_2$, 130 mM NaCl, 1 mM NaEGTA, 0.1% BSA, pH 7.4, and it was filtered and stored at 4℃. hERG-transfected HEK 293 cell membranes were prepared according the manufacturer's instructions that demonstrated by GenScript USA Inc (Cat. No. # M00355). Then, 100 µL 0.5% PEI (Polyethyleneimine Sigma-Aldrich, dissolved in Milli-Q water) was added in each well of Unifilter 96-well glass filter plates (GF/C) and incubated at 4℃ for 30-60 min. Thereafter, PEI was removed, and the plates were washed with wash buffer (2 mL/well, wash buffer contained 50 mM Tris-HCl, pH 7.4, filtered and stored at 4℃). The following were added to each well of a 24-well plate: test probe, [^{3}H] dofetilide ligand (10 nM), and membranes (10 µg/well) for a final volume of 100 µL. The assay was incubated for 2 h at 25 °C with a shaking speed of 530 RPM and terminated by rapid filtration through Millipore vacuum manifold. Then, the filter plates were washed with 2 mL/well cold wash buffer, dried at room temperature for 120 min, and radioactivity counted on TopCount NXT for 1 min/well after the addition of 50 µL of scintillant (MicroScint-20TM, Perkin Elmer). The dates were kept on the GenScript computer network and performed by Microsoft Excel program (version 2007). The data from binding experiments were converted to percent % displacement using the following formula: % displacement = [1-(sample CPM/Total binding CPM)] × 100. IC_{50} and K_i values were derived by means of the GraphPad Prism 5 using the Cheng-Prusoff equation.

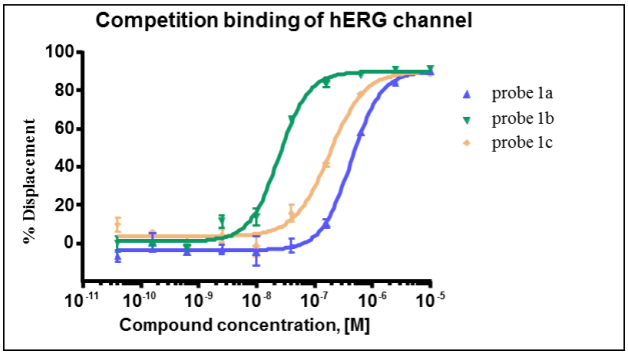


Figure S7. Biological evaluation with hERG potassium channel.

7. Cytotoxicity

The cytotoxicity effects of probes were determined by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assays, using hERG transfected HEK293 and HT-29 cells. An amount of 5×10^3 cells per well were seeded in 96-well plates in 100 μL culture medium and cultured in 5% CO_2 atmosphere at 37℃ for 24 h. Then, the cells were treated with 100 μL of different concentrations solutions of each probe (**1a**, **1b**, or **1c**) for 24 h, respectively. Subsequently, 20 μL of MTT solution was added to each well, and then the plates were incubated for 4 h at 37 ℃, the residual culture solution was removed. Then, 200 μL DMSO was added into every well to dissolve the reddish-blue crystals. After shaking for 5 min, the absorbance values of the wells were recorded using a microplate reader at 570 nm. Wells containing no probes were chosen as blanks. Then, the half maximal inhibitory concentration (IC_{50}) of each probe was calculated.

8. Fluorescence Microscopy Imaging.

Cell Culture. The fluorescent imaging was performed on hERG transfected HEK293 and an hERG potassium channel high-expressed tumor cell line, HT-29 cells. Thereinto, hERG transfected HEK293 was cultured in Phenol red-free Dulbecco's Modified Eagle's Medium (DMEM) which was added with 10% fetal bovine serum (FBS; Gibco) and 400 μg/mL G418 (Sigma), while HT-29 cells were cultured in RPMI-1640 medium supplemented with 10% FBS. When it reached 80-90% confluency in cell culture dishes, these two types of cells were seeded into confocal dishes and were cultured in an atmosphere of 5% CO_2, 95% air at 37 °C overnight.

