Supporting Information

Identification of an Orally Efficacious GPR40/ FFAR1 Receptor Agonist

Sameer Agarwal*, Santosh Sasane, Prashant Deshmukh, Bhadresh Rami, Debdutta Bandyopadhyay, Poonam Giri, Suresh Giri, Mukul Jain, and Ranjit C. Desai*

Zydus Research Centre, Cadila Healthcare Ltd. Sarkhej-Bavla N.H. No. 8 A, Moraiya, Ahmedabad – 382 210, India

Contents

Synthesis

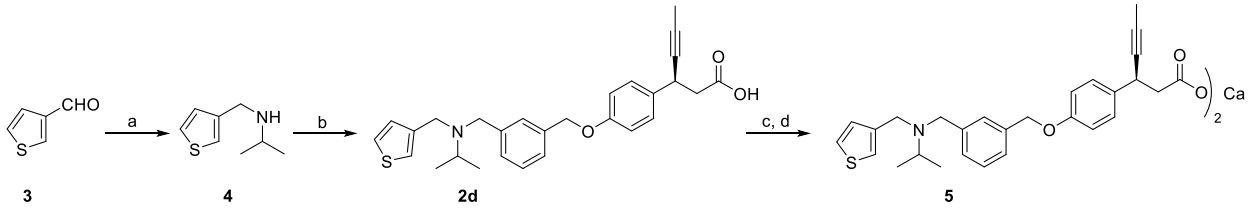
General procedures

Melting points were recorded on a scientific melting point apparatus and are uncorrected. IR spectra were recorded as neat (for oils) or on KBr pellet (for solid) on FT-IR 8300 Shimadzu and are reported in wavenumbers *v* (cm^{-1}). NMR spectra were measured on a Varian Unity 400 (^1H at 400 MHz, ^{13}C at 100 MHz), magnetic resonance spectrometer. Spectra were taken in the indicated solvent at ambient temperature. Chemical shifts (δ) are given in parts per million (ppm) with tetramethylsilane as an internal standard. Multiplicities are recorded as follows: s = singlet, d = doublet, t = triplet, q = quartet, br = broad. Coupling constants (*J* values) are given in Hz. Mass spectra are recorded on Perkin-Elmer Sciex API 3000. ESI-Q-TOF-MS measurements were performed with a micrOTOF-Q II (Bruker Daltonics) mass spectrometer. HPLC analysis were carried out at λ_{max} 220 nm using column ODS C-18, 150 mm x 4.6 mm x 4 µm on AGILENT 1100 series. Reactions were monitored using thin layer silica gel chromatography (TLC) using 0.25 mm silica gel 60F plates from Merck. Plates were visualized by treatment with UV, acidic p-anisaldehyde stain, $KMnO_4$ stain with gentle heating. Products were purified by column chromatography using silica gel 100-200 mesh and the solvent systems indicated.

All reactions involving air or moisture sensitive compounds were performed under nitrogen atmosphere in flame dried glassware. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were freshly distilled from sodium/ benzophenone under nitrogen atmosphere. Other solvents used for reactions were purified according to standard procedures. Starting reagents were purchased from commercial suppliers and used without further purification unless otherwise specified. The purity of compounds **1a**-**1c** and **2a**–**2t** was confirmed as over 95% by HPLC.

Experimental procedures

Synthesis of compound **5** is depicted in Scheme 1a. The reductive amination[1] of commercially available 3-thiophene-aldehyde (**3**) and isopropyl amine using sodium triacetoxyborohydride resulted in secondary amine intermediate **4**. Compound **4** on further reductive amination under similar conditions with aldehyde intermediate, (*S*)-3-(4-((3-formylbenzyl)oxy)phenyl)hex-4-ynoic acid (**8**), afforded **2d** in high yields. The aldehyde intermediate, **8** was obtained from (*S*)-3-(4-hydroxyphenyl)hex-4-ynoic acid (**6**) as shown in Scheme 1b. Acid **6** was synthesized via 5-step reported procedure using commercially available 4-hydroxybenzaldehyde and Meldrum's acid.[2] Resolution of racemic acid **6** was accomplished *via* diastereomeric salt formation with (1*S*,2*R*)-1-amino-2-indanol followed by salt break with aqueous acid to furnish compound **6**. Treatment of **6** with of 40% aqueous tetrabutylphosphonium hydroxide (n-Bu_4POH) in THF, followed by addition of 3-formyl benzyl bromide (**7**), afforded aldehyde intermediate **8**. Compound **2d** was further converted to its corresponding calcium salt (**5**) in two-step sequence with excellent chemical purity.



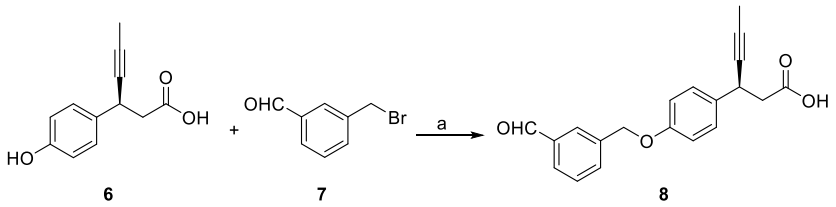
Scheme 1a. Synthesis of Compounds **2d** and **5**. *Reagent and Conditions:* (a) $CH(CH_3)_2NH_2$, $NaB(OAc)_3H$, CH_3COOH, dry THF, 0 °C to r.t., 16 h; (b) Comp **8**, $NaB(OAc)_3H$, CH_3COOH, dry THF, 0 °C to r.t., 16 h; (c) NaOH, $MeCN/H_2O$, r.t., 3 h; (d) $CaCl_2$, $MeOH/H_2O$, r.t., 16 h.

N-(thiophen-3-ylmethyl)propan-2-amine (**4**): To a stirred solution of thiophene-3-carbaldehyde (1.5 g, 13.37 mmol) in THF (10 mL), acetic acid (2.3 mL, 40.1 mmol) and solution of isopropylamine (2.3 mL, 26.7 mmol) in THF (5 mL) were added at 25 °C and the reaction mixture was further stirred for 2 h at 25 °C. After that sodium triacetoxyborohydride (7.09 g, 33.4 mmol) was added slowly at 25 °C under nitrogen gas atmosphere. Resulted mixture was then stirred for 24 h at ambient temperature. Reaction was monitored by TLC (Reaction mixture + water + EtOAc, TLC was spotted from organic layer). Excess THF was removed under reduced pressure and the residue was acidified with dil. HCl. Aqueous layer was washed with EtOAc (50 mL x 3). Aqueous layer was collected and basified with 10% $NaHCO_3$ solution. Product was extracted with EtOAc (50 mL x 3). All organic layers were mixed together brine (25 mL). Organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure at (40°C) to obtained, N-(thiophen-3-ylmethyl)propan-2-amine (2.0 g, 12.88 mmol, 96% yield) as Light yellow oil. % Yield = 96%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.44 – 7.42 (m, 1H), 7.26 – 7.25 (m, 1H), 7.08 – 7.06 (m, 1H), 3.67 (s, 2H), 2.71 (sept, *J* = 6.0 Hz, 1H), 0.98 (d, *J* = 6.0 Hz, 6H); IR ($CHCl_3$): ν = 3018, 2968, 2933, 2870, 2833, 1467, 1383, 1215, 1174, 758 cm^{-1}; MS (ESI): *m/z* (%) = 196.55 (100%) $(M+MeCN)^+$.

(S)-3-(4-((3-((isopropyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2d**). To a stirred solution of (S)-3-(4-((3-formylbenzyl)oxy)phenyl)hex-4-ynoic acid (2.5 g, 7.76 mmol) in THF (15 mL), acetic acid (1.3 mL, 23.27 mmol) and solution of N-(thiophen-3-ylmethyl)propan-2-amine (1.806 g, 11.63 mmol) in THF (10 mL) were added at 25 °C and the reaction mixture was further stirred for 2h at 25 °C. After that sodium triacetoxyborohydride (4.11 g, 19.39 mmol) was added slowly at 25 °C under nitrogen gas atmosphere. Resulted mixture was then stirred for 48 h at 25 °C. Reaction was monitored

by TLC (Reaction mixture + water + EtOAc, TLC was spotted from organic layer). Excess THF was removed under reduced pressure and the residue was diluted with EtOAc (50 mL) & water (20 mL). Organic layer was separated and aqueous layer was extracted with EtOAc (50 mL x 2). Both organic layers were mixed together & washed with water (3 x 15 mL), brine (1 x 15 mL). Organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure at 40 °C to afford crude (S)-3-(4-((3-((isopropyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (3 g). Crude product was purified by preparative HPLC. Fractions were concentrated under reduced pressure and basified with $NaHCO_3$, and then product was extracted with EtOAc (100 mL x 3). Organic layer was washed with NH_4Cl solution (50 mL), water (50 mL), and brine (50 mL), organic layer dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure at 40°C to afford (S)-3-(4-((3-((isopropyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (1.557 g, 3.34 mmol, 43.0 % yield) as wax solid. ^{1}H NMR (400 MHz, DMSO-*d6*): δ = 12.35 (br s, 1H), 7.44 (q, *J* = 3.2 Hz, 2H), 7.32 – 7.24 (m, 6H), 7.04 (d, *J* = 4.8 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 5.06 (s, 2H), 3.93 (d, *J* = 2.4 Hz, 1H), 3.51 (d, *J* = 8.8 Hz, 4H), 2.84 (sept, *J* = 6.4 Hz, 1H), 2.57 (d, *J* = 8 Hz, 2H), 1.77 (d, *J* = 2.4 Hz, 3H), 1.01 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR and DEPT: DMSO-*d*6, 100MHz):- δ = 172.35 (C), 157.63 (C), 142.13 (C), 141.44 (C), 137.42 (C), 133.93 (C), 128.73 (CH), 128.64 (CH), 128.43 (CH), 127.99 (CH), 127.73 (CH), 126.28 (CH), 122.21 (CH), 115.10 (CH), 81.16 (C), 78.52 (C), 69.69 (CH_2), 52.90 (CH_2), 48.64 (CH), 48.49 (CH_2), 43.44 (CH_2), 33.15 (CH), 17.92 (CH_3), 3.66 (CH_3); MS (EI): *m/z* (%) = 462.35 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 2960, 2918, 2810, 1712, 1608, 1510, 1383, 1240, 1174, 1109, 1018 cm^{-1}.

