Electronic Supporting Information for:

Discovery of novel retigabine derivatives as potent KCNQ4 and KCNQ5 channels agonists with improved specificity

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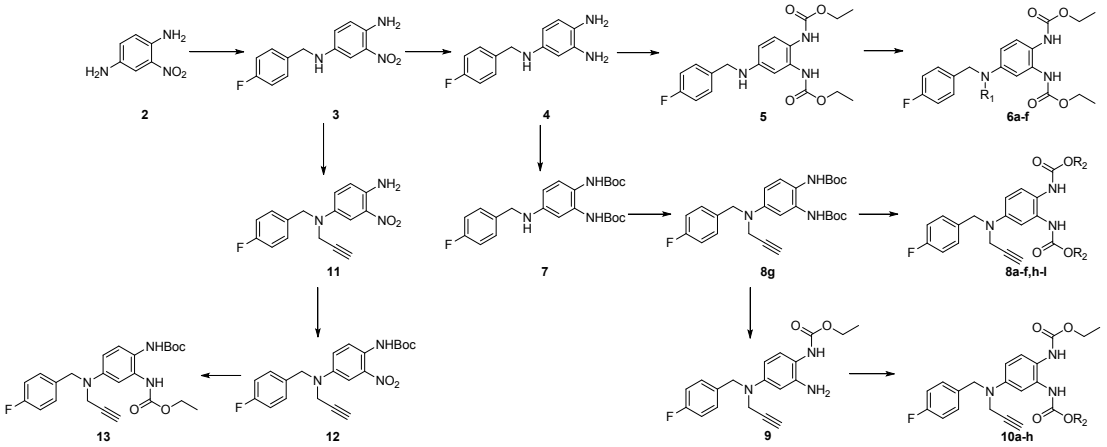
1. Materials and instruments

All reagents and solvents were purchased from the suppliers and purified/dried if anhydrous one was necessary. NMR spectra were recorded at 300 and 126 MHz or for ^{1}H and ^{13}C nuclei, respectively. ^{1}H chemical shifts were reported as δ (ppm) and spin-spin coupling constants as J (Hz) values. The ^{13}C NMR values were referenced to the residual chloroform (δ 77.16 ppm). High resolution mass spectroscopy (HRMS) was performed on a TOF instrument with ESI in positive ionization mode. HPLC analyses were performed on an HP 1100 series LC system.

2. Synthetic procedures



Representative procedure for the synthesis of compounds **6a-f**.

The starting material **5** (50 mg, 0.133 mmol), prepared according to our previous work [ref.28], was dissolved in DMF (2 mL), then corresponding alkyl bromide (0.173 mmol) and DIPEA (34.4 mg, 0.266 mmol) were added to this solution. After the mixture was stirred at 60 °C for 2 h, the mixture was poured into water and extracted with EtOAc (3×10 mL). The combined organic phases were washed with water and brine, dried over sodium sulfate and then concentrated in vacuo to give crude produce, which was further purified by column chromatography to give pure products.

(E)-diethyl(4-(but-2-en-1-yl(4-fluorobenzyl)amino)-1,2-phenylene) dicarbamate (**6a**)

Yellow oil, yield: 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (dd, *J* = 7.5, 5.7Hz, 2H), 7.06 (d, *J* = 8.7Hz, 1H), 6.99 (t, *J* = 8.7Hz, 2H), 6.42 (s, 1H), 6.42 (d, *J* = 8.7Hz, 1H), 5.48-5.62 (m, 2H), 4.45 (s, 2H), 4.18-4.22 (m, 4H), 3.88 (d, *J* = 4.5Hz, 2H), 1.67 (d, *J* = 6.0Hz, 3H), 1.24-1.32 (m, 6H). LRMS (ESI) *m/z* calcd for $C_{23}H_{29}FN_3O_4$ $[M+H]^+$: 430.2; found: 430.2.

diethyl(4-((4-fluorobenzyl)(3-methylbut-2-en-1-yl)amino)-1,2-phenylene) dicarbamate (**6b**)

Colorless oil, yield: 88%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (dd, *J* = 8.4, 5.7Hz, 2H), 7.07 (d, *J* = 8.7Hz, 1H), 6.98 (t, *J* = 8.4Hz, 2H), 6.41 (dd, *J* = 8.7, 2.4Hz, 2H), 5.23 (t, *J* = 6.3Hz, 1H), 4.43 (s, 2H), 4.12-4.24 (m, 4H), 3.90 (d, *J* = 6.3Hz, 2H), 1.71 (s, 3H), 1.62 (s, 3H), 1.28 (t, *J* = 7.2Hz, 6H). LRMS (ESI) *m/z* calcd for $C_{24}H_{31}FN_3O_4$ $[M+H]^+$: 444.2; found: 444.2.

(E)-diethyl(4-((4-fluorobenzyl)(2-methylbut-2-en-1-yl)amino)-1,2-phenylene) dicarbamate (**6c**)

Colorless oil, yield: 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 – 7.11 (m, 2H), 7.09-6.91 (m, 4H), 6.39 (dd, *J* = 8.9, 2.8 Hz, 1H), 5.38-5.22 (m, 1H), 4.46 (s, 2H), 4.27-4.11 (m, 4H), 3.83 (s, 2H), 1.64-1.52 (m, 6H), 1.37-1.20 (m, 6H). LRMS (ESI) *m/z* calcd for $C_{24}H_{31}FN_3O_4$ $[M+H]^+$: 444.2; found: 444.3.

diethyl(4-((cyclohex-1-en-1-ylmethyl)(4-fluorobenzyl)amino)-1,2-phenylene) dicarbamate (**6d**)