Staining. After the culture medium was removed from the confocal dishes, cells were washed with the corresponding culture medium without fetal bovine serum. Probes were dissolved in DMSO as a stock solution (10 mM), which was further dilute with corresponding culture medium without fetal bovine serum. Subsequently, probe **1a** (1 μM), **1b** (0.5 μM) and **1c** (1 μM) were incubated with hERG transfected HEK293 and HT-29 cells for 10 min, respectively, and then cells were washed with the corresponding culture medium without fetal bovine serum.

The imaging of hERG channel was also performed by co-incubation with inhibitor (100 μM Astemizole) incubation together with each probe (1 μM probe **1a**, 0.5 μM probe **1b** or 1μM probe **1c**) at the same conditions, using hERG- HEK293 and HT-29 cells.

Fluorescence imaging. Fluorescence imaging in live hERG-HEK293 cells and HT-29 cells were obtained on a Zeiss Axio Observer A1 fluorescent microscope.

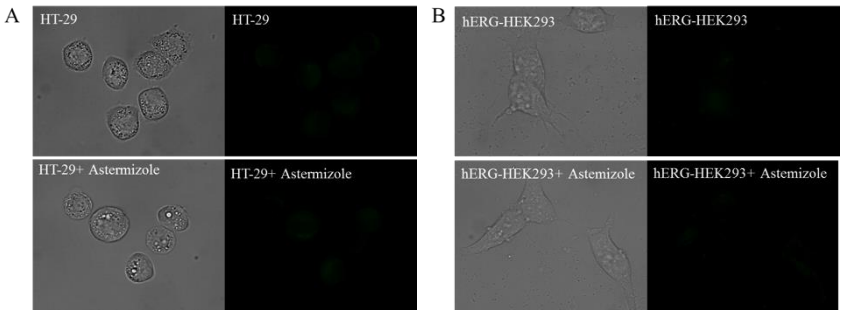


Figure S8. Fluorescence microscopic imaging of hERG transfected HEK293 and HT-29 cells in absence or presence of 100 μM Astemizole (A: HT-29 cells, left: bright field, right: GFP channel; B, hERG-HEK293 cells, left: bright field, right: GFP channel). Performed in Zeiss Axio Observer A1; Objective lens: 63×

In addition, further investigation has been done to detect whether the cells we 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 (100 μM, prepared in medium without fetal bovine serum) at 37 °C in an atmosphere of 5% CO_2, 95% air for 10min. Then, fluorescence imaging of hERG-HEK293 cells and HT-29 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 S8**), that it would not influence the imaging of cells using the obtained probes **1a~1c**.

9. ^{1}H-NMR, ^{13}C-NMR, ESI-HRMS, HPLC.

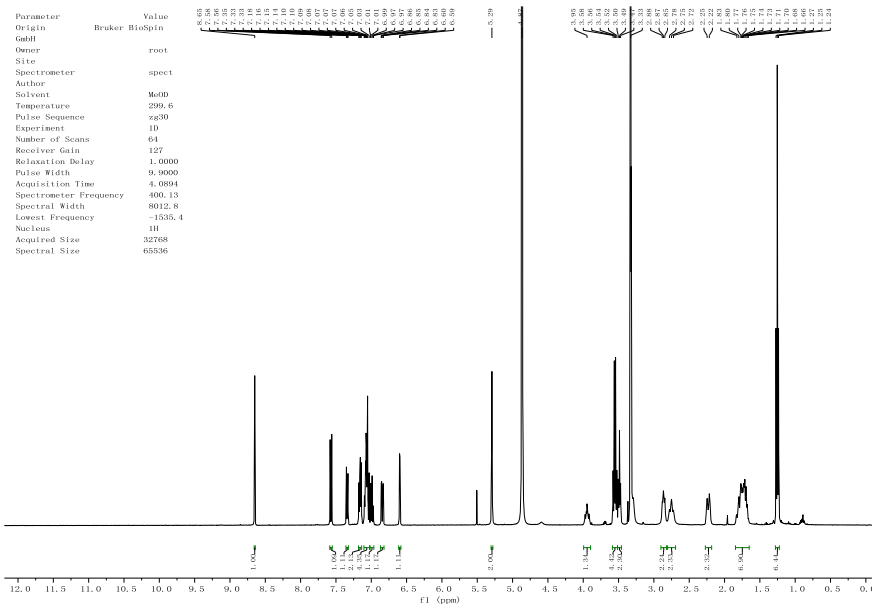


Figure S9. ^{1}H NMR spectrum of compound **1a**.