Calcium (S)-3-(4-((3-((isopropyl(thiophen-3-ylmethyl)amino)methyl) benzyl)oxy) phenyl) hex-4-ynoate (**5**): Part I: (S)-3-(4-((3-((isopropyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (1.54 g, 3.34 mmol) was taken in a mixture of MeOH (3.85 mL) & THF (3.85 mL), it was cooled to 0 °C and added aq. 5M sodium hydroxide (0.667 ml, 3.34 mmol) solution dropwise. After the addition, mixture was allowed to warm to 25 °C & stirred for 2 h. Reaction mixture was concentrated under reduced pressure at 40 °C. Part II: The residue was diluted with MeOH (3.85 mL) followed by solution of calcium chloride dihydrate (0.368 g, 2.502 mmol) in Water (3.85 mL) was added drop wise. Resulted reaction mixture was then stirred for 15 h at 25 °C. Decanted the solvent, Residue was diluted with water (4 mL). Mixture was triturated and stirred for 60 minutes, water was decanted, this process was repeated for 4 more times, finally slurry was stirred with hexane (10 mL), filtered it, washed with hexane and dried under reduced pressure at 40 °C to obtained, calcium (S)-3-(4-((3-((isopropyl(thiophen-3-yl methyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoate (1.51 g, 1.536 mmol, 46% yield) as white powder. mp: 124.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.43 – 7.42 (m, 2H), 7.28 – 7.24 (m, 6H), 7.04 (d, *J* = 4.4 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 2H), 5.02 (s, 2H), 4.02 (s, 1H), 3.50 (d, *J* = 7.2 Hz, 4H), 2.84 – 2.77 (sept, *J* = 6.4 Hz, 1H), 2.43 (dd, J_1 = 6.8 Hz, J_2 = 7.2 Hz, 1H), 2.28 (dd, J_1 = 6.8 Hz, J_2 = 7.2 Hz, 1H), 1.73 (s, 3H), 0.99 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR and DEPT (100 MHz, DMSO-d_6): δ = 177.78 (C), 157.23 (C), 142.11 (C), 141.4 (C), 137.46 (C), 135.81 (C), 128.83 (CH), 128.62 (CH), 128.40 (CH), 127.94 (CH), 127.69 (CH), 126.26 (CH), 122.18 (CH), 114.77 (CH), 83.18 (C), 77.32 (C), 69.66 (CH_2), 52.89 (CH_2), 48.59 (CH), 48.48 (CH_2), 46.86 (CH_2), 33.52 (CH), 17.88 (CH_3), 3.78 (CH_3); MS (EI): *m/z* (%) = 462.05 (100) $(M+H)^+$; ESI-Q-TOF-MS: *m/z* $[M+H]^+$ calcd for $[C_{28}H_{31}NO_3S + H]^+$: 462.6280; found: 462.4988; IR (KBr): *v* = 3435, 2960, 2918, 2868, 2818, 1608, 1550, 1508, 1440, 1383, 1359, 1240 cm^{-1}; HPLC (% Purity) = 99.38%; Calcium Content ($C_{56}H_{60}CaN_2O_6S_2$) Calcd.: 4.17%. Found: 3.99%.



Scheme 1b. Synthesis of (*S*)-3-(4-((3-formylbenzyl)oxy)phenyl)hex-4-ynoic acid (**8**). *Reagent and Conditions:* (a) nBu_4POH, THF/H_2O, 0 °C to r.t., 16 h.

(*S*)-3-(4-((3-formylbenzyl)oxy)phenyl)hex-4-ynoic acid (**8**): To a solution of (S)-3-(4-hydroxyphenyl)hex-4-ynoic acid (70 g, 343 mmol) (**6**) in THF (490 mL) was added drop wise tetrabutyl(hydroxy)phosphorane (489 mL, 699 mmol) at -5 °C, followed by drop wise addition of a solution of 3-bromomethyl) benzaldehyde (68.22 g, 343 mmol) in THF (210 mL). Reaction mixture was stirred at -5 °C to 0 °C for 30 min, and further for 16 h at ambient temperature. THF was evaporated under reduced pressure. The mixture was extracted with MTBE (5 x 800 mL), aqueous layer was acidified with dil. HCl at 0-5°C, and again extracted with MTBE (300 mL). The organic layers were combined, washed with saturated brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to furnish **8** as light yellow oil (108 g, 335 mmol, 98% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.50 (br s, 1H), 10.03 (s, 1H), 7.97 (s, 1H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.77 (d, *J* = 7.6 Hz, 2H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 5.19 (s, 2H), 3.95 – 3.92 (m, 1H), 2.58 (d, *J* = 7.6 Hz, 2H), 1.77 (s, 3H); MS (EI): *m/z* (%) = 321.00 (100%) $(M+1)^+$; IR (KBr): *v* = 3020, 2922, 1707, 1581, 1512 cm^{-1}.

Spectral Data of Novel NCEs:[3]

(S)-3-(4-((3-(((pyridin-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**1a**). solid; mp: 81 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 8.56 (s, 1H), 8.43 (d, *J* = 3.6 Hz, 1H), 7.75 (d, *J* = 7.6 Hz, 1H), 7.41 (s, 1H), 7.35 – 7.25 (m, 6H), 6.95 (d, *J* = 8.8 Hz, 2H), 5.05 (s, 2H), 3.95 – 3.91 (m, 1H), 3.68 (s, 4H), 2.58 (dd, J_1 = 8 Hz, J_2 = 16.4 Hz, 2H), 1.77 (d, *J* = 2.4 Hz, 3H); MS (EI): *m/z* (%) = 415.35 (100) $(M+H)^+$; IR (KBr): *v* = 3435, 3034, 2918, 2854, 1724, 1512, 1402 cm^{-1}.

(S)-3-(4-((3-(((thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**1b**). solid; mp: 159.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 7.47 – 7.45 (m, 2H), 7.41 (s, 1H), 7.34 – 7.25 (m, 6H), 7.09 – 7.08 (m, 1H), 6.95 – 6.93 (m, 2H), 5.06 (s, 2H), 3.96 – 3.92 (m, 1H), 3.57 (d, *J* = 8.8 Hz, 2H), 3.54 (d, *J* = 6.4, Hz, 2H), 2.61 – 2.55 (m, 2H), 1.77 (d, *J* = 2.4 Hz, 3H); MS (EI): *m/z* (%) = 419.95(100) $(M)^+$; IR (KBr): *v* = 3431, 3093, 2914, 2850, 1581, 1512, 1452, 1400, 1284, 1238. 1178 cm^{-1}.

(S)-3-(4-((3-((methyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**1c**). solid; mp: 133 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 7.49 – 7.47 (m, 1H), 7.40 (s, 1H), 7.35 – 7.31 (m, 3H), 7.29 – 7.24 (m, 3H), 7.07– 7.06 (m, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 5.07 (s, 2H), 4.03 – 4.02 (m, 1H), 3.50 (s, 2H), 3.47 (s, 2H), 2.07 (s, 3H), 1.77 (d, *J* = 2.4 Hz, 3H); MS (EI): *m/z* (%) = 434.0 (100) $(M)^+$; IR (KBr): *v* = 3433, 2924, 1722, 1612, 1510, 1327, 1246, 1176, 1128 cm^{-1}.

(S)-3-(4-((3-(((thiophen-3-ylmethyl)(2,2,2-trifluoroethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2a**). Wax solid; ^{1}H NMR (DMSO-d_6, 400MHz): 12.2 (br s, 1H), 7.51 – 7.49 (m, 1H), 7.41 (s, 1H), 7.38 – 7.27 (m, 6H), 7.05 – 7.04 (m, 1H), 6.95 (t, *J*= 2 Hz, 2H), 5.08 (s, 2H), 3.93 (br s, 1H), 3.76 (s, 2H), 3.72 (s,

2H), 3.28 (dd, J_1= 10 Hz, J_2= 10 Hz, 2H), 2.58 (d, J= 8 Hz, 2H), 1.76 (s, 3H); MS (EI): *m/z* (%) = 502.25 (100) $(M+H)^+$; IR (KBr): *v* = 3018, 2924, 2856, 1710, 1608, 1510, 1508, 1305, 1271, 1215, 1178, 1145 cm^{-1}.

5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-2-((2-phenoxyethyl)thio)-1H-imidazole (**2b**). solid; mp: 58.2 °C; ^{1}H NMR (DMSO-d_6, 400MHz): 12.30 (br s, 1H), 7.46 - 7.45 (m, 1H), 7.43 (s, 1H), 7.35 - 7.29 (m, 4H), 7.26 (d, J = 8.8Hz, 2H), 7.07 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 5.06 (s, 2H), 3.95 - 3.91 (m, 1H), 3.58 (s, 4H), 3.48 (t, J = 6.4 Hz, 2H), 2.61 - 2.54 (m, 2H), 2.48 - 2.45 (m, 2H), 1.77 (d, J = 2.4 Hz, 3H); MS (EI): *m/z* (%) = 464.25 (100) $(M+H)^+$; IR (KBr): *v* = 3419, 2916, 1724, 1608, 1583, 1442, 1408 cm^{-1}.

4-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)benzo nitrile (**2c**). Wax solid; ^{1}H NMR (DMSO-d_6, 400MHz): 12.24 (s, 1H), 7.48 - 7.46 (m, 1H), 7.42 (br s, 1H), 7.31 - 7.25 (m, 6H), 7.06 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 5.07 (s, 2H), 3.96 – 3.93 (m, 1H), 3.58 (s, 4H), 3.40 (t, J = 6.4 Hz, 2H), 3.17 (s, 3H), 2.59 - 2.51 (m, 4H), 1.76 (d, J = 2.4 Hz, 3H); MS (EI): *m/z* (%) = 478.30 (100) $(M+H)^+$; IR (KBr): *v* = 2924, 1708, 1608, 1510, 1178 cm^{-1}.

(S)-3-(4-((3-((isopropyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2d**). Wax solid. ^{1}H NMR (400 MHz, DMSO-*d6*): δ = 12.35 (br s, 1H), 7.44 (q, J = 3.2 Hz, 2H), 7.32 – 7.24 (m, 6H), 7.04 (d, J = 4.8 Hz, 1H), 6.94 (d, J = 8.4 Hz, 2H), 5.06 (s, 2H), 3.93 (d, J = 2.4 Hz, 1H), 3.51 (d, J = 8.8 Hz, 4H), 2.84 (sept, J = 6.4 Hz, 1H), 2.57 (d, J = 8 Hz, 2H), 1.77 (d, J = 2.4 Hz, 3H), 1.01 (d, J = 6.4 Hz, 6H); ^{13}C NMR and DEPT: DMSO-*d*6, 100MHz): δ = 172.35 (C), 157.63 (C), 142.13 (C), 141.44 (C), 137.42 (C), 133.93 (C), 128.73 (CH), 128.64 (CH), 128.43 (CH), 127.99 (CH), 127.73 (CH), 126.28 (CH), 122.21 (CH), 115.10 (CH), 81.16 (C), 78.52 (C), 69.69 (CH_2), 52.90 (CH_2), 48.64 (CH), 48.49 (CH_2), 43.44 (CH_2), 33.15 (CH), 17.92 (CH_3), 3.66 (CH_3); MS (EI): *m/z* (%) = 462.35 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 2960, 2918, 2810, 1712, 1608, 1510, 1383, 1240, 1174, 1109, 1018 cm^{-1}.

