Supporting Information

Discovery of a Potent and Orally Efficacious TGR5 Receptor Agonist

Sameer Agarwal*, Amit Patil, Umesh Aware, Prashant Deshmukh, Brijesh Darji, Santosh Sasane, Kalapatapu V. V. M. Sairam, Priyanka Priyadarsiny, Poonam Giri, Harilal Patel, 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

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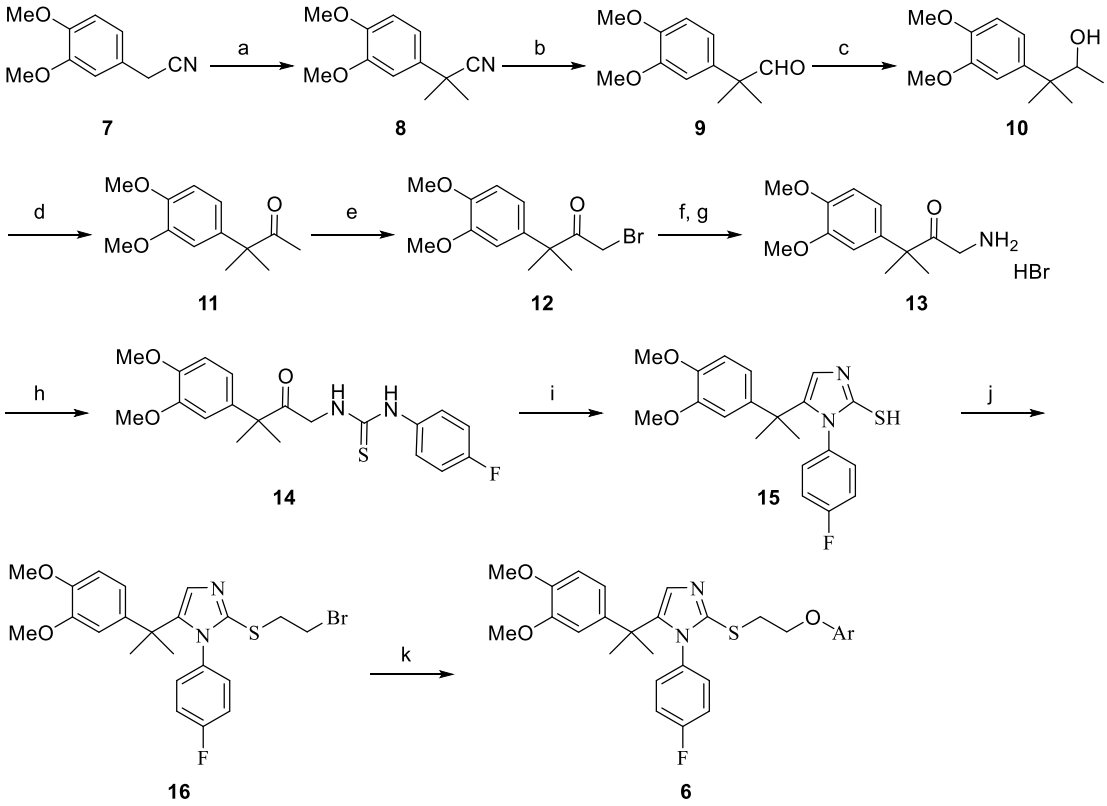
General procedures

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 (**6a**–**6l**) was confirmed as over 95% by HPLC.

Experimental procedures

The synthesis of 2-thio-imidazole derivative **6** began with the dimethylation of commercially available 2-(3,4-dimethoxyphenyl)acetonitrile (**7**) followed by selective reduction of cyano group with diisobutylaluminium hydride (DIBAL) to afford aldehyde **9**. Treatment of compound **9** with methyl magnesium bromide followed by oxidation of resulting alcohol **10** under swern[1] condition afforded **11** which in turn was brominated using tetrabutyl ammoniumtribromide to compound **12**. Further transformation of **12** under Delepine[2] reaction protocol gave amine **13** as its corresponding hydrobromide salt. The crude amine **13**, without any further purification, on treatment with 1-fluoro-4-isothiocyanatobenzene provided thiourea derivative **14**. The construction of imidazole skeleton is achieved via cyclization of **14** in boiling acetic acid to afford 2-mercapto imidazole derivative **15**. Finally, alkylation with excess of 1,2-dibromoethane gave advance intermediate **16** which on further alkylation with substituted phenol provided test compounds **6a**-**6l** in good yields and high chemical purity.[3,4]



Scheme 1. Synthesis of Compound **6**. *Reagent and Conditions:* (a) MeI, NaH, dry THF, 0 °C to r.t., 3 h, 93%; (b) DIBAL, Toluene, -78 °C, 2 h, 93%; (c) MeMgBr, dry Et_2O, 0 °C to r.t., 80%; (d) oxalyl chloride, DMSO, Et_3N, DCM, -78 °C, 2 h, 82%; (e) Bu_4NBr_3, DCM, MeOH, 0 °C to r.t., 7 h, 100%; (f) HMTA, DCM, r.t., 48 h; (g) EtOH, HCl, reflux, 3 h; (h) 1-fluoro-4-isothiocyanatobenzene, Et_3N, DCM, 0 °C to r.t., 1 h, 42%; (i) AcOH, reflux, 3 h, 73%; (j) 1,2-dibromoethane, K_2CO_3, acetone, r.t., 2 h, 73%; (k) Substituted phenol, K_2CO_3, DMF, 90 °C, 5 h.