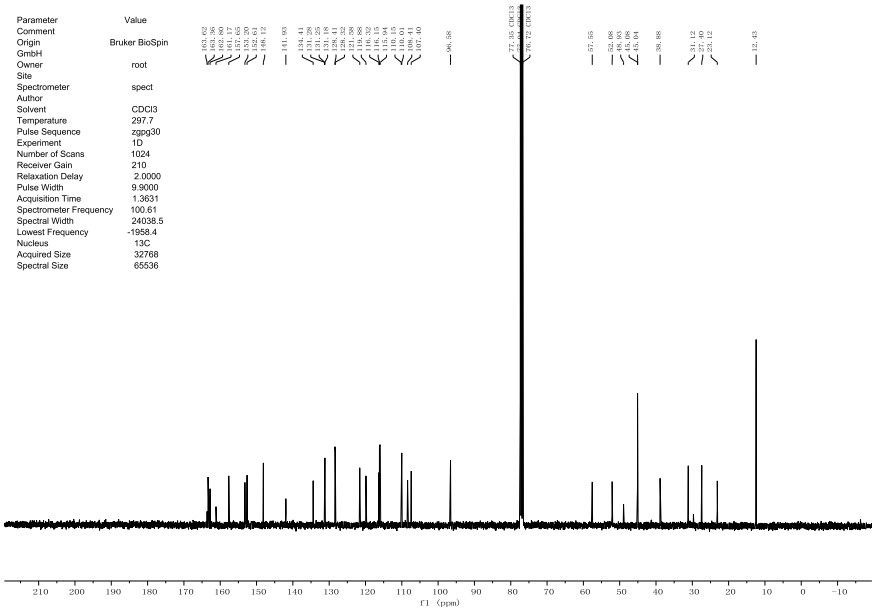


Figure S10. ^{13}C NMR spectrum of compound **1a**.

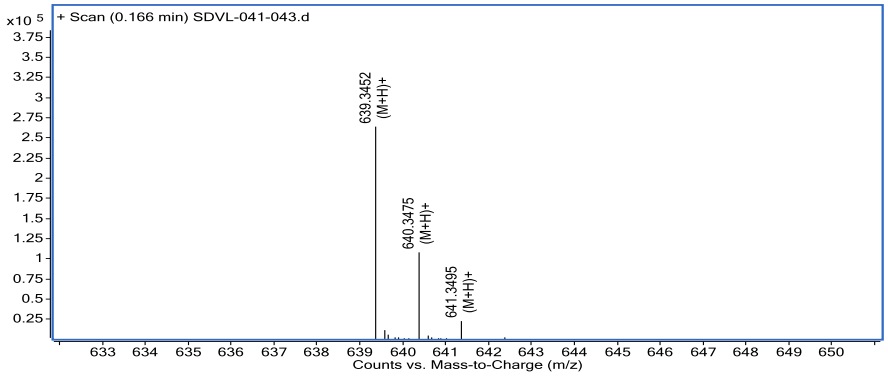


Figure S11. ESI- HRMS spectrum of compound **1a**.