Colorless oil, yield: 85%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (dd, *J* = 8.4, 5.4Hz, 2H), 7.05 (d, *J* = 8.7Hz, 1H), 6.98 (t, *J* = 9.0Hz, 2H), 6.88 (brs, 1H), 6.39 (dd, *J* = 9.0, 2.7Hz, 1H), 5.50 (s, 1H), 4.47 (s, 2H), 4.18-4.21 (m, 4H), 3.80 (s, 2H), 2.02-2.06 (m, 2H), 1.97-1.99 (m, 2H), 1.58-1.61 (m, 4H), 1.27-1.29 (m, 6H). LRMS (ESI) *m/z* calcd

for $C_{26}H_{33}FN_3O_4$ $[M+H]^+$: 470.2; found: 470.3.

diethyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene)dicarbamate (**6e**)

Colorless oil, yield: 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (t, *J* = 9.0Hz, 3H), 7.09 (d, *J* = 9.0Hz, 1H), 6.94 (t, *J* = 8.4Hz, 2H), 6.54 (d, *J* = 8.7Hz, 1H), 4.41 (s, 2H), 4.12 (q, *J* = 7.2Hz, 4H), 3.90 (t, *J* = 2.1Hz, 2H), 2.15 (t, *J* = 2.1Hz, 1H), 1.22 (t, *J* = 7.2Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.10, 161.15, 155.51, 154.23, 147.57, 133.67, 128.97, 128.90, 115.55, 115.38, 79.21, 72.48, 61.68, 61.48, 54.36, 40.00, 14.55; HRMS (ESI) *m/z* calcd for $C_{22}H_{24}FN_3NaO_4$ $[M+Na]^+$: 436.1649; found: 436.1644.

diethyl(4-((4-fluorobenzyl)(3-phenylprop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**6f**)

Colorless oil, yield: 83%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39-7.37 (m, 2H), 7.34-7.26 (m, 6H), 7.18 (d, *J* = 8.7Hz, 1H), 7.02 (t, *J* = 8.7Hz, 2H), 6.66 (dd, *J* = 8.7, 2.1Hz, 1H), 6.52(brs, 1H), 4.56 (s, 2H), 4.19-4.24 (m, 6H), 1.27-1.31 (m, 6H). LRMS (ESI) *m/z* calcd for $C_{28}H_{29}FN_3O_4$ $[M+H]^+$: 490.2; found: 490.2.

di-*tert*-butyl (4-((4-fluorobenzyl)amino)-1,2-phenylene)dicarbamate (**7**)

To a solution of **4** (5.0 g, 21.6 mmol) in THF: H_2O (60 ml, 2:1, v/v) was added Boc_2O (14.2 g, 64.8 mmol) and $NaHCO_3$ (5.5 g, 64.8 mmol). The mixture was stirred at room temperature overnight. The mixture was extracted with EtOAc (3×50 mL). The combined organic phases were dried over sodium sulfate and concentrated *in vacuo*. Purification (petroleum ether : EtOAc = 5 : 1) by silica gel column chromatography gave compound **7** as brown oil (7.93 g, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (t, *J* = 8.7 Hz, 2H), 7.00 (t, *J* = 8.7 Hz, 3H), 6.89 (s, 1H), 6.26 (d, *J* = 8.7 Hz, 1H), 4.25 (s, 2H), 1.48 (s, 18H). LRMS (ESI) *m/z* calcd for $C_{23}H_{31}FN_3O_4$ $[M+H]^+$: 432.2; found: 432.2.

di-*tert*-butyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**8g**)

To a solution of **7** (5.0 g, 11.6 mmol) in DMF (30 mL) was added 3-bromo-1-propyn (1.7 g, 13.9 mmol) and DIPEA (2.2 g, 17.4 mmol). After the mixture was stirred at 60 °C for 2 h, the mixture was poured into water and extracted with EtOAc (3×50 mL). The combined organic phases were washed with water and brine, dried over sodium sulfate and then concentrated *in vacuo* to give crude produce, which was further purified by column chromatography (petroleum ether : EtOAc = 6 : 1) to give pure **8g** as a white solid (3.3 g, 60% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.26 (m, 2H), 7.17 (d, *J* = 9.1 Hz, 1H), 7.07-6.94 (m, 2H), 6.76 (s, 1H), 6.61 (dd, *J* = 8.8, 2.9 Hz, 1H), 4.47 (s, 2H), 3.96 (d, *J* = 2.3 Hz, 2H), 2.20 (t, *J* = 2.4 Hz, 1H), 1.50 (s, 18H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.08, 161.13, 154.62, 153.41, 147.34, 133.79, 129.09, 129.02, 115.49, 115.32, 80.59, 79.30, 72.43, 54.31, 40.07, 28.30; HRMS (ESI) *m/z* calcd for $C_{26}H_{32}FN_3NaO_4$ $[M+Na]^+$: 492.2275; found: 492.2270.

Representative procedure for the synthesis of compounds **8a-f**, **h-l**.

To a solution of **8g** (100 mg, 0.21 mmol) in DCM (2.0 mL) was added TFA (1.0 mL) and the mixture was stirred at 0 ºC for 1 h. The mixture was concentrated *in vacuo*. The residue was dissolved in 1,4-dioxane (5 mL) and DIPEA (138 mg, 1.05 mmol) was added to this solution. Appropriate chloroformate (0.36 mmol) was added dropwise and the mixture was stirred at room temperature for 4 h. The mixture was evaporated to dryness to give crude produce, which was further purified by column chromatography.

dimethyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**8a**)

Brown oil, yield: 70%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.24 (m, 2H), 7.16 (d, *J* = 8.7 Hz, 1H), 7.08-6.95 (m, 3H), 6.63 (dd, *J* = 8.8, 2.8 Hz, 1H), 4.49 (s, 2H), 3.98 (d, *J* = 2.3 Hz, 2H), 3.76 (s, 6H), 2.23 (t, *J* = 2.3 Hz, 1H). LRMS (ESI) *m/z* calcd for $C_{20}H_{20}FN_3NaO_4$ $[M+Na]^+$: 408.1; found: 408.2.

diallyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene)dicarbamate (**8b**)