2-(3,4-dimethoxyphenyl)-2-methylpropanenitrile (**8**): To a solution of sodium hydride (7.2 g, 0.3 mol) in dry THF (80 mL) was added 3, 4-dimethoxy phenylacetonitrile (**7**) (10.6 g, 59.88 mmol) at 0 °C and stirred for 30 min. Methyl iodide (12.14 mL, 0.18 mol) was added drop wise at 0 °C, after 1.5 h reaction mixture was warmed to room temperature and stirred for 1 h. The mixture was extracted with ethyl acetate (2 x 200 mL) and the organic layer was washed with water (3 x 200 mL), saturated brine soln. (1 x 200 mL)

and dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford oil. Crude product was purified by flash chromatography over silica gel with 5% EtOAc/n-hexane as eluent to provide **8** (11.46 g, 93% yield) as a light yellow oil which solidifies on standing.
^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.01 – 6.97 (m, 2H), 6.86 (d, J = 8.0 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 1.71 (s, 6H); IR (KBr): ν = 3020, 2839, 2237, 1604, 1595 cm^{-1}.

2-(3,4-dimethoxyphenyl)-2-methylpropanal (**9**): To a solution of 2-(3, 4-dimethoxyphenyl)-2-methylpropanenitrile (**8**) (10 g, 48.7 mmol) in toluene (100 mL) was added drop wise DIBAL (63 mL, 63.4 mmol) (1M solution in toluene) at -78 °C. Reaction mixture was stirred at -78 °C for 2 h, followed by addition of 6N HCl (85 mL). The mixture was extracted with ethyl acetate (5 x 200 mL), and the organic layers were combined, washed with saturated brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure to furnish **9** as oil. (9.5 g, 93% yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.45 (s, 1H), 6.90 - 6.83 (m, 2H), 6.74 (d, J = 2.4 Hz, 1H), 3.88 (s, 6H), 1.46 (s, 6H); IR (KBr): ν = 2974, 2839, 2717, 1726, 1591 cm^{-1}.

3-(3,4-dimethoxyphenyl)-3-methylbutan-2-ol (**10**): To a solution of 2-(3, 4-dimethoxyphenyl)-2-methylpropanal (**9**) (10 g, 48.07 mmol) in diethyl ether (50 mL) was added drop wise methyl magnesium chloride (3M solution in THF) (53.15 mL, 144.2 mmol) under inert conditions at 0 °C. Reaction mixture was warmed to room temperature and stirred at room temperature for 1 h, followed by addition of 6N HCl (42 mL). The mixture was extracted with ethyl acetate (2 x 200 mL), and the organic layers were combined, washed with saturated brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography over silica gel with 10% EtOAc/n-hexane as eluent to furnish **10** (8.6 g, 80% yield) as a light yellow oil.
^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.94 - 6.92 (m, 2H), 6.84 (d, J = 8.8 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.84 – 3.81 (m, 1H), 1.33 (s, 3H), 1.30 (s, 3H), 1.08 (d, J = 6.0 Hz, 3H); MS (EI): m/z (%) = 241.9 (100%) $(M+NH_4)^+$; IR (KBr): ν = 3435, 2937, 1591, 1519, 1464 cm^{-1}.

3-(3,4-dimethoxyphenyl)-3-methylbutan-2-one (**11**): To a solution of oxalyl chloride (38.39 mL, 76.78 mmol) in DCM (140 mL) was added dimethyl sulfoxide (12.98 mL, 153.5 mmol) drop wise at -78 °C and stirring for 30 min. A solution of 3-(3, 4-dimethoxyphenyl)-3-methylbutan-2-ol (**10**) (8.6 g, 38.39 mmol) in DCM (40 mL) was added to the reaction mixture and allowed to stir at -78 °C for 30 min. Triethyl amine (32.1 mL, 230 mmol) was added drop wise at -78 °C and stirred for 2 h. Reaction mixture was warmed to room temperature and was diluted with DCM (100 mL) and quenched with water (150 mL). Organic phase was separated and washed with water (3 x 150 mL), saturated brine soln. (1 x 150 mL) and dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography over silica gel with 3% EtOAc/n-hexane as eluent to furnish **11** (7.0 g, 82% yield) as a light yellow oil.
^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.84 (s, 2H), 6.70 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 1.96 (s, 3H), 1.46 (s, 6H); MS (EI): m/z (%) = 222.9 (100%) $(M+H)^+$; IR (KBr): ν = 1703, 1591, 1465, 1411 cm^{-1}.

1-bromo-3-(3,4-dimethoxyphenyl)-3-methylbutan-2-one (**12**): To a solution of 3-(3, 4-dimethoxyphenyl) -3-methylbutan-2-one (**11**) (13.3 g, 60.1 mmol) in DCM (125 mL), and methanol (68 mL) was added tetra butyl ammonium tribromide (30.72 g, 63.4 mmol) at 0 °C. Reaction mixture was warmed to r.t. and stirred for 7 h. Reaction mixture was concentrated *in vacuo* to remove methanol from the reaction mixture, residue was dissolved in ethyl acetate (100 mL). Organic phase was washed with water (2 x 100 mL), dilute HCl (20 mL), saturated brine soln. (1 x 50 mL) and dried over anhydrous sodium sulfate and evaporated under reduced pressure to furnish **12** as an oil (18.0 g, 100% yield) which was used for next reaction without any further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.87 – 6.82 (m, 2H), 6.67 (d, *J* = 2 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.86 (s, 2H), 1.55 (s, 6H); MS (EI): *m/z* (%) = 302.9 (45%) $(M+H)^+$.

