Supporting Information

Identification of a Potent and Selective GPR4 Antagonist as a Drug for the Treatment of Myocardial Infarction

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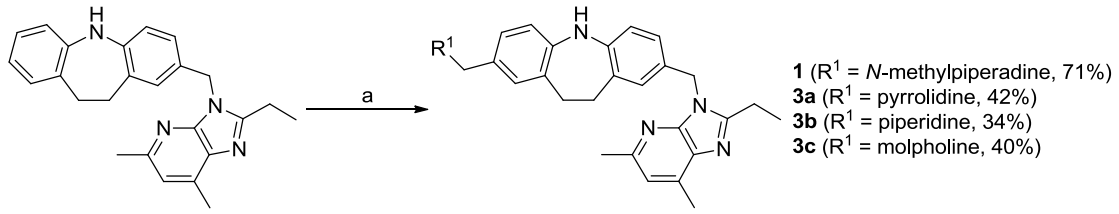
1. Chemistry

General methods and materials for synthesis. NMR spectra were measured on JEOL ECX400P (400 MHz), JEOL ECS400 (400 MHz) and JEOL ECA500 (500 MHz). Chemical shifts were reported in the δ scale relative to tetramethylsilane (TMS) as 0.00 ppm for ^{1}H ($CDCl_3$) and residual $CHCl_3$ (7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C), DMSO (2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C) as internal reference. Mass spectra (MS) were measured on Thermo Scientific Exactive (ESI). Silica gel column chromatography and flash column chromatography was carried out with Wakogel 60N (Wako Pure Chemical Industries, Ltd., neutral, 63-212 μm) and silica gel (Kishida Chemical Co., Ltd., neutral, 32-63 μm), respectively. Elemental analysis (EA) was measured on Yanaco MT-6 and J-Sience JM10. All reactions were carried out under an argon atmosphere using flame-dried or oven-dried glassware, unless otherwise noted, and monitored with analytical TLC (Merck Ltd., TLC Silica gel 60 F_{254}). Purity of all compounds was determined to be >95% by EA or HPLC (YMC-Pack SIL, 150 x 4.6 mm, UV 254 nm).



To a stirred solution of **2** (1 eq.) in $CHCl_3$/AcOH (0.1-0.2 M, 1/1) was added R^1-H (3 eq.) and 37% HCHO aq. (1.2 eq.) at room temperature. The mixture was stirred at 60 °C for 16-36 hr, evaporated. The residue was purified by silica gel column chromatography (MeOH- $CHCl_3$) to give **1**,**3a-c**.

2-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-8-((4-methylpiperazin-1-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (1) ^{1}H NMR (400 MHz, CDCl3) δ 6.99 (d, *J* = 8.2 Hz, 1H), 6.96 (s, 1H), 6.89 (s, 1H), 6.82 (m, 2H), 6.65 (d, *J* = 8.2 Hz, 1H), 6.61 (d, *J* = 8.8 Hz, 1H), 5.96 (s, 1H), 5.34 (s, 2H), 3.39 (s, 2H), 2.99 (t, *J* = 8.2 Hz, 2H), 2.98 (t, *J* = 8.2 Hz, 2H), 2.80 (q, *J* = 7.6 Hz, 2H), 2.63 (s, 3H), 2.60 (s, 3H), 2.56-2.34 (m, 8H), 2.28 (s, 3H), 1.30 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 155.9, 152.0, 147.6, 141.9, 141.2, 137.7, 132.2, 131.6, 129.1, 128.8, 128.6, 128.1, 127.8, 127.3, 125.3, 119.0, 118.0, 117.6, 62.4, 55.0, 52.9, 45.9, 44.6, 34.8, 34.7, 24.2,

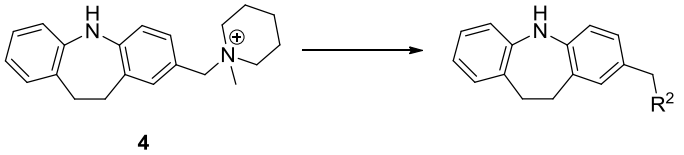
21.6, 16.2, 11.8; LRMS (EI) m/z 494 $[M]^+$; HRMS (EI) calcd for $C_{31}H_{38}N_6$ $[M]^+$ 494.3158, found 494.3152. Anal. Calcd for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42; found: C, 75.82; H, 7.44.