Brown oil, yield: 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.25 (m, 2H), 7.18 (d, *J* = 8.8 Hz, 1H), 7.11-6.88 (m, 3H), 6.64 (dd, *J* = 8.8, 2.9 Hz, 1H), 6.12-5.77 (m, 2H), 5.63-5.00 (m, 4H), 4.65 (dt, *J* = 5.7, 1.3 Hz, 4H), 4.49 (s, 2H), 3.98 (d, *J* = 2.3 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H). LRMS (ESI) *m/z* calcd for $C_{24}H_{25}FN_3O_4$ $[M+H]^+$: 438.2; found: 438.2.

dipropyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene)dicarbamate (**8c**)

Brown oil, yield: 65%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.25 (m, 2H), 7.17 (d, *J* = 9.0 Hz, 1H), 7.07-6.92 (m, 3H), 6.63 (dd, *J* = 8.8, 2.7 Hz, 1H), 4.49 (s, 2H), 4.11 (td, *J* = 6.7, 1.4 Hz, 4H), 3.98 (d, *J* = 2.3 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 1.76-1.62 (m, 4H), 0.96 (t, *J* = 7.4 Hz, 6H). LRMS (ESI) *m/z* calcd for $C_{24}H_{29}FN_3O_4$ $[M+H]^+$: 442.2; found: 442.2.

diisopropyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**8d**)

Yellow oil, yield: 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.26 (m, 2H), 7.17 (d, *J* = 8.9 Hz, 1H), 7.06-6.95 (m, 2H), 6.89 (s, 1H), 6.62 (dd, *J* = 9.0, 2.7 Hz, 1H), 5.14-4.87 (m, 2H), 4.49 (s, 2H), 3.97 (d, *J* = 2.0 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 1.28 (d, *J* = 6.2 Hz, 12H). LRMS (ESI) *m/z* calcd for $C_{24}H_{29}FN_3O_4$ $[M+H]^+$: 442.2; found: 442.3.

dibutyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene)dicarbamate (**8e**)

Brown oil, yield: 85%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.26 (m, 2H), 7.17 (d, *J* = 8.5 Hz, 1H), 7.08-6.90 (m, 3H), 6.63 (dd, *J* = 8.8, 2.8 Hz, 1H), 4.49 (s, 2H), 4.15 (td, *J* = 6.7, 1.2 Hz, 4H), 3.97 (d, *J* = 2.3 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 1.71-1.58 (m, 4H), 1.50-1.32 (m, 4H), 0.95 (t, *J* = 7.3 Hz, 6H). LRMS (ESI) *m/z* calcd for $C_{26}H_{33}FN_3O_4$ $[M+H]^+$: 470.2; found: 470.3.

diisobutyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**8f**)

Yellow oil, yield: 73%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.23 (m, 2H), 7.17 (d, *J* = 8.6 Hz, 1H), 7.07-6.90 (m, 3H), 6.63 (dd, *J* = 8.9, 2.9 Hz, 1H), 4.49 (s, 2H), 4.01-3.88 (m, 6H), 2.22 (t, *J* = 2.2 Hz, 1H), 2.05-1.88 (m, 2H), 0.95 (d, *J* = 6.7 Hz, 12H). LRMS (ESI) *m/z* calcd for $C_{26}H_{33}FN_3O_4$ $[M+H]^+$: 470.2; found: 470.2.

dicyclopropyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**8h**)

Yellow oil, yield: 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.17 (m, 3H), 7.08-6.84 (m, 3H), 6.62 (d, *J* = 6.6 Hz, 1H), 6.43 (brs, 1H), 4.49 (s, 2H), 4.23-4.03 (m, 2H), 3.97 (d, *J* = 2.3 Hz, 2H), 2.23 (t, *J* = 2.2 Hz, 1H), 0.94-0.52 (m, 8H). LRMS (ESI) *m/z* calcd for $C_{24}H_{25}FN_3O_4$ $[M+H]^+$: 438.2; found: 438.2.

dicyclobutyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**8i**)

Yellow oil, yield: 78%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.23 (m, 2H), 7.16 (d, *J* = 8.1 Hz, 1H), 7.08-6.86 (m, 3H), 6.62 (dd, *J* = 8.6, 2.8 Hz, 1H), 6.44 (brs, 1H), 5.16-4.87 (m, 2H), 4.48 (s, 2H), 3.97 (d, *J* = 2.2 Hz, 2H), 2.56-2.25 (m, 4H), 2.22 (t, *J* = 2.3 Hz, 1H), 2.19-1.93 (m, 4H), 1.89-1.72 (m, 2H), 1.70-1.58 (m, 2H). LRMS (ESI) *m/z* calcd for $C_{26}H_{29}FN_3O_4$ $[M+H]^+$: 466.2; found: 466.2.

dicyclopentyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**8j**)

Colorless oil, yield: 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.24 (m, 2H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.01 (t, *J* = 8.7 Hz, 2H), 6.90 (s, 1H), 6.62 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.39 (brs, 1H), 5.26-5.09 (m, 2H), 4.48 (s, 2H), 3.96 (d, *J* = 2.3 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 1.96-1.81 (m, 4H), 1.81-1.65 (m, 8H), 1.65-1.57 (m, 4H). LRMS (ESI) *m/z* calcd for $C_{28}H_{33}FN_3O_4$ $[M+H]^+$: 494.2; found: 494.3.

diphenyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**8k**)

White solid, yield: 73%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48-7.34 (m, 5H), 7.33-7.14 (m, 8H), 7.07-6.91 (m, 3H), 6.70 (d, *J* = 9.6 Hz, 1H), 4.51 (s, 2H), 3.99 (d, *J* = 2.1 Hz, 2H), 2.23 (t, *J* = 2.3 Hz, 1H). MS (ESI) m/z = 510.2 ($M + H^+$). LRMS (ESI) *m/z* calcd for $C_{30}H_{25}FN_3O_4$ $[M+H]^+$: 510.2; found: 510.2.

dibenzyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**8l**)

White solid, yield: 70%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54-7.13 (m, 14H), 7.01 (t, *J* = 8.6 Hz, 2H), 6.63 (d, *J* = 9.1 Hz, 1H), 5.18 (s, 4H), 4.48 (s, 2H), 3.97 (s, 2H), 2.22 (t, *J* = 2.1 Hz, 1H). LRMS (ESI) *m/z* calcd for $C_{32}H_{29}FN_3O_4$ $[M+H]^+$: 538.2; found: 538.2.