(S)-3-(4-((3-((isobutyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2e**). Wax solid; ^{1}H NMR (DMSO-d_6, 400MHz): 12.3 (br s, 1H), 7.47 (q, J = 3.2 Hz, 2H), 7.42 (s, 1H), 7.34 – 7.24 (m, 6H), 7.04 (dd, J = 1.2 Hz, 4.8 Hz, 1H), 6.94 (d, J = 8.4 Hz, 2H), 5.07 (s, 2H), 3.95 – 3.91 (m, 1H), 3.49 (s, 4H), 2.57 (d, J = 8.0 Hz, 2H), 2.08 (d, J = 7.6 Hz, 2H), 1.85 – 1.79 (m, 1H), 1.76 (d, J = 2.4 Hz, 3H), 0.8 (d, J = 6.4 Hz, 6H); MS (EI): *m/z* (%) = 476.05 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 2947, 2920, 2870, 2796, 1722, 1610, 1510, 1440, 1301, 1238, 1020 cm^{-1}.

(S)-3-(4-((3-((cyclopropyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2f**). wax solid; ^{1}H NMR (DMSO-d_6, 400MHz): 12.2 (s, 1H), 7.47 – 7.45 (m, 1H), 7.34 - 7.20 (m, 7H), 6.99 - 6.98 (m, 1H) 6.96 - 6.92 (d, J = 8.8 Hz, 2H), 5.08 (s, 2H), 3.95 – 3.91 (m, 1H), 3.60 (d, J = 6.8 Hz, 4H), 2.58 – 2.54 (m, 2H), 1.77 – 1.75 (m, 4H), 0.39 – 0.32 (m, 2H), 0.25 - 0.21 (m, 2H); MS (EI): *m/z* (%) = 459.95 (100) $(M)^+$; IR (KBr): *v* = 3433, 2918, 2852, 1722, 1608, 1583, 1510, 1240, 1018 cm^{-1}.

(S)-3-(4-((3-(((cyclopropylmethyl)(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2g**). solid; mp: 68.8 °C; ^{1}H NMR (DMSO-d_6, 400MHz): 12.3 (br s, 1H), 7.47 – 7.45 (m, 1H), 7.44 (s, 1H), 7.34 – 7.29 (m, 4H), 7.27 (d, J = 8.4 Hz, 2H), 7.07 (q, J = 4.8 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 5.07 (s, 2H), 3.95 – 3.91 (m, 1H), 3.62 (d, J = 7.2 Hz, 4H), 2.58 (d, J = 7.6 Hz, 2H), 2.25 (d, J = 6.4 Hz, 2H), 1.76 (d, J = 2.4 Hz, 3H), 0.92 – 0.85 (m, 1H), 0.43 - 0.42 (m, 2H), 0.00 – 0.02 (m, 2H); MS (EI): *m/z* (%) = 474.05 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 2916, 2852, 2800, 1710, 1608, 1510, 1240 cm^{-1}.

(S)-3-(4-((3-((cyclopentyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2h**). Wax solid; ^{1}H NMR (DMSO-d_6, 400MHz): 12.3 (s, 1H), 7.44 – 7.42 (m, 2H), 7.32 – 7.24 (m, 6H), 7.02 –

7.01 (m, 1H), 6.94 – 6.93 (m, 2H), 5.06 (s, 2H), 3.95 – 3.91 (m, 1H), 3.59 (s, 4H), 3.08 – 2.99 (m, 1H), 2.67 (d, *J* = 2 Hz, 2H), 1.77 (d, *J* = 2.4 Hz, 3H), 1.6 (m, 2H), 1.5 (m, 2H), 1.4 (m, 4H); MS (EI): *m/z* (%) = 488.10 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 2951, 2916, 2866, 1712, 1608, 1583, 1510, 1442, 1240, 1176, 1109 cm^{-1}.

(S)-3-(4-((3-((cyclohexyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2i**). Wax solid; ^1H NMR (DMSO-d_6, 400MHz): 12.2 (s, 1H), 7.43 – 7.42 (m, 2H), 7.30 – 7.24 (m, 6H), 7.02 – 7.01 (m, 1H), 6.94 (d, *J* = 6 Hz, 2H), 5.06 (s, 2H), 3.95 – 3.91 (m, 1H), 3.58 – 3.56 (m, 4H), 2.58 (d, *J* = 8 Hz, 2H), 2.45 – 2.31 (m, 1H), 1.77 – 1.76 (m, 4H), 1.71 – 1.70 (m, 2H), 1.53 (s, 1H), 1.31 – 1.30 (m, 7H); MS (EI): *m/z* (%) = 502.15 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 2924, 2852, 1722, 1612, 1510, 1444, 1249 cm^{-1}.

(S)-3-(4-((3-(((tetrahydro-2H-pyran-4-yl)(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2j**). White powder; mp: 84.7 °C; ^1H NMR (DMSO-d_6, 400MHz): 7.44 (d, *J* = 4.8 Hz, 2H), 7.32 – 7.24 (m, 6H), 7.03 (d, *J* = 4.4 Hz, 1H,), 6.93 (d, *J* = 8.8 Hz, 2H), 5.06 (s, 2H), 3.93 – 3.91 (m, 1H), 3.87 – 3.83 (m, 2H), 3.61 (d, *J* = 8.0 Hz, 4H), 3.15 – 3.08 (m, 3H), 2.61 – 2.56 (m, 2H), 1.77 (d, *J* = 2.0 Hz, 3H), 1.65 – 1.53 (m, 4H); MS (EI): *m/z* (%) = 504.35 (100) $(M+H)^+$; IR (KBr): *v* = 3429, 2943, 2918, 2848, 1726, 1608, 1510, 1442, 1383, 1240 cm^{-1}.

(S)-3-(4-((3-(((tetrahydro-2H-thiopyran-4-yl)(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2k**). White powder; mp: 74.1 °C; ^1H NMR (DMSO-d_6, 400MHz): 12.2 (s, 1H), 7.43 – 7.42 (m, 2H), 7.32 - 7.24 (m, 6H), 7.02 – 7.0 (m, 1H), 6.93 (d, *J* = 8.8 Hz, 2H), 5.06 (s, 2H), 3.95 – 3.90 (m, 1H), 3.60 (d, *J* = 9.6 Hz, 4H), 2.66 – 2.62 (m, 2H), 2.58 – 2.50 (m, 4H), 2.4 – 2.34 (m, 1H), 2.10 (d, *J* = 11.2 Hz, 3H), 1.76 (d, *J* = 2.4 Hz, 2H), 1.6 (m, 2H); MS (EI): *m/z* (%) = 520.25 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 3433, 2916, 2850, 1724, 1608, 1583, 1510, 1429, 1381, 1240, 1219 cm^{-1}.

(S)-3-(4-((3-(((1,1-dioxidotetrahydro-2H-thiopyran-4-yl)(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy) phenyl)hex-4-ynoic acid (**2l**). White powder; mp: 70.9 °C; ^1H NMR (DMSO-d_6, 400MHz): 12.3 (br s, 1H), 7.44 – 7.42 (m, 2H), 7.33 - 7.24 (m, 6H), 7.02 – 7.0 (m, 1H) 6.94 (d, *J* = 8.4 Hz, 2H), 5.06 (s, 2H), 3.95 – 3.91 (m, 1H), 3.60 (d, *J* = 11.6 Hz, 4H), 3.13 – 3.07 (m, 2H), 3.01 – 2.98 (m, 2H), 2.84 – 2.79 (m, 1H), 2.58 – 2.54 (m, 2H), 2.11 – 2.00 (m, 4H), 1.76 (s, 3H); MS (EI): *m/z* (%) = 552.4 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 2928, 2856, 1742, 1608, 1583, 1508, 1442, 1383, 1290, 1240, 1220, 1176, 1128, 1109, 1018, 846 cm^{-1}.

(S)-3-(4-((3-(((1-methylpiperidin-4-yl)(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2m**). Light yellow powder; mp: 65.7 °C; ^1H NMR (DMSO-d_6, 400MHz): 7.44 – 7.40 (m, 1H), 7.37 – 7.37 (d, *J* = 2Hz, 1H), 7.31 – 7.23 (m, 6H), 7.03 – 7.01 (m, 1H), 6.92 (d, *J* = 8.4 Hz, 2H), 5.07 (s, 2H), 3.95 – 3.91 (m, 1H), 3.57 (d, *J* = 4 Hz, 4H), 2.8 (d, *J* = 22 Hz, 2H), 2.57 – 2.54 (m, 2H), 2.38 – 2.32 (m, 1H), 2.11 (s, 3H), 1.77 (d, *J* = 2.4 Hz, 3H), 1.72 (s, 1H), 1.63 (br s, 2H), 1.57 – 1.50 (m, 3H); MS (EI): *m/z* (%) = 517.45 (100) $(M+1)^+$; IR (KBr): *v* = 3435, 2926, 2853, 1726, 1609, 1584, 1508, 1451, 1381, 1304, 1240, 1221, 1177, 1111, 1076, 1020, 833, 783, 694 cm^{-1}.

S)-3-(4-((3-((benzyl(thiophen-3-ylmethyl)amino)methyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**2n**). Wax solid; ^1H NMR (DMSO-d_6, 400MHz): 7.50 - 7.47 (m, 2H), 7.37 – 7.32 (m, 8H), 7.30 – 7.23 (m, 3H), 7.09 – 7.07 (m, 1H), 6.95 (d, *J* = 8.8 Hz, 2H), 5.09 (s, 2H), 3.93 (d, *J* = 2.8 Hz, 1H), 3.50 (d, *J* = 6.0 Hz, 6H), 2.56 – 2.53 (m, 2H) 1.76 (d, *J* = 2.4 Hz, 3H); MS (EI): *m/z* (%) = 510.15 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 2924, 2852, 1712, 1608, 1500, 1381, 1240, 1080, 1018 cm^{-1}.