2-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-8-(pyrrolidin-1-ylmethyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (3a) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (d, *J* = 8.4 Hz, 1H), 7.00 (s, 1H), 6.89 (s, 1H), 6.79 (m, 2H), 6.66 (d, *J* = 8.0 Hz, 1H), 6.61 (d, *J* = 8.4 Hz, 1H), 6.14 (s, 1H), 5.55 (s, 2H), 3.41 (s, 2H), 2.98 (t, *J* = 8.4 Hz, 2H), 2.96 (t, *J* = 8.4 Hz, 2H), 2.79 (q, *J* = 7.6 Hz, 2H), 2.63 (s, 3H), 2.60 (s, 3H), 2.56 (m, 4H), 1.80 (m, 4H), 1.30 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 155.9, 152.1, 147.7, 141.9, 141.3, 137.7, 132.2, 131.5, 129.2, 128.7, 128.2, 127.7, 127.4, 125.4, 119.0, 118.1, 117.7, 59.8, 53.9, 44.6, 34.8, 34.7, 24.3, 23.3, 21.6, 16.3, 11.9; LRMS (ESI) m/z 466 $[M+H]^+$,; Optical purity: 99.0% (Column: YMC-pack SIL 4.6 x 150 mm, eluent: $CHCl_3$: MeOH = 100:0 to 98:2 for 20 min, 1.0 mL/min, 20 ℃, 254 nm; retention time: 21.9 min).

2-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-8-(piperidin-1-ylmethyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (3b) ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (d, *J* = 8.0 Hz, 1H), 6.97 (s, 1H), 6.88 (s, 1H), 6.82 (m, 2H), 6.65 (d, *J* = 8.0 Hz, 1H), 6.61 (d, *J* = 9.0 Hz, 1H), 5.97 (s, 1H), 5.34 (s, 2H), 3.41 (s, 2H), 2.99 (t, *J* = 8.0 Hz, 2H), 2.97 (t, *J* = 8.0 Hz, 2H), 2.79 (q, *J* = 7.5 Hz, 2H), 2.63 (s, 3H), 2.60 (s, 3H), 2.39 (m, 4H), 1.60 (m, 4H), 1.43 (m, 2H), 1.30 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 155.9, 152.1, 147.7, 141.9, 141.4, 137.7, 132.2, 131.9, 129.2, 128.7, 128.2, 128.1, 127.4, 125.4, 119.0, 118.1, 117.6, 62.9, 54.1, 44.6, 34.8, 34.7, 25.5, 24.3, 24.1, 21.6, 16.3, 11.8; LRMS (ESI) m/z 480 $[M+H]^+$; Optical purity: 98.3% (Column: YMC-pack SIL 4.6 x 150 mm, eluent: $CHCl_3$: MeOH = 100:0 to 98:2 for 20 min, 1.0 mL/min, 20 ℃, 254 nm; retention time: 21.4 min).

4-((8-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-2-yl)methyl)morpholine (3c) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (d, *J* = 8.8 Hz, 1H), 6.96 (s, 1H), 6.89 (s, 1H), 6.82 (m, 2H), 6.66 (d, *J* = 8.0 Hz, 1H), 6.61 (d, *J* = 8.8 Hz, 1H), 5.97 (s, 1H), 5.35 (s, 2H), 3.38 (s, 2H), 3.00 (t, *J* = 8.4 Hz, 2H), 2.98 (t, *J* = 8.4 Hz, 2H), 2.80 (q, *J* = 7.6 Hz, 2H), 2.63 (s, 3H), 2.60 (s, 3H), 2.42 (m, 4H), 1.64 (m, 4H), 1.30 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 152.0, 147.6, 141.9, 141.3, 137.7, 132.2, 131.6, 129.1, 128.6, 128.1, 128.1,

127.8, 127.3, 125.3, 119.0, 118.0, 117.6, 66.8, 62.8, 53.5, 44.6, 34.8, 34.7, 24.2, 21.5, 16.2, 11.8; LRMS (ESI) m/z 504 $[M+Na]^+$; Optical purity: 95.7% (Column: YMC-pack SIL 4.6 x 150 mm, eluent: $CHCl_3$: MeOH = 100:0 to 98:2 for 20 min, 20 °C, 254 nm; retention time: 20.0 min).