1-amino-3-(3,4-dimethoxyphenyl)-3-methylbutan-2-one HBr (**13**): To a solution of 3-(3, 4-dimethoxyphenyl) -3-methylbutan-2-one (**12**) (18.0 g, 60.0 mmol) in DCM (108.6 mL) as added hexamethyl tetra amine (9.0 g, 63.4 mmol) and stirred for 48 h at room temperature. Dichloromethane was distilled out and residue was dissolved in ethanol (180 mL) and cooled to 0 °C. Concentrated hydrochloric acid (90 mL) was added slowly. Reaction mixture was refluxed for 3 h. Reaction mixture was diluted with ethyl acetate (400 mL). Combined organic layer was concentrated *in vacuo* to furnish **13** as oil, which was used for next reaction without any further purification.

1-(3-(3,4-dimethoxyphenyl)-3-methyl-2-oxobutyl)-3-(4-fluorophenyl)thiourea (**14**): To a solution of 1-amino-3-(3, 4-dimethoxyphenyl)-3-methylbutan-2-one hydrobromide (**13**) (14 g, 44 mmol) in DCM (140 mL) and triethyl amine (24 mL, 176 mmol) was added 4-fluorophenyl isothiocyanate (8.07 g, 52.78 mmol) at 0 °C and stirred at room temperature for 1 h. Reaction mixture was diluted with DCM (100 mL) and quenched with water (150mL). Organic phase was separated and washed with water (3 x 100 mL), saturated brine soln. (1 x 100 mL) and dried over Na_2SO_4 and evaporated under reduced pressure. The residue was triturated in hexane to furnish **14** as a white solid (7.3 g, 42% yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.39 (br s, 1H), 7.30 – 7.16 (m, 2H), 7.13 – 7.07 (m, 2H), 6.90 (br s, 1H), 6.83 (s, 2H), 6.72 (s, 1H), 4.39 (d, *J* = 4 Hz, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 1.54 (s, 6H); MS (EI): *m/z* (%) = 391.0 (80%) $(M+H)^+$.

5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazole-2-thiol (**15**): A suspension of 1-(3-(3, 4-dimethoxyphenyl)-3-methyl-2-oxobutyl)-3-(4-fluorophenyl) thiourea (**14**) (7.3 g, 18.69 mmol) and acetic acid (70 mL), was refluxed for 3 h. Reaction mixture was cooled to room temperature and poured onto chipped ice (100 mL) with vigorous stirring. The resulting precipitate was collected by vacuum filtration and washed with n-hexane (100 mL) and di-isopropyl ether (50 mL). The resulting solid was dried in *vacuo* to furnish **15** as a white powder (5.1 g, 73% yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.76 (s, 1H), 6.87 (t, *J* = 8.8 Hz, 2H), 6.70 (d, *J* = 4 Hz, 1H), 6.67 (d, *J* = 7.6 Hz, 1H), 6.62 - 6.58 (m, 2H), 6.48 - 6.45 (m, 2H), 3.87 (s, 3H), 3.74 (s, 3H), 1.44 (s, 6H); MS (EI): *m/z* (%) = 373 (100%) $(M)^+$; IR (KBr): *v* = 800, 1263, 1601, 2600, 2791, 2930, 3104, 3431 cm^{-1}.

2-((2-bromoethyl)thio)-5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazole (**16**): 1,2-dibromoethane (2.53 g, 13.45 mmol) was added to a solution of **13** in anhydrous acetone (3 mL) and was stirred at room temperature under nitrogen atmosphere for 2 h. Filtration over a short path of silica (20% EtOAc/hexane) and removal of solvent provided **16** (470 mg, 73% yield) as light yellow solid.
^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.02 (s, 1H), 6.94 (t, *J* = 8.8 Hz, 2H), 6.80 - 6.77 (m, 2H), 6.73 - 6.70 (m, 1H), 6.56 - 6.54 (m, 2H), 5.11 (t, *J* = 7.6 Hz, 2H), 4.29 (t, *J* = 7.6 Hz, 2H), 3.88 (s, 3H), 3.81 (s, 3H), 1.58 (s, 6H); IR (KBr): *v* = 3425, 3356, 3057, 2933, 2837, 2127, 1600, 1585, 1464 cm^{-1}.

General procedure for synthesis of 6.
To a solution of **16** (1 mmol) in DMF/MeCN (1:1) (4 mL) was added substituted phenol (1 mmol) and potassium carbonate (3 mmol) at room temperature. The reaction mixture was heated at 70 °C under nitrogen atmosphere for 5 h. The cooled mixture was partitioned between diethyl ether and water. The organic layer was separated, and aqueous layer was extracted with diethyl ether. The combined organic layers were washed with 0.5 *N* NaOH aqueous solutions and further with water and brine, dried over

Na_2SO_4 and concentrated in *vacuo*. The residual oil was loaded on silica gel column and eluted with ethylacetate/n-hexane to furnish **6**.