(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene)dicarbamate (**9**)

To a solution of **8g** (3.0 g, 6.39 mmol) in DCM (30 mL) was added TFA (10 mL) and the mixture was stirred at 0 ºC for 1 h. The mixture was concentrated *in vacuo*. The residue was dissolved in 1,4-dioxane (20 mL) and DIPEA (2.5 g, 19.2 mmol) , ethyl chloroformate (0.7 g, 6.39 mmol) were added dropwise to this solution

in sequence. After the mixture was stirred at room temperature for 4 h, the mixture was poured into water and extracted with EtOAc (3×50 mL). The combined organic phases were washed with water and brine, dried over sodium sulfate and then concentrated *in vacuo* to give crude produce, which was further purified by column chromatography (petroleum ether : EtOAc = 3 : 1) to give pure **9** as a brown solid (1.1 g, 50% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.22 (m, 2H), 7.09-6.93 (m, 3H), 6.38-6.20 (m, 2H), 6.09 (brs, 1H), 4.45 (s, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.94 (d, *J* = 2.3 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 1.29 (t, *J* = 7.1 Hz, 3H).

Representative procedure for the synthesis of compounds **10a-h**.

To a solution of **9** (50 mg, 0.15 mmol) in 1,4-dioxane (2.0 mL) was added DIPEA (28.4 mg, 0.22 mmol) and appropriate chloroformate (0.18 mmol) in sequence. After the mixture was stirred at room temperature for 4 h, the mixture was evaporated to dryness to give crude produce, which was further purified by column chromatography.

ethyl methyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**10a**)

White solid, yield: 85%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.26 (m, 3H), 7.15 (d, *J* = 8.9 Hz, 1H), 7.06-6.96 (m, 2H), 6.62 (dd, *J* = 8.8, 2.9 Hz, 1H), 4.49 (s, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.98 (d, *J* = 2.3 Hz, 2H), 3.76 (s, 3H), 2.23 (t, *J* = 2.4 Hz, 1H), 1.30 (t, *J* = 7.1 Hz, 3H). LRMS (ESI) *m/z* calcd for $C_{21}H_{23}FN_3O_4$ $[M+H]^+$: 400.2; found: 400.1.

allyl ethyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**10b**)

White solid, yield: 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.23 (m, 3H), 7.16 (d, *J* = 8.6 Hz, 1H), 7.06-6.94 (m, 2H), 6.63 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.05-5.86 (m, 1H), 5.46-5.14 (m, 2H), 4.65 (d, *J* = 5.7 Hz, 2H), 4.49 (s, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.98 (d, *J* = 2.2 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 1.30 (t, *J* = 7.1 Hz, 3H). LRMS (ESI) *m/z* calcd for $C_{23}H_{25}FN_3O_4$ $[M+H]^+$: 426.2; found: 426.1.

ethyl propyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**10c**)

White solid, yield: 95%.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.25 (m, 3H), 7.17 (d, *J* = 9.0 Hz, 1H), 7.08-6.96 (m, 2H), 6.67-6.59 (m, 1H), 4.49 (s, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.11 (t, *J* = 6.8 Hz, 2H), 3.98 (d, *J* = 2.2 Hz, 2H), 2.22 (t, *J* = 2.2 Hz, 1H), 1.76-1.61 (m, 2H), 1.30 (t, *J* = 7.1 Hz, 3H), 0.96 (t, *J* = 7.4 Hz, 3H). LRMS (ESI) *m/z* calcd for $C_{23}H_{26}FN_3O_4$ $[M+H]^+$: 428.2; found: 428.2.

ethyl isopropyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**10d**)

Colorless oil, yield: 80%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.25 (m, 3H), 7.18 (d, *J* = 8.6 Hz, 1H), 7.07-6.97 (m, 2H), 6.87 (s, 1H), 6.62 (dd, *J* = 8.8, 2.7 Hz, 1H), 5.07-4.91 (m, 1H), 4.49 (s, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.97 (d, *J* = 2.3 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 1.33-1.25 (m, 9H). LRMS (ESI) *m/z* calcd for $C_{23}H_{26}FN_3O_4$ $[M+H]^+$: 428.2; found: 428.2.

butyl ethyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**10e**)

White solid, yield: 92%.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31-7.25 (m, 3H), 7.17 (d, *J* = 8.7 Hz, 1H), 7.06-6.97 (m, 2H), 6.63 (dd, *J* = 8.8, 2.8 Hz, 1H), 4.49 (s, 2H), 4.27-4.11 (m, 4H), 3.97 (d, *J* = 2.3 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 1.71-1.59 (m, 2H), 1.47-1.36 (m, 2H), 1.30 (t, *J* = 7.1 Hz, 3H), 0.95 (t, *J* = 7.3 Hz, 3H). LRMS (ESI) *m/z* calcd for $C_{24}H_{29}FN_3O_4$ $[M+H]^+$: 442.2; found: 442.2.

ethyl isobutyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**10f**)

White solid, yield: 96%.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.26 (m, 3H), 7.18 (d, *J* = 8.7 Hz, 1H), 7.06-6.97 (m, 2H), 6.63 (dd, *J* = 8.9, 2.2 Hz, 1H), 4.49 (s, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.98 (d, *J* = 2.2 Hz, 2H), 3.93 (d, *J* = 6.7 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 2.02-1.89 (m, 1H), 1.30 (t, *J* = 7.1 Hz, 3H), 0.95 (d, *J* = 6.7 Hz, 6H). LRMS (ESI) *m/z* calcd for $C_{24}H_{29}FN_3O_4$ $[M+H]^+$: 442.2; found: 442.2.