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5a (R^2 = 2-ethylbenzimidazole, 73%)
5b (R^2 = 2-methylbenzimidazole, 73%)
5c (R^2 = benzimidazole, 32%)
5d (R^2 = indole, 25%)
5e (R^2 = carbazole, 36%)
5f (R^2 = 2-ethylimidazole, 72%)
5g (R^2 = 2-methylimidazole, 63%)
5h (R^2 = imidazole, 65%)

To a stirred solution of R^2-H (1 eq.) in DMF (0.1-0.2 M) was added $LiOH \cdot H_2O$ (1.5 eq.) at room temperature. The mixture was stirred at the same temperature for 15 min. **4** (1.2 eq.) was added to the reaction mixture and the solution was stirred at 40 °C overnight, quenched by addition of H_2O and extracted with AcOEt. The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography to give **5a-h**.

2-((2-Ethyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (5a) ^{1}H NMR (400 MHz, CDCl3) δ 7.77 (d, *J* = 7.2 Hz, 1H), 7.27-7.18 (m, 3H), 7.06 (t, *J* = 7.8 Hz, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.79-6.70 (m, 4H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.04 (s, 1H), 5.22 (s, 2H), 3.01 (t, *J* = 8.4 Hz, 2H), 2.99 (t, *J* = 8.4 Hz, 2H), 2.86 (q, *J* = 7.6 Hz, 2H), 1.43 (t, *J* = 7.6 Hz, 3H),; ^{13}C NMR (100 MHz, CDCl3) δ 156.3, 142.5, 142.1, 142.1, 135.5, 130.6, 128.9, 128.7, 128.6, 126.9, 126.5, 124.8, 122.2, 121.9, 119.7, 119.1, 118.3, 117.9, 109.5, 46.3, 34.9, 34.7, 21.0, 11.7; LRMS (ESI) m/z 376 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{23}N_3 \cdot 0.6H_2O$: C, 79.13; H, 6.70; N, 11.54; found: C, 78.97; H, 6.62; N, 11.28.

2-((2-Methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (5b) ^{1}H NMR (500 MHz, CDCl3) δ 7.72 (d, *J* = 7.0 Hz, 1H), 7.27-7.19 (m, 3H), 7.06 (t, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 7.5 Hz, 1H), 6.78-6.74 (m, 3H), 6.71 (d, *J* = 7.5 Hz, 1H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.04 (s, 1H), 5.21 (s, 2H), 3.01 (t, *J* = 8.8 Hz, 2H), 2.99 (t, *J* = 8.8 Hz, 2H), 2.58 (s, 1H),; ^{13}C NMR (125 MHz, CDCl3) δ 151.9, 142.6, 142.2, 142.1, 135.5, 130.6, 128.9, 128.8, 128.6, 126.9, 126.4, 124.9, 122.2, 121.9, 119.8, 119.0, 118.3, 118.0, 109.4, 46.6, 34.9, 34.7, 14.1; LRMS (ESI) m/z 362 $[M+Na]^+$; Anal. Calcd for $C_{23}H_{21}N_3 \cdot 0.6H_2O$: C, 78.87; H, 6.39; N, 12.00; found: C, 78.96; H, 6.42; N, 12.29.