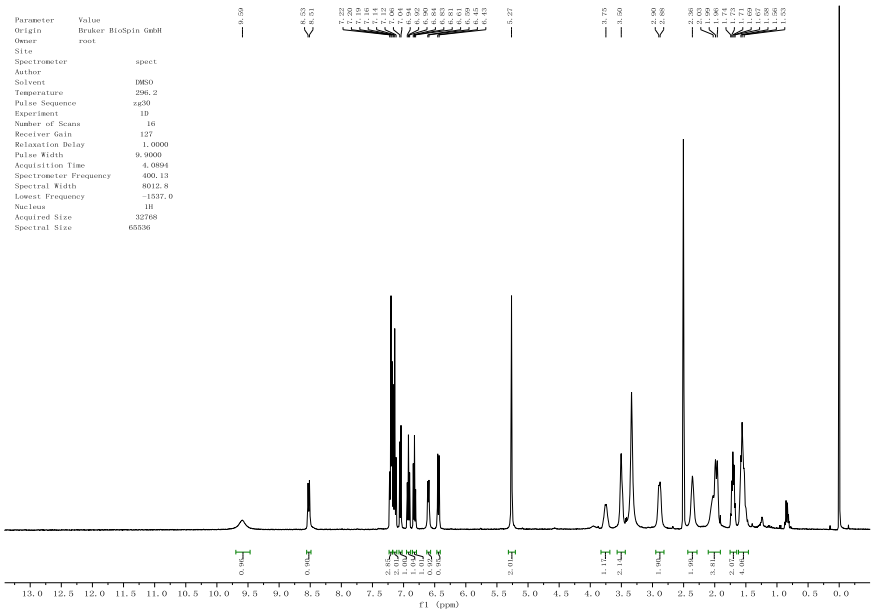


Figure S12. ^{1}H NMR spectrum of compound **1b**.

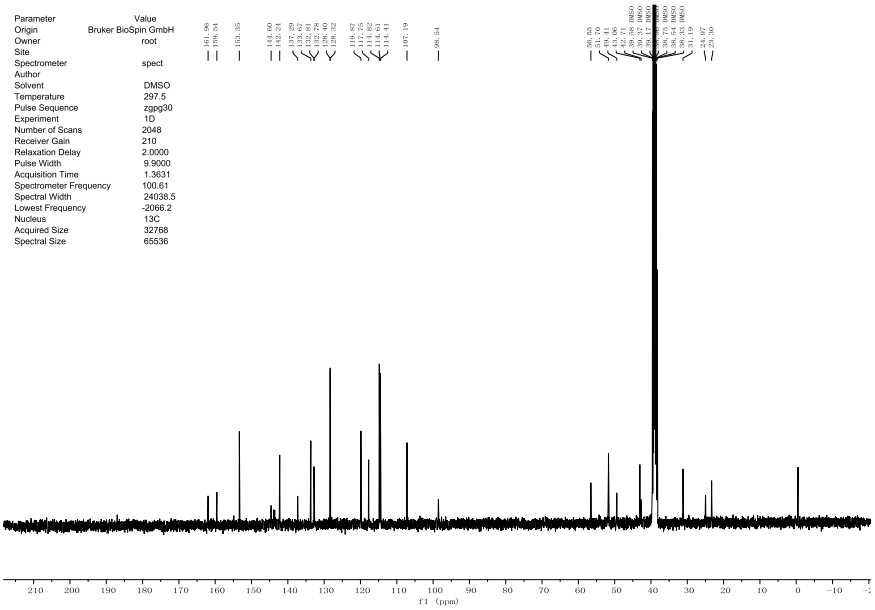


Figure S13. ^{13}C NMR spectrum of compound **1b**.

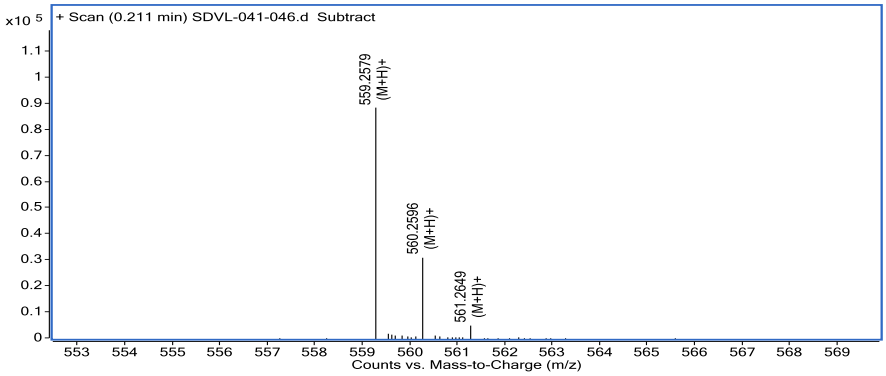


Figure S14. ESI- HRMS spectrum of compound **1b**.