5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-2-((2-phenoxyethyl)thio)-1H-imidazole (**6a**). % Yield = 78%. Waxy solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.28 - 7.27 (m, 1H), 7.25 - 7.24 (m, 1H), 7.14 (s, 1H), 6.95 - 6.91 (m, 1H), 6.88 - 6.85 (m, 2H), 6.83 - 6.79 (m, 2H), 6.64 (d, *J* = 8.8 Hz, 1H), 6.53 - 6.47 (m, 4H), 4.21 (t, *J* = 6.4 Hz, 2H), 3.85 (s, 3H), 3.71 (s, 3H), 3.41 (t, *J* = 6.4 Hz, 2H), 1.48 (s, 6H); MS (EI): *m/z* (%) = 493.1 (100% $(M)^+$; IR (KBr): *v* = 3020, 2927, 1600, 1589, 1215 cm^{-1}.

4-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)benzo nitrile (**6b**). % Yield = 73%. White powder; mp: 107.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58 (dd, J_1 = 2 Hz, J_2 = 6.8 Hz, 2H), 7.14 (s, 1H), 6.98 (dd, J_1 = 2 Hz, J_2 = 6.8 Hz, 2H), 6.84 - 6.80 (m,2H), 6.65 (d, *J* = 9.2 Hz, 1H), 6.53 - 6.48 (m, 4H), 4.32 (t, *J* = 6.8 Hz. 2H), 3.85 (s, 3H), 3.71 (s, 3H), 3.41 (t, *J* = 6.8 Hz, 2H), 1.49 (s, 6H); MS (EI): *m/z* (%) = 518.1 (100%) $(M)^+$; IR (KBr): *v* = 3416, 3119, 2972, 2834, 1035, 1502, 1256, 1236, 1173, 1024, 836 cm^{-1}.

2-((2-(4-(1H-pyrrol-1-yl)phenoxy)ethyl)thio)-5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazole (**6c**). % Yield = 58%. Light green powder; mp: 131.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29 - 7.27 (m, 2H), 7.20 (s, 1H), 6.99 (t, *J* = 2 Hz, 2H), 6.92 (d, *J* = 8.0 Hz, 2H), 6.85 - 6.80 (m, 2H), 6.65 - 6.63 (m, 1H), 6.54 - 6.52 (m, 2H), 6.49 - 6.47 (m, 2H), 6.31 (t, *J* = 2 Hz, 2H), 3.84 (s, 3H), 3.71 (s, 3H), 3.49 - 3.44 (m, 2H), 1.49 (s, 6H); MS (EI): *m/z* (%) = 558.0 (100%) $(M)^+$; IR (KBr): *v* = 3020, 2974, 1512, 1404, 1215 cm^{-1}.

1-(4-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy) phenyl)-1H-pyrazole (**6d**). % Yield = 62%. Pale yellow powder; mp: 130.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 (dd, J_1 = 0.4 Hz, J_2 = 2.4 Hz, 1H), 7.68 (d, *J* = 1.2 Hz, 1H), 7.58 (dd, J_1= 2.4 Hz, J_2 = 7.2 Hz, 2H), 7.15 (s, 1H), 6.97 (dd, J_1 = 2 Hz, J_2 = 7.2 Hz, 2H), 6.82 (t, *J* = 8.4 Hz, 2H), 6.64 (d, *J* = 8.8 Hz, 2H), 6.54 - 6.48 (m, 4H), 6.43 (t, *J* = 2.4 Hz, 1H), 4.26 (t, *J* = 6.4 Hz, 2H), 3.84 (s, 3H), 3.71 (s, 3H) 3.43 (t, *J* = 6.4 Hz, 2H), 1.49 (s, 6H); MS (EI): *m/z* (%) = 559 (100%) $(M)^+$; IR (KBr): *v* = 3418, 3154, 3109, 2969, 2636, 2835, 1599, 1393, 1170, 1030, 849 cm^{-1}.

1-(4-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy) phenyl)-1H-1,2,4-triazole (**6e**). % Yield = 66%. Pale yellow powder; mp: 60.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (s, 1H), 8.07 (s, 1H), 7.57 - 7.52 (m, 2H), 7.15 (s, 1H), 7.04 - 6.99 (m, 2H), 6.85 - 6.80 (m, 2H), 6.66 - 6.64 (m, 1H), 6.55 - 6.51 (m, 4H), 4.29 (t, *J* = 6.4 Hz, 2H), 3.84 (s, 3H), 3.72 (s, 3H), 3.43 (t, *J* = 6.4 Hz, 2H), 1.49 (s, 6H); MS (EI): *m/z* (%) = 560.0 (100%) $(M)^+$; IR (KBr): *v* = 3433, 3119, 2932, 1734, 1464, 1254, 1150, 1026, 841 cm^{-1}.

2-(4-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy) phenyl)thiazole (**6f**). % Yield = 62%. Waxy solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.88 (d, *J* = 8.8 Hz, 2H), 7.80 (d, *J* = 3.2 Hz, 1H), 7.26 - 7.25 (m, 1H), 7.15 (s, 1H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.82 (t, *J* = 8.8 Hz, 2H), 6.65 (d, *J* = 9.2 Hz, 1H), 6.54 - 6.48 (m, 4H), 4.28 (t, *J* = 6.4 Hz, 2H), 3.84 (s, 3H), 3.71 (s, 3H), 3.43 (t, *J* = 6.4 Hz, 2H), 1.49 (s, 6H); MS (EI): *m/z* (%) = 576.2 (100%) $(M+H)^+$; IR (KBr): *v* = 2974, 2937, 1606, 1487, 1464, 1438, 1411 cm^{-1}.