2-((1*H*-Benzo[*d*]imidazol-1-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (5c) ^{1}H NMR (500 MHz, CDCl3) δ 7.92 (s, 1H) 7.82 (d, *J* = 6.5 Hz, 1H), 7.34 (m, 1H), 7.29-7.25 (m, 2H), 7.07 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.90 (s, 2H), 6.78 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.73 (d, *J* = 7.5 Hz, 1H), 6.68 (d, *J* = 9.0 Hz, 1H), 6.13 (s, 1H), 5.22 (s, 2H), 3.03 (t, *J* = 8.2 Hz, 2H), 3.02 (t, *J* = 8.2 Hz, 2H); ^{13}C NMR (125 MHz, CDCl3) δ 144.0, 143.1, 142.5, 142.0, 133.9, 130.6, 129.9, 128.75, 128.69, 126.9, 126.0, 125.8, 122.9, 122.1, 120.3, 119.8, 118.3, 118.0, 110.1, 48.3, 34.9, 34.7; LRMS (EI) m/z 325 $[M]^{+}$; HRMS (EI) calcd for CHNO Na^{+} $[M]^{+}$ 325.15790, found 325.15717. Optical purity: 98.9% (Column: YMC-pack SIL 4.6 x 150 mm, eluent: $CHCl_3$: MeOH = 100:0 to 98:2 for 20 min, 1.0 mL/min, 20 ℃, 254 nm; retention time: 21.6 min).

2-((1*H*-Indol-1-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (5d) ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H) 7.53 (d, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 3.2 Hz, 1H), 7.09 (dd, *J* = 8.0, 7.2 Hz, 1H), 7.01-6.86 (m, 7H), 6.62 (dd, *J* = 7.6, 7.2 Hz, 1H), 6.43 (d, *J* = 3.2 Hz, 1H), 5.23 (s, 2H), 2.91-2.86 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 142.7, 142.2, 135.6, 130.2, 129.5, 128.9, 128.2, 127.7, 127.6, 127.5, 126.6, 125.8, 121.0, 120.3, 118.9, 118.3, 117.9, 117.8, 110.1, 100.6, 48.6, 34.9, 34.7; LRMS (EI) m/z 324 $[M]^{+}$; HRMS (EI) calcd for CHNO Na^{+} $[M]^{+}$ 324.16265, found 324.16224. Anal. Calcd for $C_{23}H_{20}N_2$ · $0.1H_2O$: C, 84.68; H, 6.24; N, 8.59; found: C, 84.81; H, 6.38; N, 8.30.

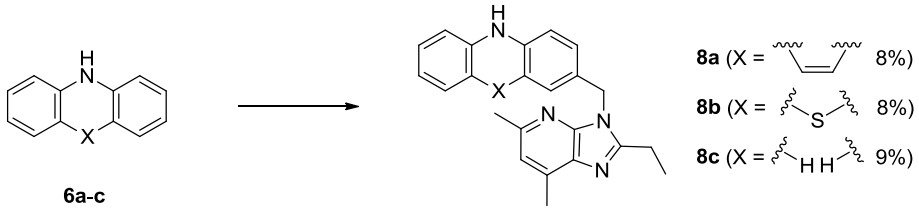
2-((9*H*-Carbazol-9-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (5e) ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (s, 1H) 8.16 (d, *J* = 7.6 Hz, 2H), 7.65 (d, *J* = 8.2 Hz, 2H), 7.43 (dd, *J* = 8.2, 7.4 Hz, 2H), 7.19 (dd, *J* = 7.6, 7.4 Hz, 2H), 7.00-6.84 (m, 6H), 6.61 (dd, *J* = 7.6, 7.2 Hz, 1H), 5.48 (s, 2H), 2.85 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 142.7, 142.1, 140.1, 130.2, 129.1, 127.6, 127.5, 127.3, 126.5, 125.7, 125.4, 122.1, 120.3, 118.8, 118.3, 118.0, 117.8, 109.6, 45.2, 35.0, 34.7; LRMS (EI) m/z 374 $[M]^{+}$; HRMS (EI) calcd for CHNO Na^{+} $[M]^{+}$ 374.17830, found 374.17820. Anal. Calcd for $C_{27}H_{22}O_2$: C, 86.60; H, 5.92; N, 7.48; found: C, 86.36; H, 6.09; N, 7.23.