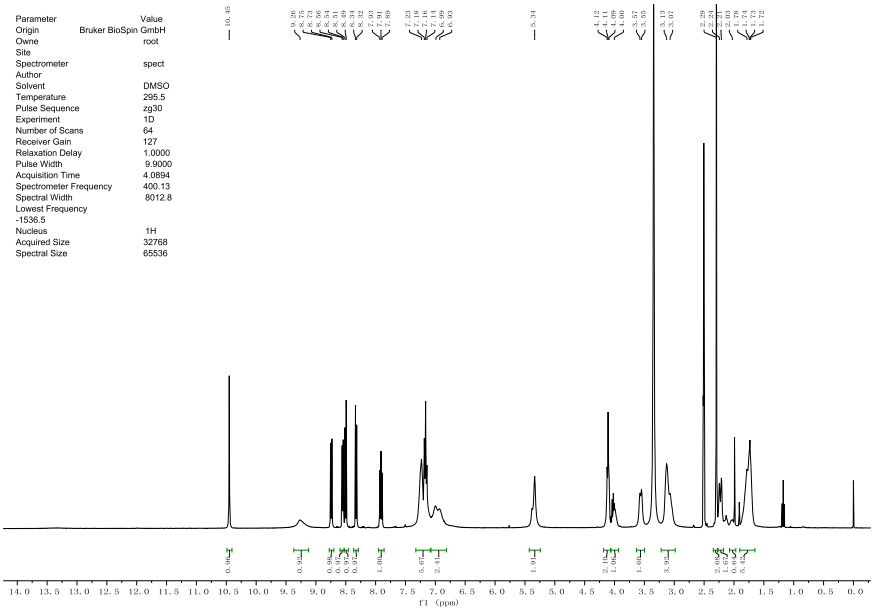


Figure S15. ^{1}H NMR spectrum of compound **1c**.

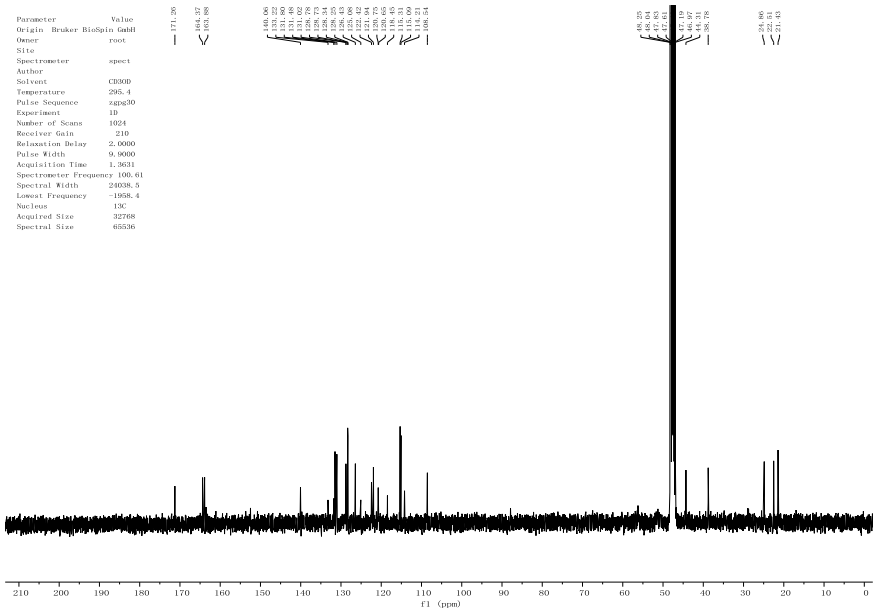


Figure S16. ^{1}H NMR spectrum of compound **1c**.