tert-butyl ethyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**10g**)

White solid, yield: 94%.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.24 (m, 3H), 7.07-6.94 (m, 2H), 6.70 (s, 1H), 6.63 (d, *J* = 8.7 Hz, 1H), 4.48 (s, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.97 (d, *J* = 2.3 Hz, 2H), 2.21 (t, *J* = 2.3 Hz, 1H), 1.50 (s, 9H), 1.29 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.09, 161.14, 155.47, 153.42, 147.46, 133.76,

129.04, 128.98, 115.52, 115.35, 80.83, 79.27, 72.47, 61.51, 54.31, 40.02, 28.30, 14.57; HRMS (ESI) *m/z* calcd for $C_{24}H_{28}FN_3NaO_4$ $[M+Na]^+$: 464.1962; found: 464.1959.

benzyl ethyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**10h**)

White solid, yield: 75%.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.32 (m, 4H), 7.33-7.21 (m, 3H), 7.16 (d, *J* = 8.6 Hz, 1H), 7.11-6.90 (m, 3H), 6.63 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.38 (brs, 1H), 5.19 (s, 2H), 4.48 (s, 2H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.97 (d, *J* = 2.1 Hz, 2H), 2.22 (t, *J* = 2.3 Hz, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). LRMS (ESI) *m/z* calcd for $C_{27}H_{27}FN_3O_4$ $[M+H]^+$: 476.2; found: 476.2.

N^1-(4-fluorobenzyl)-3-nitro-*N*^{1}-(prop-2-yn-1-yl)benzene-1,4-diamine (**11**)

To a solution of **3** (500 mg, 1.91 mmol) in DMF (5 mL) was added 3-bromo-1-propyn (270 mg, 2.30 mmol) and DIPEA (370 mg, 2.87 mmol). After the mixture was stirred at 65 °C overnight, the mixture was poured into water and extracted with EtOAc (3×20 mL). The combined organic phases were washed with water and brine, dried over sodium sulfate and then concentrated *in vacuo* to give crude produce, which was further purified by column chromatography (petroleum ether : EtOAc = 4 : 1) to give pure **11** as a brown oil (480 mg, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, *J* = 2.9 Hz, 1H), 7.35 – 7.28 (m, 2H), 7.15 (dd, *J* = 9.0, 2.9 Hz, 1H), 7.02 (t, *J* = 8.7 Hz, 2H), 6.76 (d, *J* = 9.0 Hz, 1H), 5.85 (brs, 2H), 4.35 (s, 2H), 3.87 (d, *J* = 2.3 Hz, 2H), 2.26 (t, *J* = 2.3 Hz, 1H). LRMS (ESI) *m/z* calcd for $C_{16}H_{15}FN_3O_2$ $[M+H]^+$: 300.1; found: 300.1.

tert-butyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-2-nitrophenyl)carbamate (**12**)

The mixture of compound **11** (300 mg, 1.0 mmol), Boc_2O (260 mg, 1.2 mmol) and DMAP (180 mg, 1.5 mmol) in THF (5 mL) was refluxed for 12 h under nitrogen. After cooled to room temperature, the mixture was poured into water and extracted with EtOAc (3×20 mL). The combined organic phases were washed with water and brine, dried over sodium sulfate and then concentrated *in vacuo* to give crude produce, which was further purified by column chromatography (petroleum ether : EtOAc = 6 : 1) to give pure **12** as a red solid (140 mg, 35% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.34 (d, *J* = 9.3 Hz, 1H), 7.63 (d, *J* = 3.0 Hz, 1H), 7.34-7.22 (m, 2H), 7.18 (dd, *J* = 9.3, 3.1 Hz, 1H), 7.03 (t, *J* = 8.5 Hz, 2H), 4.48 (s, 2H), 4.01 (d, *J* = 2.2 Hz, 2H), 2.26 (t, *J* = 2.1 Hz, 1H), 1.52 (s, 9H). LRMS (ESI) *m/z* calcd for $C_{21}H_{23}FN_3O_4$ $[M+H]^+$: 400.2; found: 400.2.

tert-butyl ethyl(4-((4-fluorobenzyl)(prop-2-yn-1-yl)amino)-1,2-phenylene) dicarbamate (**13**)

To a stirred solution of compound **12** (100 mg, 0.25 mmol) in methanol (3 ml), zinc powder (82 mg, 1.25 mmol) was added, followed by dropwise addition of ammonium chloride solution (67 mg, 1.25 mmol) in H_2O (1 ml). After being stirred at room temperature for 3 h, DIPEA (48 mg, 0.38 mmol) and ethyl chloroformate (33 mg, 0.30 mmol) were added to the reaction mixture at 0°C and the stirring was continued for another 2 h at room temperature. After consumption of the starting material, the reaction mixture was diluted with water (10 ml) and extracted with EtOAc (3×10 mL). The combined organic phases were washed with water and brine, dried over sodium sulfate and then concentrated *in vacuo* to give crude produce, which was further purified by column chromatography (petroleum ether : EtOAc = 5 : 1) to give pure **13** as a yellow oil (66 mg, 60% yield in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.21 (m, 2H), 7.13 (d, *J* = 8.7 Hz, 1H), 7.07-6.91 (m, 3H), 6.61 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.24 (brs, 1H), 4.48 (s, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.96 (d, *J* = 2.3 Hz, 2H), 2.21 (t, *J* = 2.2 Hz, 1H), 1.50 (s, 9H), 1.29 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.08, 161.13, 154.69, 154.24, 147.41, 133.75, 133.73, 129.01, 128.95, 115.51, 115.34, 80.83, 79.25, 72.45, 61.35, 54.36, 40.04, 28.29, 14.55; HRMS (ESI) *m/z* calcd for $C_{24}H_{28}FN_3NaO_4$ $[M+Na]^+$: 464.1962; found: 464.1956.