2-((2-Ethyl-1*H*-imidazol-1-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (5f) ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (s, 1H) 7.05-6.90 (m, 5H), 6.82 (m, 2H), 6.74 (s, 1H), 6.64 (t, *J* = 7.4 Hz, 1H), 4.95 (s, 2H), 2.90 (m, 4H), 2.58 (q, *J* = 7.6 Hz, 2H), 1.12 (t, *J* = 7.6 Hz, 3H),; ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.2, 142.7, 142.3, 130.3, 129.4, 127.7, 127.6, 127.0, 126.6, 125.7, 120.0, 118.4,

118.1, 117.9, 47.9, 34.9, 34.8, 19.5, 12.1; LRMS (ESI) m/z 326 $[M+Na]^+$; Anal. Calcd for $C_{20}H_{21}N_3 \cdot 0.4H_2O$: C, 77.34; H, 7.07; N, 13.53; found: C, 77.17; H, 7.08; N, 13.40.

2-((2-Methyl-1*H*-imidazol-1-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (5g) ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32 (s, 1H) 7.06-6.90 (m, 5H), 6.84 (m, 2H), 6.71 (s, 1H), 6.64 (t, *J* = 6.8 Hz, 1H), 4.93 (s, 2H), 2.91 (m, 4H), 2.24 (s, 3H),; ^{13}C NMR (100 MHz, DMSO-d_6) δ 143.7, 142.7, 142.3, 130.3, 129.5, 127.8, 127.6, 126.9, 126.6, 125.8, 120.1, 118.4, 118.1, 117.9, 48.2, 35.0, 34.8, 12.9; LRMS (ESI) m/z 312 $[M+Na]^+$; Anal. Calcd for $C_{19}H_{19}N_3 \cdot 0.4H_2O$: C, 76.95; H, 6.73; N, 14.17; found: C, 76.93; H, 6.75; N, 14.25.

2-((1*H*-Imidazol-1-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (5h) ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.69 (s, 1H), 7.14 (s, 1H), 7.00-6.86 (m, 7H), 6.64 (t, *J* = 7.0 Hz, 1H), 4.99 (s, 2H), 2.91 (m, 4H),; ^{13}C NMR (125 MHz, DMSO-d_6) δ 142.6, 142.5, 137.1, 130.2, 129.9, 128.5, 127.8, 127.5, 127.1, 126.6, 126.2, 119.3, 118.4, 118.0, 117.9, 49.1, 34.9, 34.7; LRMS (ESI) m/z 298 $[M+Na]^+$; Anal. Calcd for $C_{18}H_{17}N_3 \cdot 0.2H_2O$: C, 77.50; H, 6.29; N, 15.06; found: C, 77.68; H, 6.35; N, 14.80.



To a stirred solution of phenothiazine (1.0 eq.) in $CHCl_3$/AcOH (0.1-0.15 M, 1/1) was added piperidine (3.0 eq.) and 37% HCHO aq. (1.2 eq.) at room temperature. The mixture was stirred at 60 °C for 18-20 hr, evaporated, quenched by addition of 2 M NaOH aq. and extracted with $CHCl_3$. The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (MeOH- $CHCl_3$) to give amine.

To a stirred solution of amine (1.0 eq.) in $CHCl_3$ (0.25-0.38 M) was added MeI (1.3 eq.) at room temperature. The mixture was stirred at 40 °C overnight and concentrated to give crude ammonium.

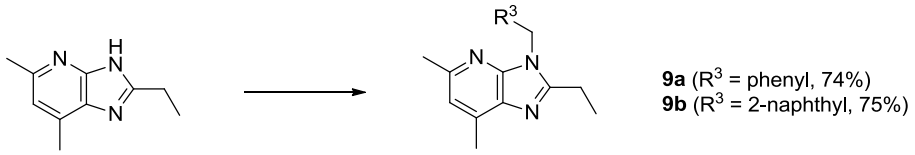
To a stirred solution of **2** (1.0 eq.) in DMF (0.12-0.13 M) was added $LiOH \cdot H_2O$ (1.5 eq.) at room temperature. The mixture was stirred at the same temperature for 20 min. The reaction mixture was added to ammonium (1.2 eq.) in DMF via cannula and stirred at 40 °C for 21-27 hr, quenched by addition of H_2O and extracted with AcOEt. The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (AcOEt - hexane) to give **8a-c**.