2-((2-(4-(1H-imidazol-1-yl)phenoxy)ethyl)thio)-5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluoro phenyl)-1H-imidazole (**6g**). % Yield = 73%. White powder; mp: 115.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.14 (s, 1H), 7.65 (s, 1H), 7.53 (d, *J* = 8.8 Hz, 2H), 7.12 (s, 1H), 7.09 – 6.99 (m, 5H), 6.73 (d, *J* = 8.4 Hz,

1H), 6.64 – 6.61 (m, 2H), 6.44 – 6.42 (m, 2H), 4.21 (t, *J* = 6.4 Hz, 2H), 3.68 (s, 3H), 3.55 (s, 3H), 3.36 (t, *J* = 6.4 Hz, 2H), 1.43 (s, 6H); ^{13}C NMR and DEPT (100 MHz, DMSO-d_6): δ = 163.34 (C), 160.89 (C), 157.26 (C), 148.71 (C), 147.69 (C), 143.38 (C), 139.90 (C), 132.58 (C), 131.59 (CH), 131.50 (CH), 130.94 (C), 129.96 (CH), 126.13 (CH), 126.13 (CH), 122.37 (CH), 122.37 (CH), 118.71 (CH), 115.92 (CH), 115.92 (CH), 115.86 (CH), 115.63 (CH), 112.10 (CH), 112.10 (CH), 110.57 (CH), 67.05 (CH_2), 56.06 (CH_3), 55.86 (CH_3), 38.06 (C), 31.54 (CH_2), 29.84 (CH_3), 29.84 (CH_3); MS (EI): *m/z* (%) = 559.45 (100%) $(M+H)^+$; ESI-Q-TOF-MS: *m/z* $[M+H]^+$ calcd for $[C_{31}H_{31}FN_4O_3S + H]^+$: 559.2134; found: 559.2244; IR (KBr): *v* = 3495, 3367, 3063, 2931, 2829, 1602, 1462 cm^{-1}.

5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-2-((2-(4-(2-methyl-1H-imidazol-1-yl)phenoxy) ethyl)thio)-1H-imidazole (**6h**). % Yield = 76%. Pale yellow powder; mp: 51.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.20 (dd, J_1 = 2.4 Hz, J_2 = 6.8 Hz, 2H), 7.14 (s, 1H), 7.05 (s, 1H), 7.01 - 7.69 (dd, J_1 = 2 Hz, J_2 = 6.8 Hz, 2H), 6.97 (s, 1H), 6.85 - 6.81 (m, 2H), 6.65 (d, *J* = 9.2 Hz, 1H), 6.65 - 6.52 (m, 2H), 6.50 - 6.48 (m, 2H), 4.30 (t, *J* = 6.8 Hz, 2H), 3.85 (s, 3H), 3.72 (s, 3H), 3.44 (t, *J* = 6.8 Hz, 2H), 2.86 (s, 3H), 1.49 (s, 6H); MS (EI): *m/z* (%) = 573.4 (100%) $(M)^+$; IR (KBr): *v* = 841, 1464, 1605, 2833, 2932, 2967, 3115, 3433 cm^{-1}.

5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-2-((2-(2-fluoro-4-(1H-imidazol-1-yl)phenoxy)ethyl)thio)-1-(4-fluorophenyl)-1H-imidazole (**6i**). % Yield = 69%. White powder; mp: 60 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (s, 1H), 7.20 (t, *J* = 7.6 Hz, 3H), 7.14 - 7.09 (m, 3H), 6.85 - 6.80 (m, 2H), 6.66 - 6.64 (m, 1H), 6.56 - 6.50 (m, 4H), 4.37 (t, *J* = 6.4 Hz, 2H), 3.85 (s, 3H), 3.72 (s, 3H), 3.43 (t, *J* = 6.4 Hz, 2H), 1.49 (s, 6H); MS (EI): *m/z* (%) = 577.1 (100%) $(M)^+$; IR (KBr): *v* = 3410, 3021, 2974, 2934, 1519, 1439, 1134, 1028 cm^{-1}.

5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-2-((2-(2-fluoro-4-(2-methyl-1H-imidazol-1-yl)phenoxy)ethyl) thio)-1-(4-fluorophenyl)-1H-imidazole (**6j**). % Yield = 64%. White powder; mp: 62 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.20 (t, *J* = 8.8 Hz, 1H), 7.14 (s, 1H), 7.04 - 7.01 (m, 3H), 6.95 (s, 1H), 6.84 (t, *J* = 8.8 Hz, 2H), 6.65 (d, *J* = 8.8 Hz, 1H), 6.58 - 6.48 (m, 4H), 4.39 (t, *J* = 6.4 Hz, 2H), 3.85 (s, 3H), 3.72 (s, 3H), 3.44 (t, *J* = 6.4 Hz, 2H), 2.34 (s, 3H), 1.49 (s, 6H); MS (EI): *m/z* (%) = 591.2 (100 %) $(M+)^+$; IR (KBr): *v* = 3406, 3019, 2972, 2938, 2939, 1599, 1514, 1132, 1026 cm^{-1}.