5-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)-1H-indazole (**2o**). White powder; mp: 61.3 °C; ^1H NMR (DMSO-d_6, 400MHz): 12.15 (br s, 1H), 8.54 (d, *J* = 1.6Hz, 1H), 8.45 (dd, J_1 = 4.8 Hz, J_2 = 1.6 Hz, 1H), 7.74 (d, *J* = 8 Hz, 1H), 7.51 – 7.49 (m, 1H), 7.46 (s, 1H),

7.36 – 7.29 (m, 5H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.09 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 5.09 (s, 2H), 3.95 – 3.91 (m, 1H), 3.52 (s, 6H), 2.62 - 2.49 (m, 2H), 1.76 (s, 3H); MS (EI): *m/z* (%) = 511.15 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 3099, 2850, 1712, 1608, 1583, 1425 cm^{-1}.

5-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)-1H-indazole (**2p**). White powder; mp: 137.4 °C; ^{1}H NMR (DMSO-d_6, 400MHz): 7.50 – 7.48 (m, 2H), 7.46 (s, 1H), 7.35 – 7.30 (m, 5H), 7.26 (d, *J* = 8.8 Hz, 2H), 7.09 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 5.09 (s, 2H), 3.96 – 3.93 (m, 1H), 3.50 (s, 6H), 2.55 - 2.50 (m, 2H), 1.76 (d, *J* = 2.4 Hz, 3H); MS (EI): *m/z* (%) = 516.30 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 3099, 2850, 1716, 1608, 1583, 1408 cm^{-1}.

5-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)-1H-indazole (**2q**). White powder; mp: 74.7 °C; ^{1}H NMR (DMSO-d_6, 400MHz): 12.41 (br s, 1H), 7.54 - 7.45 (m, 1H), 7.39 – 7.29 (m, 3H), 7.27 - 7.24 (m, 3H), 7.17 - 7.12 (m, 1H), 6.99 (d, *J* = 5.2 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 2H), 5.06 (d, *J* = 9.2 Hz, 2H), 4.50 (d, *J* = 13.2 Hz, 2H), 4.42 (s, 2H), 3.95 – 3.31 (m, 1H), 2.61 – 2.49 (m, 2H), 2.08 (d, *J* = 37.2 Hz, 3H), 1.76 (d, *J* = 2.4 Hz, 3H); MS (EI): *m/z* (%) = 462.15 (100) $(M+H)^+$; IR (KBr): *v* = 3433, 2918, 1724, 1641, 1610, 1429 cm^{-1}.

5-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)-1H-indazole (**2r**). Wax solid; ^{1}H NMR (DMSO-d_6, 400MHz): 12.24 (br s, 1H), 7.48 - 7.46 (m, 1H), 7.34 - 7.21 (m, 7H), 7.00 – 6.98 (m, 1H), 6.93 (d, *J* = 8.8 Hz, 2H), 5.06 (s, 2H), 4.32 (s, 2H), 4.26 (s, 2H), 4.32 – 3.91 (m, 1H), 3.08 (q, *J* = 7.4 Hz, 2H), 2.59 - 2.54 (m, 2H), 1.76 (d, *J* = 2.4 Hz, 3H), 1.15 (t, *J* = 7.4 Hz, 3H); MS (EI): *m/z* (%) = 529.35 (100) $(M+NH_4)^+$, 510.35 (100) $(M-H)^+$; IR (KBr): *v* = 3400, 3020, 2922, 2401, 1710, 1608, 1510, 1215 cm^{-1}.

5-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)-1H-indazole (**2s**). Wax solid; ^{1}H NMR (DMSO-d_6, 400MHz): 7.47 - 7.45 (m, 1H), 7.34 - 7.31 (m, 3H), 7.30 - 7.29 (m, 1H), 7.26 - 7.22 (m, 3H), 7.08 (dd, J_1 = 5.2 Hz, J_2 = 1.6 Hz, 1H), 6.90 (d, *J* = 8.8 Hz, 2H), 5.05 (s, 2H), 4.34 (s, 2H), 4.28 (s, 2H), 3.92 – 3.84 (m, 1H), 2.59 - 2.52 (m, 1H), 2.45 - 2.39 (m, 2H), 1.75 (d, *J* = 2.4 Hz, 3H), 0.99 – 0.91 (m, 4H); MS (EI): *m/z* (%) = 524.00 (20%) $(M+H)^+$; IR (KBr): *v* = 3365, 3099, 3032, 1708, 1608, 1583 cm^{-1}.

5-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)-1H-indazole (**2t**). White powder; mp: 80-82 °C; ^{1}H NMR (DMSO-d_6, 400MHz):- 7.49 - 7.47 (m, 1H), 7.40 (s, 1H), 7.33 - 7.27 (m, 3H), 7.24 - 7.21 (m, 3H), 7.07 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 5.09 (s, 2H), 3.92 – 3.88 (m, 1H), 3.75 (s, 2H), 3.72 (s, 2H), 3.05 (s, 2H), 2.54 - 2.52 (m, 2H), 1.76 (d, *J* = 2.4 Hz, 3H); MS (EI): *m/z* (%) = 478.30 (100) $(M+H)^+$; IR (KBr): *v* = 3020, 1215, 760, 669 cm^{-1}.

Biological studies:

In vitro assays

5XSRE based Luciferase assay:[4]

GPR40 agonists were routinely screened in SRE (Serum Response Element) based Luciferase transactivation assay by transient transfection of 5X SRE (5 concomitant repeats of Serum Response Element) in stably expressing HEK-*h*GPR40 cell line (HTS038L, Millipore). Briefly, HEK-GPR40 (HTS038L, Merck-Millipore) cells were maintained as monolayer in complete DMEM:F12 (1:1) medium in presence of 400 µg/mL G418 (CalBioChem) and 1 µg/mL puromycin (Sigma). Day before transfection, 35000 cells were seeded in poly-D-Lysine (Sigma) coated 96 well cell culture plate in 100 µL antibiotic free medium and incubated at 37 °C in 5% CO2 containing humidified chamber O/N. Prior to transfection, cells were fed with fresh complete growth medium and incubated until the addition of transfection complex.

50 µL of transfection complex (200 ng/µl pGL2-promoter-5XSRE-Luc, 40 ng/µL β-gal plasmid, and 0.5 µl/well Lipofectamine2000) were added in 100 µL of complete medium to respective wells, mixed gently and plates were incubated for 5 h at 37 °C in 5% CO_2 containing humidified chamber. After 5 h of transfection, content of the wells were aspirated and cells were treated with increasing concentration of GPR40 agonist in medium devoid of serum with a final DMSO concentration of 0.2% for 18-20 h at 37 °C in 5% CO_2 containing humidified chamber. 16-20 h post treatment, cells were lysed and lysates were assayed for luciferase and β-gal activity using luciferase assay system (Promega, Inc.). Luciferase signals were normalized with β-gal and % activity was determined with respect to the activity obtained by 10 nM control agonist (TAK875). EC50 was determined by non linear regression analysis of % activity, plotted against NCE concentration.

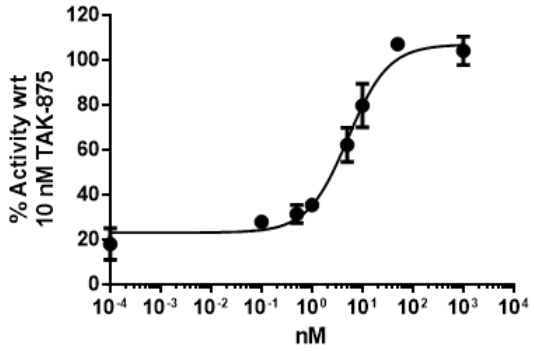


Figure 1a. Effect of Compound **5** in hGPR40 Luciference Assay. EC_{50} = 5.8 nM

Intracellular Calcium Flux Assay:[5]

HEK293 cells stably express GPR40 (HTS038L, Millipore) were maintained in DMEM high Glucose (Himedia) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Biowest), 400 µg/mL G418 (Goldbio), 1 µg/mL puromycin (Sigma). Cells were seeded at a density of 50,000 cells/well in black clear bottom 96-well plates (Nunc; Thermo fisher, USA), and cultured overnight before experiment. Media

was removed and cells were loaded with 100 µL Fluo-4 NW dye (Invitrogen, Molecular probe, USA) at 37 °C for 30 minutes, and then at room temperature for 40 minutes. Fluorescence intensities were detected at 494nm excitation and 516°nm emission in multimode detection microplate reader (Infinite® M1000 PRO, Tecan). The time course of Fluo-4 in HEK293 stable cell line was recorded as the ratio of the fluorescent signals without stimulus for 60 s and with stimulus of test compound for 180 s. The [Ca^{2+}] response was determined by baseline subtraction and plotted as change in fluorescence intensity (%Δ*F/F)*, expressed as follows: %Δ/*F* = ($F_{(t)}$ − F_{min})/(F_{max} − F_{min}) × 100.

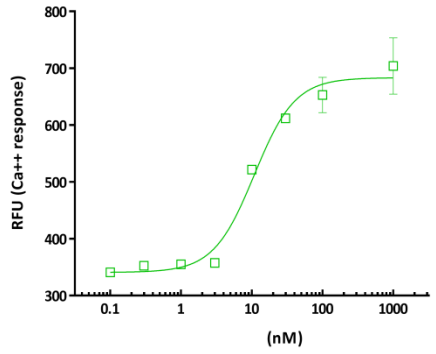


Figure 1b. Effect of Compound **5** in hGPR40 Intracellular Calcium Flux Assay. EC_{50} = 11.6 nM.

IP1 Measurement assay:[6]

HTS038L cells were maintained in DMEM (high Glucose) containing 10% heat-inactivated fetal bovine serum with 400 µg/mL G418 & 1 µg/mL puromycine. Cells were seeded into 24-well plate (400,000 cells per well) and cultured o/n at 37 °C in a humidified 5% CO_2/95% air environment. Media was removed from the cells, 100 µL of stimulation buffer (provided in kit) plus or minus compound was added in respective wells and the plate was returned to the incubator for 1 h. After incubation 50 µL (2.5% lysis reagent) was added in each well. Plate was kept at 37 °C incubator for 30 min then the cell lysis solution collected and centrifuged at 13000RPM for 10 min at 4 °C. Supernatant was used for IP1 measurement by CIS BIO IP1 ELISA kit.