2-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-5*H*-dibenzo[*b*,*f*]azepine (8a) ^{1}H NMR (400 MHz, CDCl3) δ 6.96-6.92 (m, 1H), 6.81 (s, 1H), 6.75 (m, 2H), 6.67 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.55 (d, *J* = 1.6 Hz, 1H), 6.38 (d, *J* = 8.4 Hz, 1H), 6.31 (d, *J* = 8.0 Hz, 1H), 6.19 (d, *J* = 12.0 Hz, 1H), 6.09 (d, *J* = 12.0 Hz, 1H), 2.69 (q, *J* = 7.6 Hz, 2H), 2.55 (s, 3H), 2.51 (s, 3H), 1.24 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.7, 152.1, 148.1, 147.8, 147.5, 137.8, 132.5, 132.2, 131.6, 131.2, 130.6, 129.9, 129.5, 129.4, 128.9, 127.7, 122.9, 119.5, 119.2, 119.1, 44.3, 24.2, 21.5, 16.2, 11.8; LRMS (ESI) m/z 403 $[M+Na]^+$; Optical purity: 93.2% (Column: YMC-pack SIL 4.6 x 150 mm, eluent: $CHCl_3$: MeOH = 100:0 to 98:2 for 20 min, 20 ℃, 254 nm; retention time: 16.1 min).

3-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-10*H*-phenothiazine (8b) ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H) 6.95 (ddt, *J* = 7.6, 7.6, 1.2 Hz, 1H), 6.92 (s, 1H), 6.85 (m, 1H), 6.75-6.70 (m, 3H), 6.64 (d, *J* = 7.6 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 5.23 (s, 2H), 2.75 (q, *J* = 7.2 Hz, 2H), 2.49 (s, 3H), 2.48 (s, 3H), 1.21 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.3, 151.2, 147.2, 141.8, 141.3, 137.0, 131.7, 130.7, 127.6, 126.2, 126.1, 124.7, 121.8, 118.4, 116.6, 115.8, 114.5, 114.4, 43.5, 23.9, 20.6, 15.8, 11.1; LRMS (EI) m/z 386 $[M]^+$; HRMS (EI) calcd for CHNO Na^+ $[M]^+$ 386.15652, found 386.15621. Optical purity: 95.5% (Column: YMC-pack SIL 4.6 x 150 mm, eluent: $CHCl_3$: MeOH = 100:0 to 98:2 for 20 min, 20 ℃, 254 nm; retention time: 23.2 min).

4-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-*N*-phenylaniline (8c) ^{1}H NMR (500 MHz, CDCl3) δ 7.25-7.23 (m, 2H) 7.04 (t, *J* = 8.5 Hz, 4H), 6.97 (d, *J* = 8.5 Hz, 2H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.89 (s, 1H), 5.68 (s, 1H), 5.39 (s, 2H), 2.82 (q, *J* = 7.5 Hz, 2H), 2.63 (s, 3H), 2.60 (s, 3H), 1.32 (t, *J* = 7.5 Hz, 3H),; ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.8, 152.1, 147.6, 142.8, 142.6,

137.8, 132.2, 129.3, 128.9, 127.9, 121.0, 119.1, 117.7, 117.6, 44.8, 24.2, 21.6, 16.2, 11.8; LRMS (ESI) m/z 379 $[M+Na]^+$; Optical purity: 98.7% (Column: YMC-pack SIL 4.6 x 150 mm, eluent: $CHCl_3$: MeOH = 100:0 to 98:2 for 20 min, 20 ℃, 254 nm; retention time: 18.3 min).



7 **9a, b**

To a stirred solution of **7** (1.0 eq.) and R^3CH_2Br (1.2 eq.) in DMF (0.3 M) was added $LiOH \cdot H_2O$ (1.5 eq.) at room temperature. The mixture was stirred at 40 °C overnight and quenched by addition of H_2O and extracted with AcOEt. The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (AcOEt - hexane) to give **9a**,**b**.