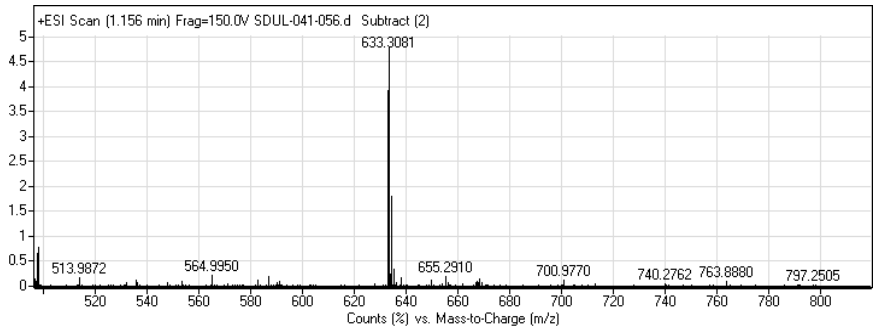
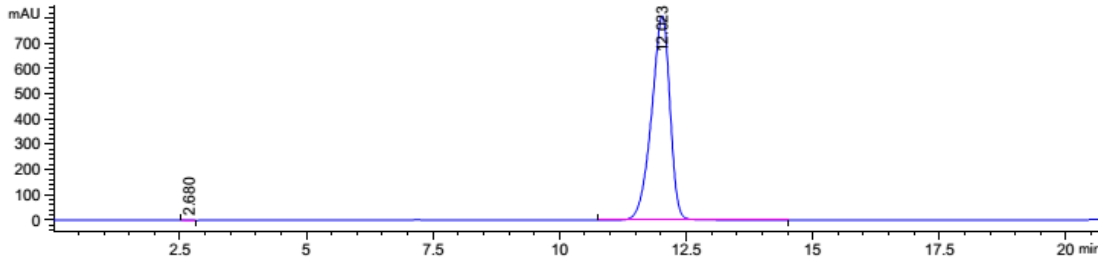


Figure S17. ESI- HRMS spectrum of compound **1c**.

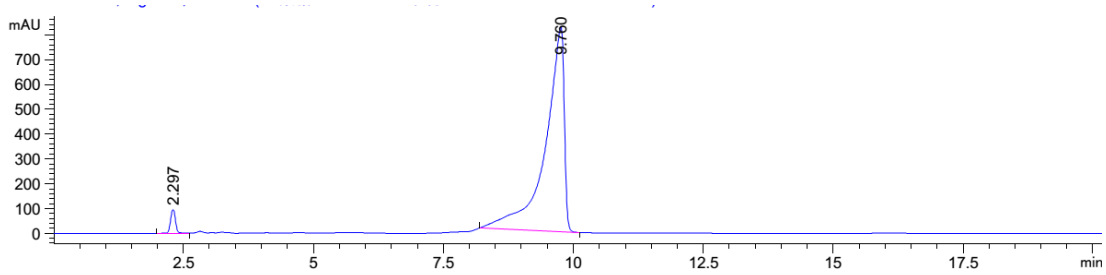
HPLC assessment of compound purity.

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.