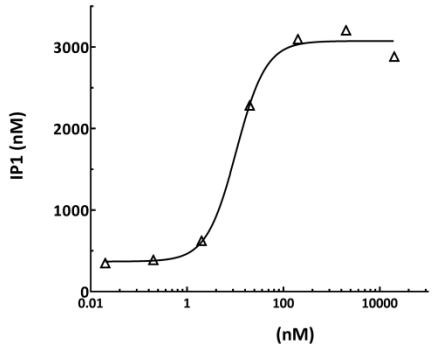


Figure 1c. Effect of Compound **5** in hGPR40 IP1 Measurement Assay. EC_{50} = 10.5 nM.

RINm insulin secretion assay:

All reagents are from sigma except RPMI 1640, FBS, fatty acid free BSA, DMSO (HiMedia), Insulin Kit (Crystal chem). RINm cells were maintained in RPMI1640 (HiMedia) containing 10% heat-inactivated fetal bovine serum. Cells were seeded into 24-well plate (100,000 cells per well) and cultured for 72 h at 37 °C in a humidified 5% CO_2/95% air environment. Media was removed from the cells and they were washed twice with glucose-free Krebs (NaCl 119 mM, KCl 4.74 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.19 mM, KH_2PO_4 1.19 mM, $NaHCO_3$ 12.5 mM and HEPES (pH 7.4) 10 mM) containing 0.05% (w/v) fatty acid-free bovine serum albumin (BSA). 200 µL of the above Krebs solution was added to each well and the plate was returned to the incubator for 2 h. After incubation 200 µL of Krebs containing glucose at the desired concentration, plus or minus compound, was added. Following 1 h incubation supernatants from each well were collected and insulin content was determined using crystal chem Insulin ELISA kit.

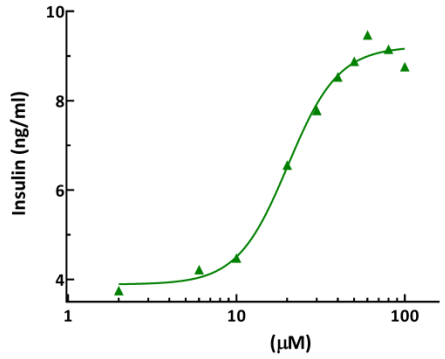


Figure 1d. Effect of Compound **5** in hGPR40 RINm Insulin secretion Assay. EC_{50} = 20 µM

ADME Profile

All the animals used in the study were procured from the Animal Breeding Facility of Zydus Research Center. All animal studies were conducted according to protocols reviewed and approved by the Institutional Animal Care and Ethics Committee at the Zydus Research Centre.

Pharmacokinetic studies[7]

Oral bioavailability of compound **5** was evaluated in male Sprague–Dawley rats. Rats were divided into two groups (weight range 270–370 g) each group containing four rats. Non-fasted Group-1 rats were received compound **5** solution formulation (prepared using 10% NMP, 5% Solutol and 85% citric acid (0.1% w/v)) through intravenous (i.v.) route at a dose of 1 mg/kg; whereas over-night fasted Group-2 received compound **5** suspension formulation (prepared using 0.45% Tween-80 and 89.55% of Na-CMC (0.5% w/v)) through oral gavage at 3 mg/kg doses. Serial blood samples (100 µL) were collected from retro-orbital plexus at pre-dose, 0.08 (i.v. only) 0.25, 0.5, 1, 2, 4, 8 and 24 h. Blood samples were collected in tubes containing Na-heparin as the anticoagulant and centrifuged for 15 min at 4,000 rpm in a refrigerated centrifuge maintained at 4 °C for plasma separation and stored frozen at -70 ± 10 °C until analysis.

The mean plasma concentration vs time profiles for compound **5** following single i.v. and oral administration is depicted in Fig. 1. The relevant pharmacokinetic parameters are summarized in Table 1. Animals were well tolerated following oral and i.v. administration of compound **5** and no abnormal behavior was observed. Following i.v. administration, compound **5** plasma concentrations declined in a mono-exponential fashion with a terminal half-life ($t_{1/2}$) of 1.78 ± 0.16 h. Following i.v. administration, the clearance and volume of distribution were found to be 13.83 ± 1.56 mL/min/kg and 1.05 ± 0.09 L/kg, respectively. Following single oral dose administration of 3 mg/kg, compound **5** was quickly and well absorbed from rat gastrointestinal tract, reaching maximum concentrations (Cmax) in plasma between 0.25 to 1 h (Tmax). Absolute oral bioavailability of compound **5** was 100%.

Table 1. Pharmacokinetic parameters of compound **5** in Sprague–Dawley rats (Mean ± S.D, n = 4)

Dose, Route	PK parameters	Units	Values
1 mg/kg, IV	Vss	(L/kg)	1.05 ± 0.09
	Cl	(mL/min/kg)	13.83 ± 1.56
	T1/2	(h)	1.78 ± 0.16
	MRT	(h)	1.27 ± 0.05
3 mg/kg, PO	Tmax	(h)	0.25-1
	C_{max}	(µg/mL)	2.78 ± 0.68
	AUC (0-t)	(h.µg/mL)	6.61 ± 1.00
	AUC (0-inf)	(h.µg/mL)	6.85 ± 1.14
	T1/2	(h)	2.01 ± 0.19
	MRT	(h)	2.69 ± 0.55
	Bioavailability	%F	100

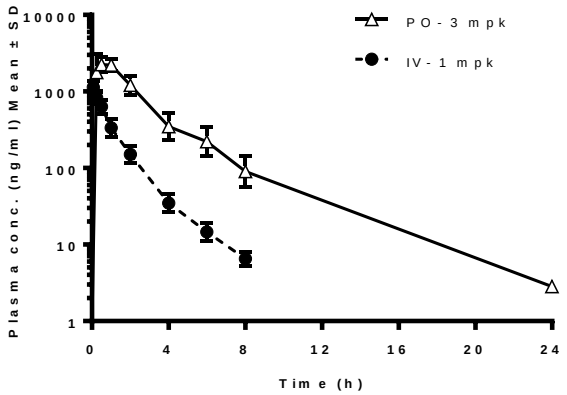


Figure 2: Plasma concentration–time profiles (mean ± S.D, n = 4) of compound **5** after oral (3 mg/kg, fasted) and intravenous (1 mg/kg, non-fasted) administration in male Sprague–Dawley rats

Aqueous solubility

Compound **5** was incubated separately with (A) water, (B) phosphate buffer pH 6.8, (C) ammonium acetate buffer pH 4.5 and (D) 0.1 N hydrochloric acid in triplicate at 1 mg/mL concentration in a shaking water bath for 24 hours, at 37 °C and 100 rpm. Following incubation, the samples were centrifuged at 10000 rpm for 5 min at room temperature, and supernatants were filtered through a 0.45 µ filter membrane. The filtrates were analyzed for the concentration of **5** with an HPLC-UV method.
Compound **5** showed good solubility; mean solubility was 48 µg/mL in purified water, 293 µg/mL in pH 6.8 buffer, 471 µg/mL in pH 4.5 buffer and 781 µg/mL in 0.1 N hydrochloric acid. Results are summarized in Table 2.

Table 2. Aqueous solubility data of compound **5**

Solvents	**Aqueous solubility (µg/mL)**, Mean n=2
Water	1.06
PBS pH6.8	0.81
Acetate buffer pH 4.5	1.21
0.1 N HCl	1.50

CYP inhibition

Compound (at 2 and 10 µM concentration, with a final DMSO concentration of 0.2 %) was incubated with human liver microsomes (protein, 0.025, 0.15, 0.025, 0.2, 0.2, 0.2 and 0 .05 mg/mL concentration for CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4 with two substrate testosterone and midazolam, respectively) 100 mM potassium phosphate buffer (pH 7.4), and 1 mM NADPH in the presence of CYP-specific substrates (CYP1A2: phenacetin; CYP2C8: paclitaxel; CYP2C9: diclofenac; CYP2C19: S-mephenytoin; CYP2D6: dextromethorphan; and CYP3A4/5: testosterone and midazolam). Reactions were quenched with acetonitrile containing internal standard. The reaction mixtures (obtained from the above studies) were extracted, processed, and analyzed on LC–MS/MS for peak area ratio of CYP-specific metabolites (CYP1A2: acetaminophen; CYP2C8: 6'-hydroxy paclitaxel, CYP2C9: 4'-hydroxy diclofenac; CYP2C19: 4'-

hydroxy S- mephenytoin; CYP2D6: dextromethorphan; and CYP3A4/5: 6 β- hydroxy testosterone and 1' - hydroxy midazolam). Inhibition by positive control inhibitors (CYP1A2: α naphtha-flavone, 2C8: rosiglitazone, 2C9: sulfaphenazole, 2C19: ticlopidine, 2D6: quinidine and 3A4/5: ketoconazole) was similarly determined (data not shown). CYP450 inhibition data of reference compounds (**TAK-875** & **AMG-837**) and compounds **2a**-**2t** are tabulated in Table 3. Compound **2d** and its corresponding calcium salt (compound **5**) showed no significant inhibitory activity against CYP450 isoforms (Table 4).

Table 3. CYP450 inhibition data of reference compounds (**TAK-875** & **AMG-837**) and compounds **2a**-**2t**.*

Compound	% Inhibition											
	CYP 1A2		CYP 2C8		CYP 2C9		CYP 2C19		CYP 2D6		CYP 3A4 (STO)	
	2 uM	10uM	2 uM	10uM	2 uM	10uM	2 uM	10 uM	2 uM	10 uM	2 uM	10uM
TAK-875	NI	4.4	10.9	53.4	20.7	57.7	5.7	16.2	0.1	NI	NI	NI
AMG-837	14.3	56.2	1.9	37.9	25.7	82.4	NI	5.5	NI	NI	1.6	3.4
2a	5.6	78.6	36.5	81	71.8	97.1	15.8	41	26.5	70.3	11.4	30.9
2b	NI	NI	2	NI	10.6	28.1	NI	NI	21.7	63.6	NI	NI
2c	NI	NI	23.3	63	25.7	63.6	NI	5.2	9.8	17	3.3	4.9
2d	14.5	36.3	21.8	37.7	10.8	24.7	NI	2.3	NI	3.6	NI	NI
2e	NI	37.4	45	72.2	33.9	79.3	53.5	60.6	7.3	14.5	3.3	2.2
2f	NI	NI	27.9	63.7	38.4	79.3	0.9	6.2	NI	7.1	5.1	7.6
2g	NI	NI	21.2	53.2	37	48	1.2	NI	NI	10.2	5.4	0.8
2h	1.4	NI	36	73.2	25.6	72.8	1.3	20.9	NI	18.5	NI	0.6
2i	13.7	39.2	9.6	50.7	54.1	86.2	0.2	16.1	0.7	14.5	NI	NI
2j	NI	5.7	70.1	88.5	43.8	81.3	34.9	46.8	5.2	7.5	1.6	0.5
2k	NI	35.7	88.7	96.7	76.8	96	15.2	48.3	NI	13.7	NI	6
2l	NI	NI	17	60.6	24.8	63.8	7.6	14.7	NI	49.8	3.5	12.5
2m	76.3	91	97	99.3	99.1	99.7	97.5	100	98.7	99.4	94.6	96.1
2n	19.5	85.3	46	80.1	68.2	94.9	4.7	29.1	1.1	1.1	NI	0.4
2o	57.6	91.6	97.2	100	88.5	98.6	72.6	94.4	20.8	50.5	73.6	91.5
2p	5.5	80.2	48.8	90	67.6	97.7	NI	5.9	15.6	16.5	10.8	14.1
2r	12.1	17.7	40.1	77.9	36.5	68.2	2.8	24.6	18.6	57.6	27.7	55.7
2s	69.3	84.7	95.8	99	98.7	99.7	96.1	100	97.3	98.7	93.5	95.7
2t	NI	5.3	NI	15.9	11.3	17.9	NI	0.2	10.2	9.8	NI	6.3

* NI = No inhibition.