3. Plasmid construction

Plasmids encoding human Kv7.1, rat Kv7.2, rat Kv7.3, human Kv7.4 (GenBank accession numbers: NM000218, NM_133322, AF091247 and NM_004700, respectively) were subcloned into pcDNA3.1 vector. Plasmids encoding human Kv7.5 were gifted from Kenneth L. Byron (Loyola University Chicago) and were subcloned into

pEGFP-N3 vector. KCNQ2/3 dimers were constructed by DNA In-Fusion clone (Takara, In-Fusion cloning Kit) and verified by sequencing. To ensure the currents function of the constructed dimer, we designed 8 glycine as linker between KCNQ2 and KCNQ3. The expression vector pcDNA3.1(+) carrying KCNQ2 cDNAs were double digested with Nel1 and EcoR1 generating a linearized vector. Amplified KCNQ3 cDNAs with the following primers: F, 5'-GGGAGACCCAAGCTGGCTAGCATGGCATTGGAGTTCCCGGGCTTG-3'; R, 5'-CATGCCTCCGCCTCCGAATTCTCCACCGCCTCCAGTGGGCTTGTTGGAAGGGGTCCA-3'.

4. Cell culture

Chinese hamster ovary (CHO) cells were grown in DMEM/F-12 (Gibco^{TM}, Life Technologies, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS). To overexpress KCNQs channels, cells were split and plated in 60-mm dishes, and after 24h transfected with Lipofectamine 2000^{TM} reagent (Invitrogen^{TM}, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. At 24h after transfection, cells were split and replated onto coverslips coated with poly-L-lysine (Sigma-Aldrich, St Louis, MO, USA).

5. Electrophysiological recording

For KCNQs channels current measurements in CHO cells, a standard whole-cell voltage-clamp technique was used. Pipettes were pulled from borosilicate glass capillaries (TW150-4, World Precision Instruments, Sarasota, FL, USA). and had resistances of 3-5 megaohms when filled with the intracellular solution. During the recording, the extracellular solution was constantly perfused by a BPS perfusion system (ALA Scientifc Instruments). The pipettes solution containing the following (in mM): 145 KCl, 1 $MgCl_2$, 5 EGTA, 10 HEPES and 5 K_2-ATP (adjustment pH 7.3 with 1 mM KOH; extracellular solution contained (in mM): 140 NaCl, 3 KCl, 2 $CaCl_2$, 1.5 $MgCl_2$, 10 HEPES and 10 glucose, adjustment pH 7.3 with 1 mM NaOH. Current and voltage were recorded using an Axopatch-200B amplifer, fltered at 2 kHz, and digitized using a DigiData 1440A with Clampex 10.4 software (Axon Instruments, Sunnyvale, CA, USA). Series resistance compensation was used and set to 60%-80%. Particularly for KCNQ4 and KCNQ5 recording, those cells with serial resistance less than 10 MΩ and very good compensation (~80%) were used.

6. Compounds application

All compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain a 20 mM stock solution, from which the appropriate volumes were added to the external solutions to produce the desired concentrations. DMSO (less than 0.1% in the final dilution) elicited no observable effect on the K^+ currents. The external solution containing the drugs was delivered to the recorded cell using ALA 8 Channel Solution Exchange System (ALA Scientific Instruments Inc, Farmingdale, NY, USA).

7. Data analysis

Patch clamp data were processed using Clampfit 10.2 (Molecular Devices, Sunnyvale, CA, USA) and then analyzed in GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Voltage-dependent activation curves were fitted using the Boltzmann equation, $G=G_{min} + (G_{max}-G_{min})/(1+\exp(V-V_{1/2})/S)$, where G_{max} is the maximum conductance, G_{min} is the minimum conductance, $V_{1/2}$ is the voltage for reaching 50% of maximum conductance, and S is the slope factor. The voltage errors caused by series resistance were corrected by Ohm's law: $V_c = V_{hold} - (I*R_s)$, V_c stand for the Calibrated voltage, V_{hold} stand for holding voltage, R_s stand for series resistance. Dose-response curves were fitted with the Hill equation, $E=E_{max}/ (1-(EC_{50}/C) P)$, where EC_{50} is the drug concentration producing half of the maximum response, and P is the Hill coefficient. The data are presented as the mean ± SEM. Significance was estimated using Student's t test, where $P<0.05$ was considered significant,

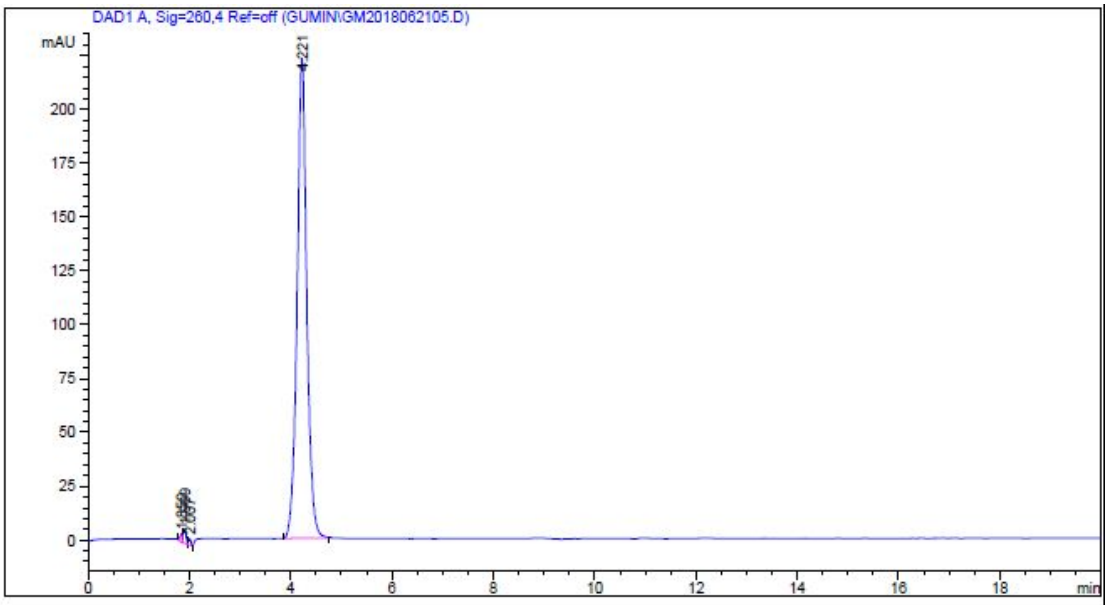
*** means p<0.0001, ** means p<0.005, * means p<0.05, ns means no significant.