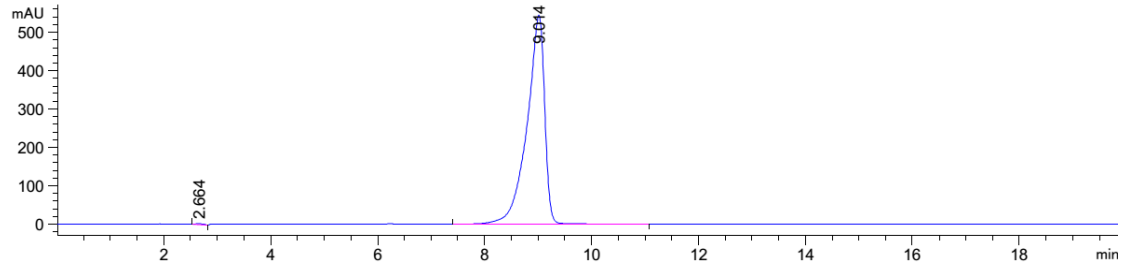
1a, 99.9%



1b, 96.9%



1c, 99.8%



10. References

1. Fiser, A.; Sali, A. Modeller: generation and refinement of homology-based protein structure models. *Methods Enzymol* **2003,** 374, 461-91.

2. Stewart, J. J. MOPAC: a semiempirical molecular orbital program. *J Comput Aided Mol Des* **1990,** 4, 1-105.

3. Jakalian, A.; Bush, B. L.; Jack, D. B.; Bayly, C. I. Fast, Efficient Generation of High-Quality Atomic Charges. AM1-BCC Model: I. Method. *J. Comput. Chem.* **2000,** 21, 132-146.

4. Jakalian, A.; Jack, D. B.; Bayly, C. I. Fast, Efficient Generation of High-Quality Atomic Charges. AM1-BCC Model: II. Parameterization and Validation. *J. Comput. Chem.* **2002,** 23, 1623-41.

5. Tsai, K.-C.; Wang, S.-H.; Hsiao, N.-W.; Li, M.; Wang, B. The Effect of Different Electrostatic Potentials on Docking Accuracy: A Case Study Using DOCK5.4. *Bioorg. Med. Chem. Lett.* **2008**, 10.1016/j.bmcl.2008.05.026.

6. Moustakas, D. T.; Lang, P. T.; Pegg, S.; Pettersen, E.; Kuntz, I. D.; Brooijmans, N.; Rizzo, R. C. Development and validation of a modular, extensible docking program: DOCK 5. *J Comput Aided Mol Des* **2006,** 20, 601-19.

7. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* **1983,** 4, 187-217.

8. Im, W.; Feig, M.; Brooks, C. L., 3rd. An implicit membrane generalized born theory for the study of structure, stability, and interactions of membrane proteins. *Biophys. J.* **2003,** 85, 2900-18.

9. DeLano, W. L. *The PyMOL Molecular Graphics System*, 0.99; DeLano Scientific, San Carlos, CA, USA: 2006.

10. Stierand, K.; Maass, P. C.; Rarey, M. Molecular complexes at a glance: automated generation of two-dimensional complex diagrams. *Bioinformatics* **2006,** 22, 1710-6.

11. HJ, Y. A Guide to Recording Fluorescence Quantum Yields. HORIBA, Jobin Yvon Ltd, Stanmore,. *Middlesex, UK* **2012**.

12. Huang, X. P.; Mangano, T.; Hufeisen, S.; Setola, V.; Roth, B. L. Identification of human Ether-a-go-go related gene modulators by three screening platforms in an academic drug-discovery setting. *Assay Drug Dev Technol* **2010,** *8*, 727-742.

13. Diaz, G. J.; Daniell, K.; Leitza, S. T.; Martin, R. L.; Su, Z.; McDermott, J. S.; Cox, B. F.; Gintant, G. A. The [3H]dofetilide binding assay is a predictive screening tool for hERG blockade and proarrhythmia: Comparison of intact cell and membrane preparations and effects of altering [K+]o. *J Pharmacol Toxicol Methods* **2004,** *50*, 187-199.

14. Piper, D. R.; Duff, S. R.; Eliason, H. C.; Frazee, W. J.; Frey, E. A.; Fuerstenau-Sharp, M.; Jachec, C.; Marks, B. D.; Pollok, B. A.; Shekhani, M. S.; Thompson, D. V.; Whitney, P.; Vogel, K. W.; Hess, S. D. Development of the predictor HERG fluorescence polarization assay using a membrane protein enrichment approach. *Assay Drug Dev Technol* **2008,** *6*, 213-223.