Table 4. CYP450 inhibition data of compound **5**

CYP Isoforms	% Inhibition of controls	
	2μM	10μM
1A2	3.2	0.0
2C8	24.8	49.7
2C9	11.2	47.5
2C19	0.0	20.2
2D6	12.8	13.5
3A4 (Substrate: testosterone)	0.6	9.0
3A4 (Substrate: midazolam)	0.0	0.7

Metabolic stability

A standard incubation mixture (final volume 1,000 μL) contained 100 mM potassium phosphate buffer (pH 7.4), 0.5 mg/mL protein of rat liver microsomes and compound **5** (1 μM). Testosterone (1 μM) was used as a positive control. An aliquot of 100 μL was aliquoted into different vials labeled as 0, 5, 10, 15 and 30 min. The vials were preincubated in a 37 °C water bath for 5 min. The reactions were initiated by adding 20 μL of 6 mM NADPH. Reactions without NADPH (0 and 30 min) were also incubated to rule out non-NADPH metabolism or chemicalinstability in the incubation buffer. All reactions were terminated using 300 μL of ice-cold acetonitrile containing internal standard at specified time points. The vials were centrifuged at 10,000 rpm for 5 min. The supernatants, thus, obtained were analyzed on LC–MS/MS to monitor the disappearance of compound **5**.

Compound **5** was found to be stable in rat liver microsomes. The % metabolized was 67.7% in rat liver microsomes. Metabolic stability of compound **5** in rat liver microsomes is presented in Table 5.

Table 5. Metabolic stability data of compound **5** at 1 μM in rat liver microsomes

Species	Percent metabolized	t1/2 (min)	mClint (μL/min/mg)
Rat	67.7	18.6	74.4

Plasma protein binding

Compound **5** was spiked at 1 μM concentration in rat plasma (anticoagulant: K_2EDTA) and loaded into the donor compartments of a 96-well equilibrium dialyzer (HTD) and dialyzed against human plasma ultrafiltrate. Human plasma ultrafiltrate used for the dialysis was obtained by ultrafiltration of blank plasma across a 10 kDa molecular weight cutoff membrane. The dialysis membrane between the donor and receiver compartment was a 12-14 kDa molecular weight cutoff membrane. To determine non-specific binding, human plasma ultrafiltrate was spiked with compound **5** to get 1 μM concentration and added to both, donor and receiver compartment. To determine equilibrium between donor and receiver compartment, human ultra-filtrate spiked with compound **5** to get 1 μM concentration and was added to donor compartment while plain human ultra-filtrate to receiver compartment of the well. Finally 96-

Well Teflon plate was sealed with adhesive sealing film and dialysis was performed for 6 h at 37 °C at 200 rpm. After dialysis, compound **5** concentrations in samples from the donor and receiver compartments was measured by LC-MS/MS. Warfarin and atenolol were used as high and low binding control compounds, respectively.
Compound **5** was found to be highly bound in rat plasma. Plasma protein binding of compound **5** at 1 µM was 99.65% in rat plasma. Equilibrium was achieved between donor and receiver compartment over 6 h of dialysis. Non-specific binding was not significant (<12%). Data are presented in Table 6.

Table 6. Plasma protein binding data of compound **5** in rat plasma at 1 µM concentration 96-well high throughput dialysis (HTD).

Matrix	Method	Incubation period	Plasma protein binding	Mass recovery	Stability in plasma
Rat plasma	Equilibrium dialysis	6 h	99.65%	89.92%	89.59%

Bi-directional Caco-2 permeability assay

The in vitro bi-directional permeability of Compound **5** (2 µM) across Caco2 cell monolayers was determined. Caco-2 cells (passage 27) were seeded onto 24 well filter membranes and cultured for 21 days. The trans-epithelial electrical resistance (TEER) was measured prior to the experiment and was in the range of 567 to 670 Ωcm^2. For assessment of permeability in the apical to basolateral direction, 1 mL transport medium (Hanks balanced salt solution with 10 mM HEPES, pH 7.4) was added to the basal compartment, and 0.5 mL transport medium containing test compound was added to the apical compartment. For assessment of permeability in the basolateral to apical direction, 0.5 mL transport medium was added to the apical compartment and 1 mL of transport medium containing test compound was added to the basal side. Following incubation (37^{o}C, 95% RH, 5% CO2 and 75 rpm for 120 min) samples were collected from both apical and basal side and diluted 2-fold with acetonitrile and stored at 2-8^{o}C until analysis by a liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Monolayer integrity at the end of the experiment was confirmed using Lucifer yellow with fluorescence detection. Control studies were performed similarly and concurrently with propranolol (5 µM) a high permeability compound, and rosuvastatin (10 µM) a substrate for efflux transporters.
Compound **5** showed high apical to basal permeability with a Papp value of 339.3 nm/sec. The basal to apical permeability was 40.4 nm/sec. The efflux ratio of Compound **5** was 0.1, indicating that it was not to be a substrate of efflux transporters expressed in CaCo-2 cells including P-gp (P-glycoprotein) and/or BCRP (breast cancer related polypeptide). The permeability data for the control compounds propranolol, and rosuvastatin matched historical in-house data (data not presented).

Table 7. Bi-directional permeability of Compound **5** using Caco2 cell monolayers

	Papp (nm/sec)		
	A-to-B	B-to-A	Ratio (B/A)
Compound 5	339.3	40.4	0.1

In Vivo Studies of Compound 5[8-9]

All the animals used in the study were procured from the Animal Breeding Facility of Zydus Research Center. Institutional Animal Ethical Committee approved all the study protocols.

Evaluation of anti-diabetic activity for GRP40 agonist in n-STZ rat model:

Wistar rat pups of 1-2 day old injected with Streptozotocin (STZ) at 120 mg/kg dose by intraperitoneal route. All pups allowed grow normally and at the age of 12-14 week they were screen for glucose intolerance by performing the oral glucose tolerance test by tail clip method using glucometer. Animals showing glucose intolerance were selected for evaluation of test compound. Three to seven days of rest period animals were kept on overnight fasting. Next day morning blood glucose levels measured using glucometer and animals were grouped such that their pretreatment glucose levels were not significantly different between groups. Animals were administered with test compound and then then 60 min after the compound administration "0" min blood glucose levels were measured and immediately glucose load at 2 g/kg was administered orally. Blood glucose levels were measured at 30, 60 and 120 min after glucose load using by tail clip method using glucometer. Blood was also collected at 10 min after glucose load for measurement of insulin levels. Glucose area under the curve (AUC) was calculated using Graph Pad Prism software and % reduction in AUC-glucose Vs Vehicle treated control was calculated.

Evaluation of anti-diabetic activity for GRP40 agonist in db/db mice model:

The db/db mice are a genetic model for Type 2 diabetes mellitus and commonly used for evaluation of anti-diabetic activity. The db/db mice of 10-11 week of age were used for this study. Animals were issued and kept for 2-3 days acclimatization. On Day-0, animals were bled by tail vein for blood glucose measurement using glucometer and grouped such that their pretreatment glucose levels were not significantly different between groups and were kept on overnight fasting. On next day animals were administered with test compound and then then 60 min after the compound administration "O" min blood glucose levels were measured and immediately glucose load at 0.75 g/kg/10 mL was administered orally. Blood glucose levels were measured at 30, 60 and 120 min after glucose load using by tail clip method using glucometer. Glucose area under the curve (AUC) was calculated using Graph Pad Prism software and % reduction in AUC-glucose Vs Vehicle treated control was calculated (Figure 3).

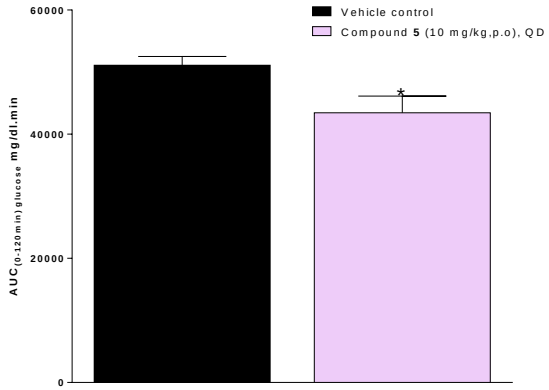


Figure 3. In vivo efficacy studies of Comp. **5** in db/db mice at 10 mg/Kg dose. Each bar represents mean ± s.e.m. * indicates statistical significance, $p<0.05$.