8. Table S1. The $V_{1/2}$ (mV) of KCNQ2 and KCNQ4 channels before and after application of RTG, 6e, 10g

	KCNQ2			KCNQ4		
	Ctrl	Drug	$\Delta V_{1/2}$	Ctrl	Drug	$\Delta V_{1/2}$
RTG(10 μM)	-8.4±1.1	-45.6±3.2	-37.2±2.1***	-19.0±2.2	-36.3±1.7	-17.4±0.5***
6e(10 μM)	-9.7±1.1	-17.6±4.3	-8.0±3.2*	-10.8±2.1	-15.9±3.8	-5.1±1.7ns
10g(10 μM)	-13.5±1.8	-23.2±1.8	-9.7±0.02**	-12.3±2.3	-15.7±3.4	-3.4±1.0ns

9. HPLC spectrums of 6e, 8g, 10g and 13

Figure S1. HPLC Spectrum of **6e**

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=====================================================================
                      Area Percent Report
=====================================================================

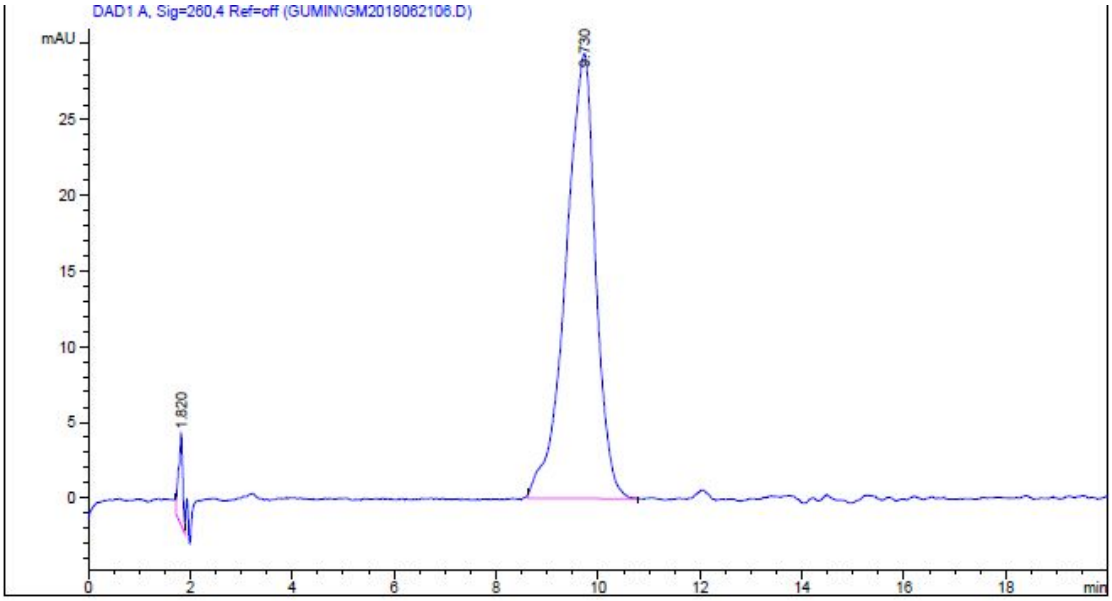
Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=260,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.850 BV    0.0486   13.08484    3.95505   0.4367
   2   1.909 VV    0.0575   24.90187    6.67176   0.8311
   3   2.007 VV    0.0510    9.14817    2.88319   0.3053
   4   4.221 BB    0.1963 2949.08569  223.02243  98.4269

Totals :                   2996.22057  236.53243
```

Figure S2. HPLC Spectrum of **8g**

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=====================================================================
                      Area Percent Report
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Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=260,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.820 BV    0.0790   33.70421    6.04669   2.7252
   2   9.730 BB    0.5554 1203.05359   29.40294  97.2748

Totals :                   1236.75780   35.44963


=====================================================================
```
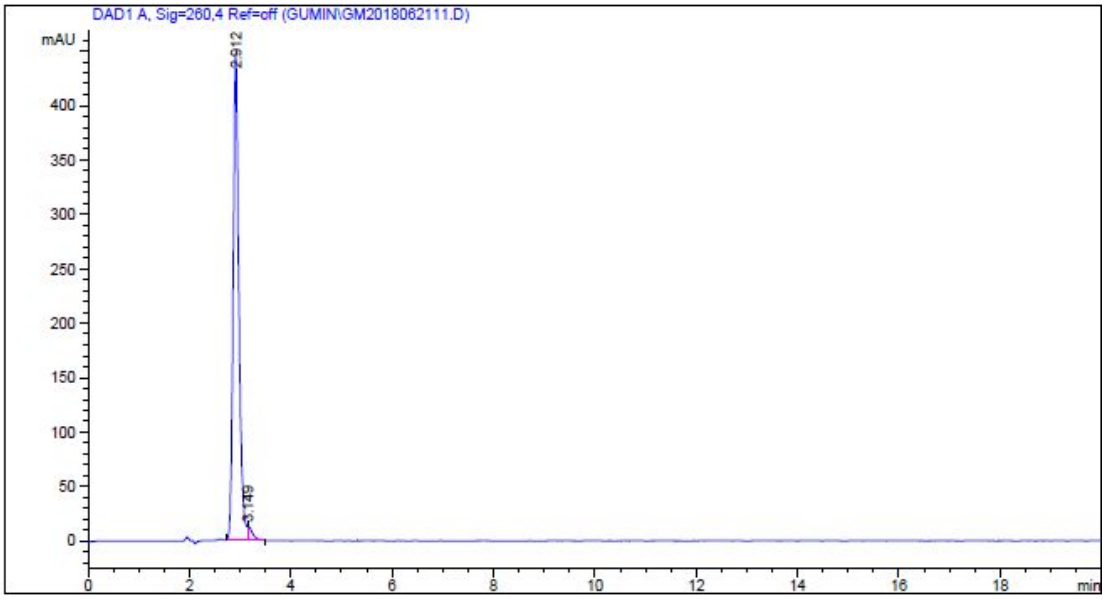