3-Benzyl-2-ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridine (9a) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.26 (m, 3H) 7.12 (m, 2H), 6.89 (s, 1H), 5.46 (s, 2H), 2.77 (q, *J* = 7.6 Hz, 2H), 2.64 (s, 3H), 2.59 (s, 3H), 1.29 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 152.0, 147.6, 137.7, 136.7, 132.1, 128.6, 127.4, 126.6, 119.0, 45.0, 24.1, 21.4, 16.1, 17.7; LRMS (EI) m/z 265 $[M]^+$; HRMS (EI) calcd for $C_{17}H_{19}N_3$ $[M]^+$ 265.1579, found 265.1574. Anal. Calcd for $C_{17}H_{19}N_3$: C, 76.95; H, 7.22; N, 15.84; found: C, 76.74; H, 7.29; N, 15.67.

2-Ethyl-5,7-dimethyl-3-(naphthalen-2-ylmethyl)-3*H*-imidazo[4,5-*b*]pyridine (9b) ^{1}H NMR (400 MHz, CDCl3) δ 7.81-7.71 (m, 3H) 7.48-7.43 (m, 3H) 7.29-7.26 (m, 1H) 6.92 (s, 1H), 5.46 (s, 2H), 2.80 (q, *J* = 7.6 Hz, 2H), 2.66 (s, 3H), 2.60 (s, 3H), 1.29 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 155.9, 152.2, 147.7, 137.9, 134.3, 133.2, 132.7, 132.3, 128.7, 127.72, 127.66, 126.3, 126.0, 125.2, 124.8, 119.2, 45.3, 24.3, 21.6, 16.3, 11.8; LRMS (EI) m/z 315 $[M]^+$; HRMS (EI) calcd for $C_{21}H_{21}N_3$ $[M]^+$ 315.1736, found 315.1743. Anal. Calcd for $C_{21}H_{21}N_3$: C, 79.97; H, 6.71; N, 13.32; found: C, 80.10; H, 6.78; N, 13.30.

2. Biological assay

Luciferase assay. HEK293 cells were plated (1 x 10^5 cells/well) on 24-well plates coated with poly-L lysine and cultured overnight. CRE-Luc and NFAT-Luc (50 ng/well) together with pRL-TK (10 ng/well) and pcDNA3 encoding pH-sensing GPCRs tagged with HA at their N-termini (HA-GPR4, HA-TDAG8, and HA-OGR1, 10 ng/well) were transfected into these cells using X-tremeGENE 9 (Roche Diagnostics) according to the manufactural protocol. Twelve hours after transfection, cells were starved with DMEM without FBS buffered with 25 mM HEPES (pH 7.7) for 16 hours. Then, the medium was changed to DMEM without FBS buffered with 25 mM HEPES (pH 7.2) containing a compound adjusted to appropriate concentration and the cells were incubated for 6 hours. Medium (pH 7.2 and pH 7.7) without compound was used as a positive control and negative control, respectively. The cells were lysed with passive lysis buffer (Promega) and luciferase activity was analyzed using Dual-Luciferase Reporter Assay System (Promega). The activity of firefly luciferase (CRE-Luc and NFAT-Luc) was normalized to the *Renilla* luciferase activity (pRL-TK). Responses to medium (pH 7.2 and pH 7.7) without compound were taken as 100% and 0%, respectively, and those to medium (pH 7.2) containing a compound were calculated by application of the following formula:

$$response(\%) = \frac{response(compound) - response(pH7.7)}{response(pH7.2) - response(pH7.7)} \times 100$$

Sigmoid curves were fitted to data and IC_{50} of each compound were calculated using GraphPad Prism 5.

Surgical procedures of MI operation. We purchased C57BL/6J mice from Charles River Japan. Male mice (8-10 weeks old) were subjected to permanent left anterior descending coronary artery ligation in anesthetized condition (50 mg/kg sodium pentobarbital). Vehicle (a cocktail of 10% ethanol, 40% polyethylene glycol 300, and 50% H_2O) and compound **3b** (6.7 or 20 mg/kg/day) were intraperitoneally injected to MI-operated mice twice a day from 1 day before to 7 day after MI without the operation day. All animal experiments were approved by Animal Care and Use Committee, Kyushu University.