5-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)-1H-indole (**6k**). % Yield = 71%. White powder; mp: 152.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.9 (s, 1H), 7.28 - 7.26 (m, 1H), 7.24 (s, 1H), 7.11 (s, 1H), 7.03 - 6.99 (m, 3H), 6.71 (d, *J* = 8.8 Hz, 1H), 6.67 - 6.62 (m, 3H), 6.44 - 6.42 (m, 2H), 6.31 (s, 1H), 4.13 (t, *J* = 6.4 Hz, 2H), 3.69 (s, 3H), 3.43 (s, 3H), 3.35 - 3.29 (m, 2H), 1.43 (s, 6H); MS (EI): *m/z* (%) = 532.3 (100%) $(M+H)^+$; IR (KBr): *v* = 3421, 3020, 2945, 1604 cm^{-1}.

5-(2-((5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1H-imidazol-2-yl)thio)ethoxy)-1H-indazole (**6l**). % Yield = 74%. White powder; mp: 160.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.97 (s, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.16 (s, 1H), 7.12 (d, *J* = 2.4 Hz, 1H), 7.02 (dd, J_1 = 2Hz, J_2 = 8.8 Hz, 1H), 6.18 (t, *J* = 8.8 Hz, 2H), 6.64 - 6.62 (m, 1H), 6.54 - 6.47 (m, 4H), 4.26 (t, *J* = 6.4 Hz, 2H), 3.84 (s, 3H), 3.70 (s, 3H), 3.44 (t, *J* = 6.4 Hz, 2H), 1.49 (s, 6H). MS (EI): *m/z* (%) = 533.2 (100%) $(M)^+$; IR (KBr): *v* = 3313, 3061, 2833, 1629, 1589, 1465, 1433 cm^{-1}.

Biological studies:

In vitro assays

hTGR5 Reporter Gene Assay:
Chinese Hamster Ovarian (CHO) K1 cells were plated in 24 well tissue culture plate at a density of 4 X 10^4 cells/well in a Nutrient Mixture F-12 HAM containing 10% Fetal Bovine Serum, cultured for 24 h at 37 °C, 5% CO_2, and then transfected with 50 ng of human (h) TGR5 expression plasmid (pCMV SPORT6 - hTGR5), 300 ng of cAMP-responsive element (CRE)-driven luciferase reporter plasmid (pCRE-Luc) and 100 ng of *β*-galactosidase reporter vector in each well using Polyfect Transfection Reagent (QIAGEN, Cat. No.: 301107) according to the manufacturer's instructions. After 4 h of incubation, cells were washed once with phosphate-buffered saline (PBS) and medium was exchanged to Nutrient Mixture F-12 HAM containing 0.5% Fatty acid free bovine serum albumin (FAFBSA) and 1 mM Sodium Pyruvate Solution. After incubation for another 18 h, cells were treated for 5 h with different concentrations of each compound. After treatment, the cells were lysed with 100 μL of Glo Lysis buffer (Promega, Cat. No.: E2661) and subjected to Luciferase and *β*-Galactosidase assays as described below.

Luciferase and *β*-Galactosidase Assays:
For luciferase assays, 20 μL of cell lysate was mixed with 100 μL of Luciferase Assay Substrate (Promega, Cat. No.: E1501) & Luminescence was measured in HIDEX Multitechnology Plate Reader.
For galactosidase assays, 30 μL of cell lysate was mixed with 30 μL of 2X ONPG Buffer [20 mM sodium phosphate buffer - pH 7.3, 2 mM $MgCl_2$, 100 mM β-mercaptoethanol, and 1.33 mg/mL *o*-nitrophenyl-*β*-D-galactopyranoside (ONPG)] and incubated at 37 °C for 2-10 minutes. The optical density at 415 nm was determined in SpectraMax 190. Normalized luciferase values were determined by dividing the luciferase activity by the galactosidase activity and expressed as fold induction with respect to (w.r.t.) DMSO control.

Reporter Gene Assay: Compound **6g** mediated hTGR5 activity was measured in luciferase based reporter gene assay. CHO-K1 cells were seeded; transiently co-transfected with (a) human or (b) mouse TGR5, CRE-Luc & control β-gal plasmid DNA and treated in presence & absence of the compound **6g** (Figure 1a and Figure 1b). Luciferase activity obtained were normalized with the β-Galactosidase activity and presented as Mean fold induction with respect to DMSO control. EC_{50} of the compound was calculated using Graph Pad Prism.