Figure S3. HPLC Spectrum of **10g**

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=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

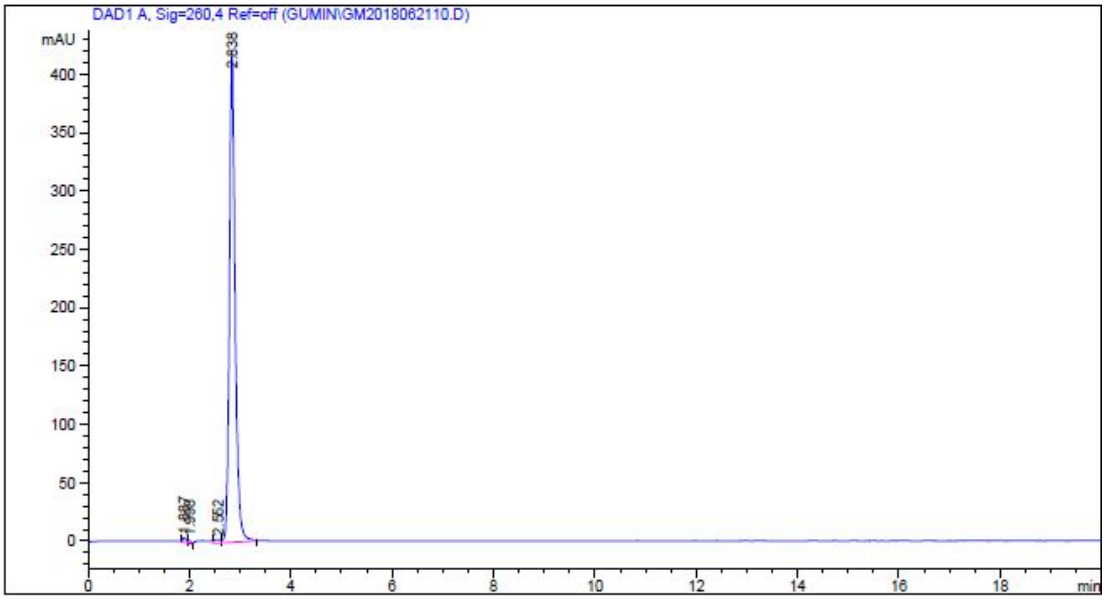

Signal 1: DAD1 A, Sig=260,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.912 MF R  0.1310 3504.20605  445.92978  97.7070
   2   3.149 FM R  0.1080   82.23641   12.68992   2.2930

Totals :                  3586.44247  458.61970


=====================================================================
```

Figure S4. HPLC Spectrum of **13**

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=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=260,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.887 BV    0.0773   23.02152    4.11216   0.6881
   2   1.998 VV    0.0487    8.29458    2.63842   0.2479
   3   2.552 BV    0.1280   23.48204    2.48944   0.7019
   4   2.838 VB    0.1160 3290.69092  417.18414  98.3620

Totals :                   3345.48906  426.42416
```
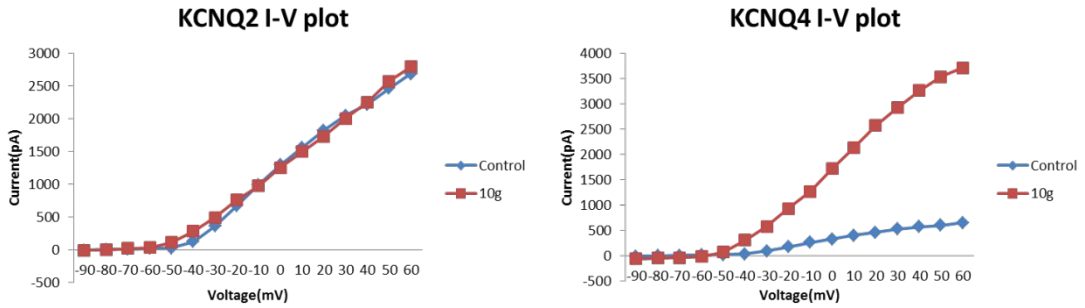

10. Figure S5. Current-voltage (*I-V*) relationships of KCNQ2 and KCNQ4 channels showing drug 10g does not induce channel opening at -60 mV or more negative membrane potentials.



11. Table S2. The membrane potentials before and after voltage correction in KCNQ4 recordings.

V_{hold}(mV)	I(pA)		V_c(mV)	
	control	10g	control	10g
-90	-5.1	-52.5	-90	-90
-80	3.2	-41.2	-80	-80
-70	8.2	-33.3	-70	-70
-60	17.2	-15.0	-60	-60
-50	26.5	76.0	-50	-50
-40	40.7	314.9	-40	-39
-30	96.8	583.2	-30	-29
-20	177.6	938.4	-20	-18
-10	264.2	1271.4	-9	-7
0	332.9	1723.9	-0.7	-3
10	409.5	2141.1	9	6
20	463.2	2572.3	19	15
30	534.5	2923.0	29	24
40	578.3	3266.0	39	33
50	601.8	3533.0	49	43
60	654.4	3712.2	59	53

Note: Taking into account series resistance voltage errors, we have checked and re-analyzed our data to ensure that it did not affect the final conclusion with or without voltage correction.