References

1. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxyborohydride. Studies on Direct and Indirect Reductive Amination Procedures. *J. Org. Chem.*, **1996**, *61* (11), 3849–3862.
2. Walker, S. D.; Borths, C. J.; DiVirgilio, E.; Huang, L.; Liu, P.; Morrison, H.; Sugi, K.; Tanaka, M.; Woo, J. C. S.; Faul, M. M. Development of a Scalable Synthesis of a GPR40 Receptor Agonist. *Org. Process Res. Dev.* **2011**, *15*, 570–580.
3. Desai, R. C., Agarwal, S. Novel Heterocyclic Compounds, Pharmaceutical Compositions and Uses Thereof. Indian Pat. Appl. 2025/MUM/2015, 25 May **2015**.
4. Cheng, Z., Garvin, D., Paguio, A., Stecha, P., Wood, K., & Fan, F. Luciferase Reporter Assay System for Deciphering GPCR Pathways. *Current Chemical Genomics*, **2010**, *4*, 84–91. http://doi.org/10.2174/1875397301004010084
5. Arkin, M. R., Connor, P. R., Emkey, R., et al. FLIPR™ Assays for GPCR and Ion Channel Targets. 2012 May 1 [Updated 2012 Oct 1]. In: Sittampalam, G. S., Coussens, N. P., Nelson, H., et al., editors. Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004. Available from: http://www.ncbi.nlm.nih.gov/books/NBK92012/
6. Garbison, K. E., Heinz, B. A., Lajiness, M. E. IP-3/IP-1 Assays. 2012 May 1. In: Sittampalam, G. S., Coussens, N. P., Nelson, H., et al., editors. Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004. Available from: http://www.ncbi.nlm.nih.gov/books/NBK92004/
7. Milić, A., Mihaljević, V.B., Ralić, J. et al. A comparison of in vitro ADME properties and pharmacokinetics of azithromycin and selected 15-membered ring macrolides in rodents. *Eur J Drug Metab Pharmacokinet*, **2014**, *39*, 263. doi:10.1007/s13318-013-0155-8
8. Bell, R. H.; Hye, R. J. Animal models of diabetes mellitus: physiology and pathology. *J. Surg. Res.* **1983**, *35*, 433-460.
9. Shafrir, E. Animal models of non insulin dependent diabetes. *Diabetes Metab Rev.* **1992**, *8*, 179-208.

Supporting Information

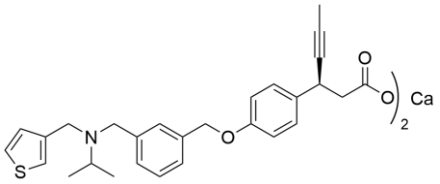
Identification of an Orally Efficacious GPR40/ FFAR1 Receptor Agonist

Sameer Agarwal*, Santosh Sasane, Prashant Deshmukh, Bhadresh Rami, Debdutta Bandyopadhyay, Poonam Giri, Suresh Giri, Mukul Jain, and Ranjit C. Desai*

Zydus Research Centre, Cadila Healthcare Ltd. Sarkhej-Bavla N.H. No. 8 A, Moraiya, Ahmedabad – 382 210, India

Contents

Spectra's of Compound **5**



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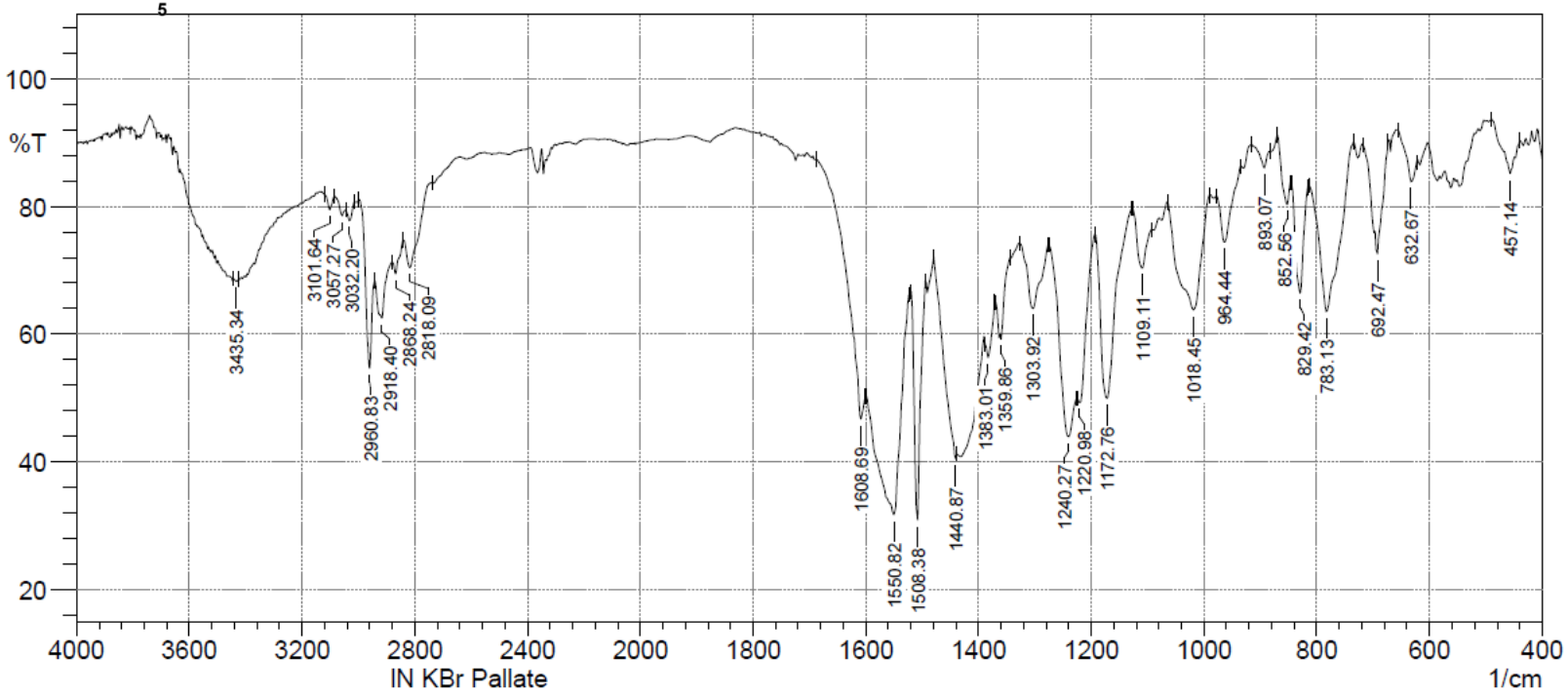

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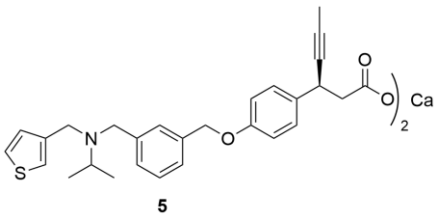

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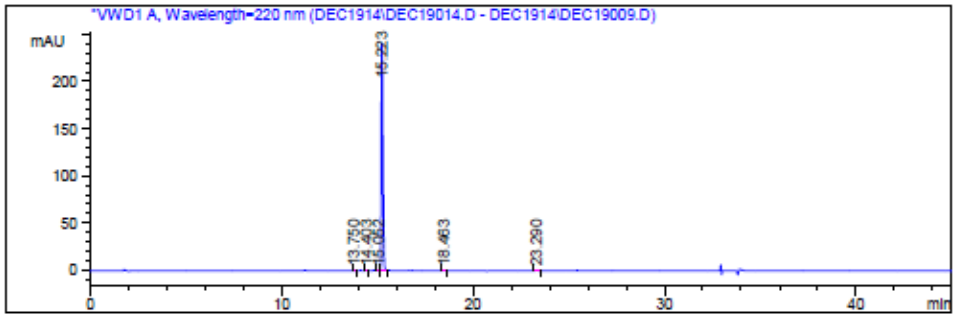