Figure 1a. Effect of Compound **6g** in hTGR5 Reporter Gene Assay

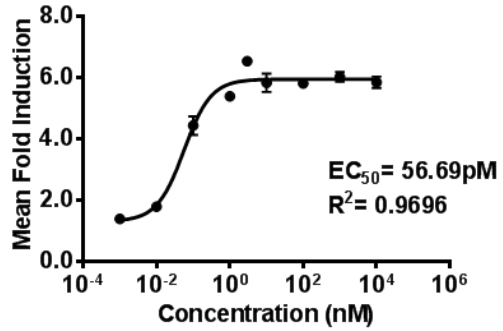
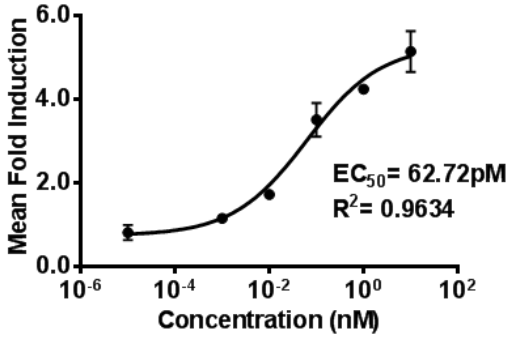
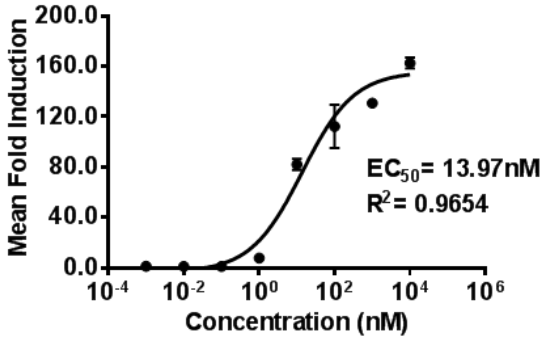


Figure: 1b. Effect of Compound **6g** in mTGR5 Reporter Gene Assay



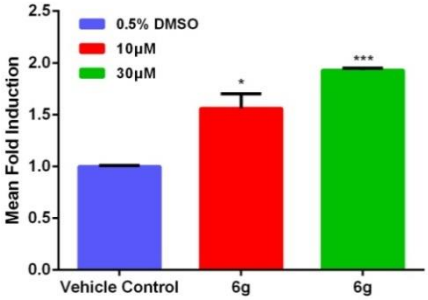
hTGR5 cAMP Assay: Compound **6g** was tested at various concentrations ranging from 1 pM to 10 µM against human TGR5 receptor expressing CHO-K1 cells in cAMP Assay. Briefly cells were seeded into 24 well tissue culture plates, pretreated with the IBMX in serum free medium and then incubated for 30 minutes in presence & absence of compound. Cells were then lysed & cAMP was quantified using Arbor Assay's DetectX® Enzyme immuno Assay kit for direct cAMP measurement. EC_{50} of the compounds were calculated using Graph Pad Prism (Figure 2).

Figure: 2. Effect of Compound **6g** against hTGR5 in cAMP Assay



GLP-1 Secretion Assay: Human NCI-H716 cells were seeded into 96- well culture plate pre-coated with Collagen type I. Twenty-four hours later, the supernatants were replaced by KRB containing 0.18% Glucose & 0.2% BSA and stimulated in presence & absence of compound **6g** for 2 h at 37 °C, 5%CO_2. GLP-1 was measured by EDI™ Total GLP1 ELISA Kit (Epitope Diagnostic Inc.) and normalized to total protein content (Figure 3).

Figure 3. Effect of Compound **6g** in GLP-1 Secretion Assay.[a]



[a]*P < 0.05; ***P < 0.001 versus control. Error bar indicates SEM.

Pharmacokinetic Studies

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.

The goal of these studies were to evaluate the Pharmacokinetic profile of compound **5** in C57Bl/6 mice and of compound **6g** in C57Bl/6 mice and Sprague-Dawley rats following a single intravenous bolus (iv; 1 mg/kg), oral gavage (po; 3 mg/kg). Sprague Dawley (SD) rats (8 adult males), and C57Bl/6 mice (36 adult females), were obtained from Zydus Research Centre, Ahmedabad India, AAALAC Accreditation. All animals were housed in temperature-control rooms with appropriate light/dark cycles. The animals were overnight fasted before oral gavage dosing but were given access to water *ad libitum*. Food was provided 4 h after dosing. Compounds **5** and **6g** were dosed as a solution in 10% NMP and 10% solutol in normal Saline (i.v.) and homogenous suspension (p.o.) in 0.5% Tween 80 in 0.5% Na-CMC as a single dose in both rats (**6g** only) and mice. For mouse composite PK profile was generated. Blood samples were collected at 0.08 (i.v. only), 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post-dose in Na-heparin coated micro centrifuge tubes. Blood samples were centrifuged to separate plasma which were then stored at -70 °C until analysis. PK parameters were calculated by non-compartmental analysis using WinNonlin program, version 5.3 (Pharsight Corp., Mountain View, California). A model was selected based on the vascular (i.v. bolus) or extravascular (p.o.) routes of administration. For the p.o. route, concentration at time zero was assumed to be zero. Plasma concentrations below the limit of quantitation were treated as zero concentration for the purpose of calculating the mean plasma concentration values.

Table 1. Pharmacokinetics Properties of 5 and 6g[a].

Parameters	**5**	**6g**	
Species	Mouse	Mouse	Rat
PO Dose, mg/kg	3	3	3
Tmax (h)	1	0.25	0.5
Cmax (ng/mL)	51.75	788.84	147.71 ± 72.53
AUC (0-t) (ng.h/mL)	73.48	754.21	207.13 ± 98.15
AUC (0-∞) (ng.h/mL)	75.21	759.41	221.10 ± 94.79
T½,po (h)	0.65	0.54	0.68 ± 0.20
MRT(h)	1.18	0.98	1.23 ± 0.35
i.v. Dose, mg/kg	1	1	1
C0 (ng/mL)	682.51	1149.24	584.76 ± 324.44
AUC (0-t) (ng.h/mL)	361.11	349.89	240.00 ± 114.31
AUC (0-∞) (ng.h/mL)	368.14	356.03	240.82 ± 114.53
Vss (L/kg)	1.54	0.83	2.05 ± 0.97
CL (mL/min/kg)	45.52	47.28	79.73± 30.92
T½, iv (h)	0.76	0.33	0.32 ± 0.05
MRT (h)	0.56	0.29	0.41± 0.05
%F	7	71	31

[a] Rat PK data is Mean±SD, N=4, Mouse PK data is mean data because of composite study design, N=3/time point; formulation: PO: 0.5% Tween 80 + 99.5% (0.5%) Na-CMC in water; IV: 10% NMP + 10% solutol + 80% Normal Saline.