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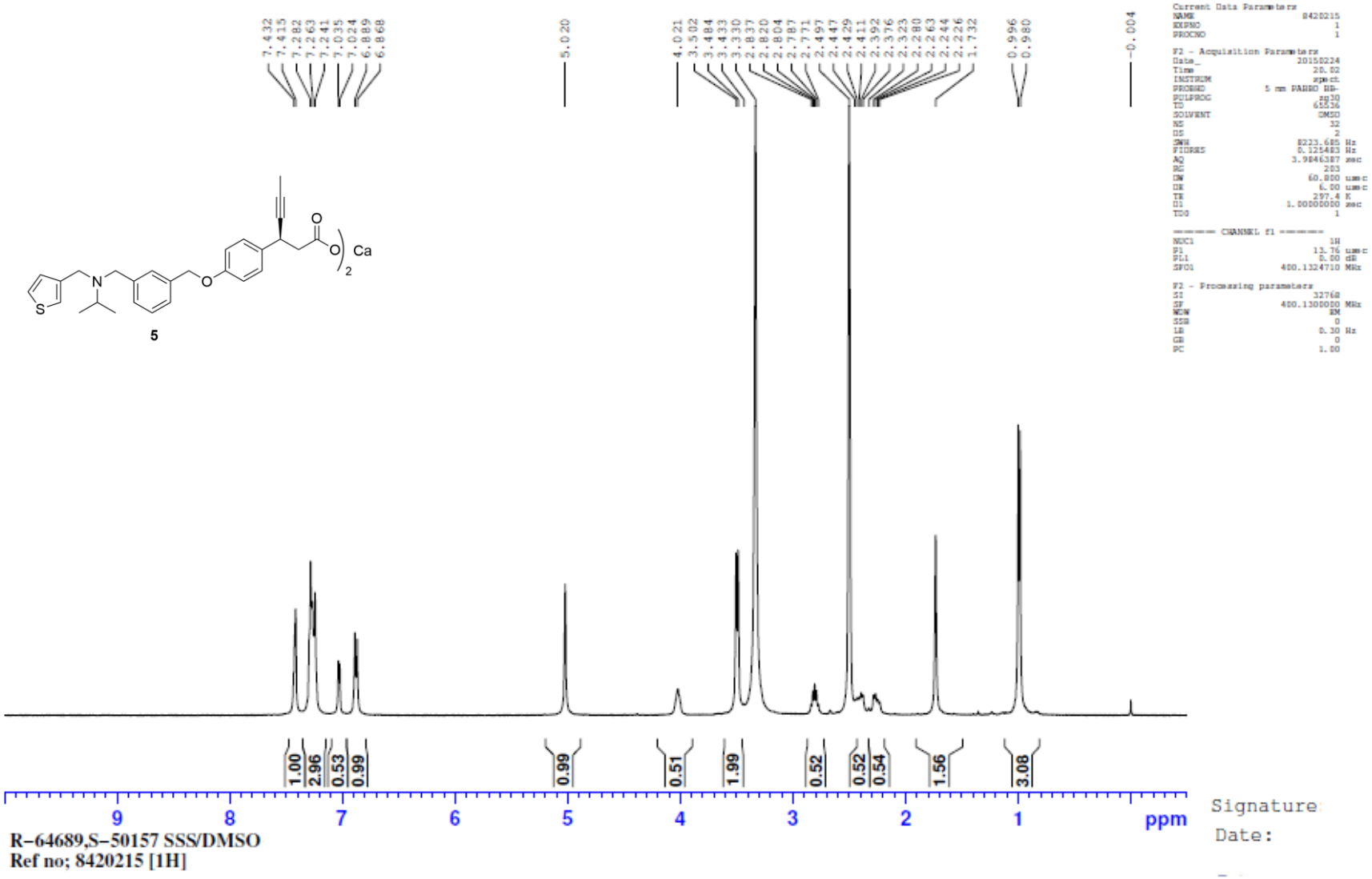
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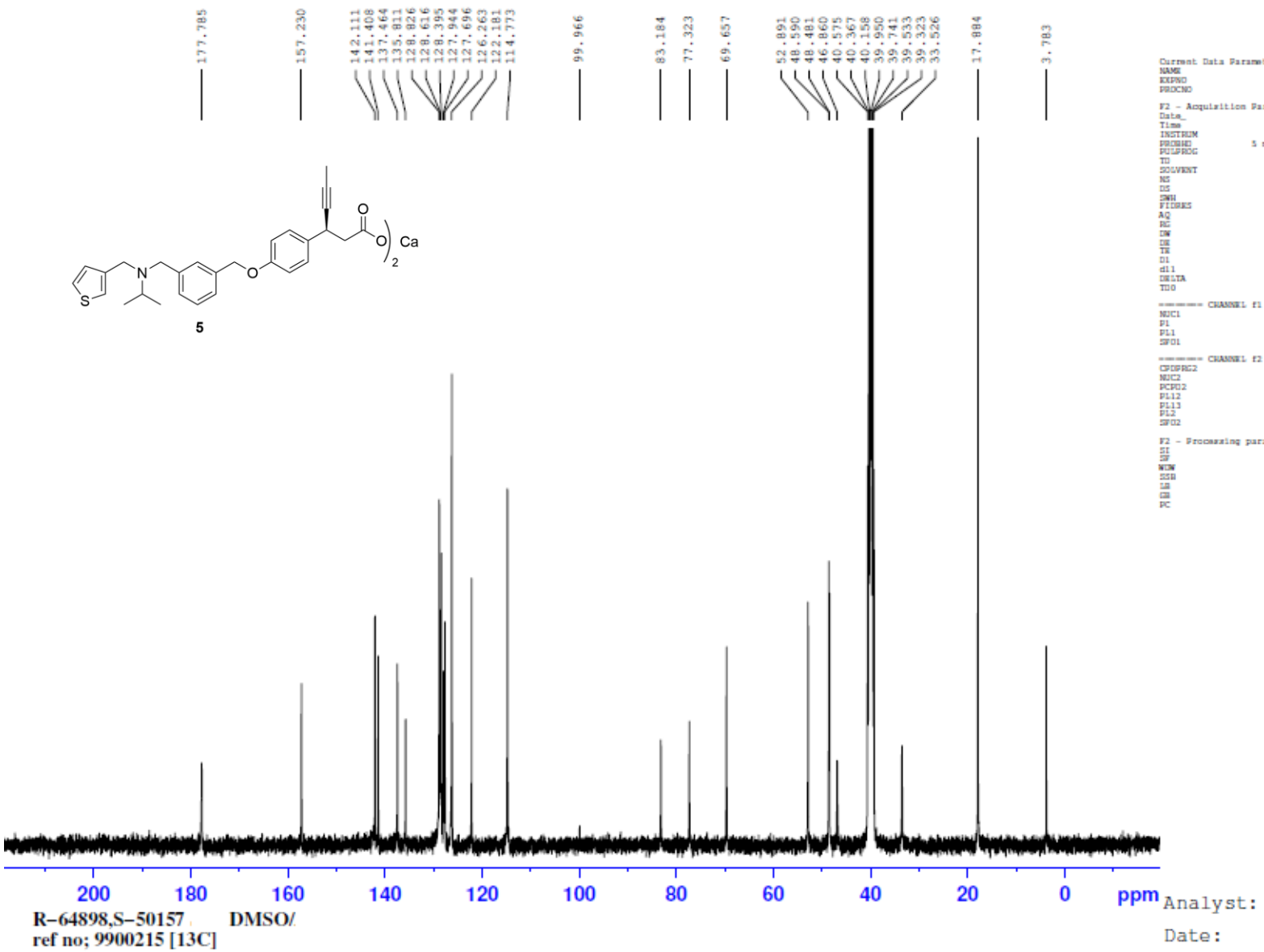
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ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

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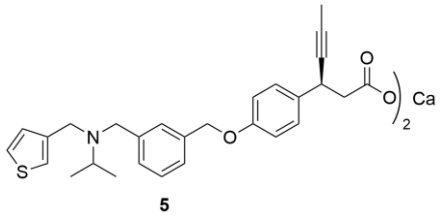

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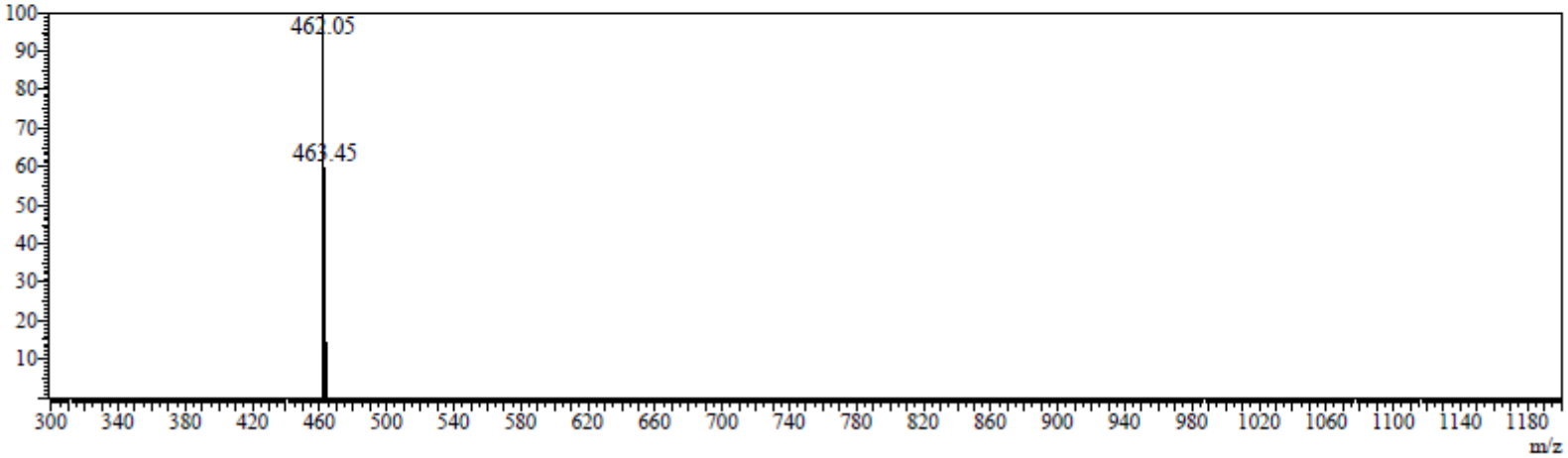

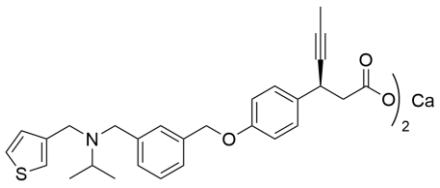
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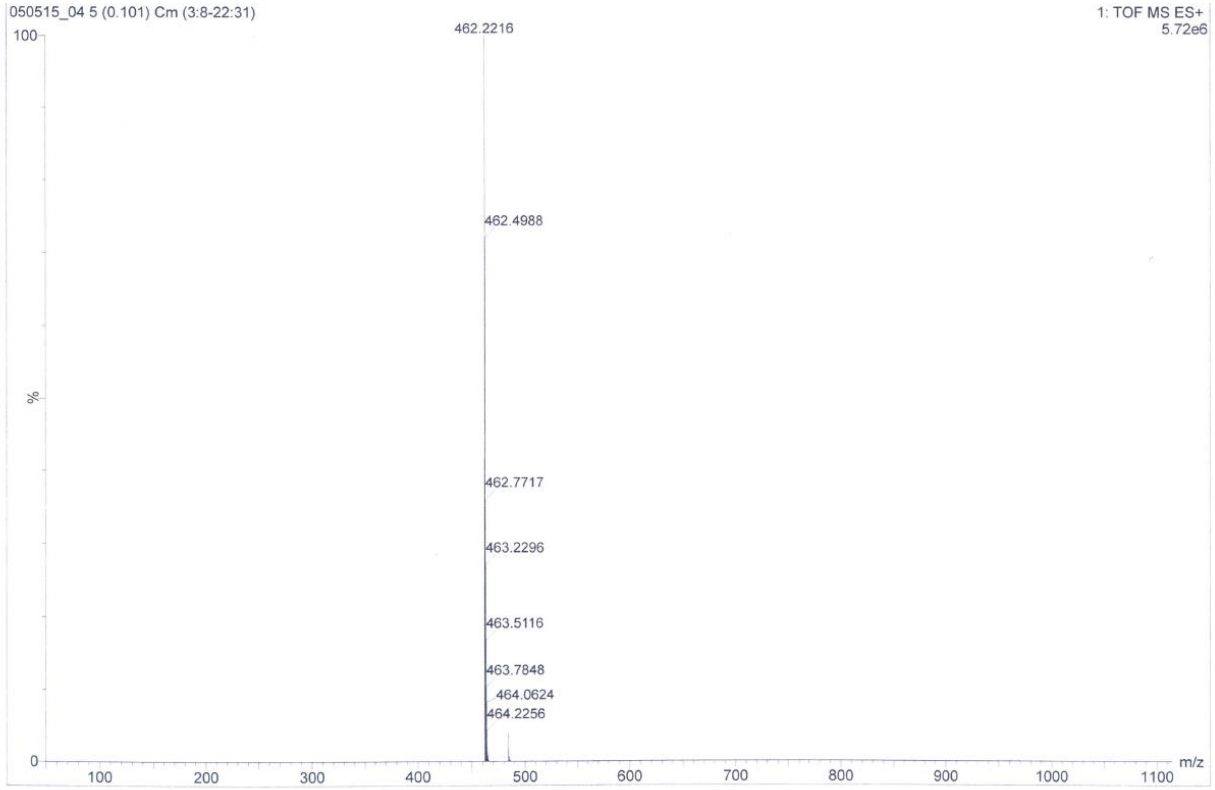
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