Bioanalysis: Concentrations of Compounds **5** and **6g** in plasma were determined using LC-MS/MS methods (Micromass Quattro Micro API, Waters, Milford, MA). Separation was achieved on ACE C18 50 x 4.6mm, 5µ, 100 Å analytical columns. The on-column flow rate was 1 mL/min, with split flow before the detector with approximately half of the flow (0.5 mL/min) going to waste. Sample injection volume was 15 µL. Calibration standards were prepared from 5.00-5000 ng /mL concentration range by adding Compounds **5** or **6g** to control plasma obtained from Sprague-Dawley rats or C57Bl/6 mice. Alprazolam was used as an internal standard in the LC-MS/MS method. Detection of ions was done in positive ionization mode with the following transitions in multiple reaction monitoring mode (MRM): m/z 559.02 → 399.16 for compound **6g**, m/z 515.00 → 357.00, 515.00 → 372.04 for compound **5** and 309.06 → 205.11, 309.06 → 274.06, 309.06 → 281.05 for alprazolam.

Physicochemical Properties:

Plasma Protein Binding

In vitro plasma protein binding of compound **6g** was determined in mouse plasma by the equilibrium dialysis method using liquid chromatography tandem mass spectrometry (LC-MS/MS) detection. **6g** was spiked at 1 µM concentration in mouse plasma (anticoagulant: Na-heparin) and loaded into the donor compartments of a 96-well equilibrium dialyzer (HTD) and dialyzed against human plasma ultra-filtrate (n=3). Human plasma ultra-filtrate 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. Dialysis was performed for 6 h at 37 °C at 100 rpm. After dialysis, ZY12201 concentration in samples from the donor and receiver compartments was measured by LC-MS/MS.

Compound **6g** was found to be highly bound in mouse plasma. Plasma protein binding of **6g** at 1 µM was 99.5% in mouse plasma and 99.98% in rat.

Log P and Log D

The partition co-efficient (Log P) and distribution co-efficient (Log D)[5] of Compound **6g** was determined in purified water: 1-octanol and phosphate buffer pH 7.4: 1-octanol using HPLC-UV detection.

For determination of partition co-efficient (Log P), Compound **6g** was spiked in water: 1-octanol (1:1, v/v) to obtain 50 µM final concentration in a 15 mL tube (n=3). Samples were equilibrated for 16 hours at room temperature by shaking in a multi-vortex (1500 rpm). Following equilibration, the samples were centrifuged at 3000 rpm for 10 min at room temperature. The aqueous and organic phases were separated and analyzed using a high-performance liquid chromatography ultraviolet detection (HPLC-UV) method.

The distribution co-efficient (Log D) was determined similarly in phosphate buffer pH 7.4: 1-octanol (1:1, v/v).

The Log P and Log D of compound **6g** was 4.70 and 4.69 respectively.

Metabolic Stability: Test compound was incubated with 0.5 mg/mL protein of liver microsomes of mouse, rat, and human in phosphate buffer pH 7.4 in the presence of 1 mM NADPH at 37^{o}C, 100 rpm in shaking water bath. After incubation, reactions were terminated by addition of acetonitrile-containing analytical internal standard at 0, 5, 10, 15 and 30 min. The 0 min samples were made in the absence of NADPH and used as control. The metabolic stability in incubated samples was assessed with respect to zero min control samples. Controls included an NADPH- free incubation at 30 min as negative control for chemical instability/ non-NADPH dependent metabolism. All samples were centrifuged and analyzed by liquid chromatography tandem mass spectrometry method.

Table 2. In-vitro ADME Profile of **5** and **6g**.

Parameters	5	6g
Caco2 Permeability	0.38 x 10^{-6} cm/sec	6.1 ± 1.52 x 10^{-6} cm/sec
Solubility (PBS pH 6.8)	> 1 µg/mL	< 1 µg/mL
Solubility (0.1 N HCl)	216 µg/mL	>500 µg/mL

Table 3. Profile of Compound **6g**.

hTGR5 Reporter Gene Assay (EC_{50}):	57 pM
mTGR5 Reporter Gene Assay (EC_{50}):	62 pM
hTGR5 cAMP Assay (EC_{50}):	14 nM
FXR coactivator functional assay (EC_{50}):	6.3 µM
TNF-α Inhibition IC_{50}: (Ex-vivo LPS induced cytokine release in Human whole blood)	251 nM
Molecular Weight:	558.67
cLogP (experimental):	4.7
Log $D_{7.4}$:	4.69
Plasma protein binding:	Rat: 99.98% Human: 95.90%
In vitro Metabolic stability: (% Metabolized)	MLM: 69% RLM: 52% HLM: 74%

In Vivo Studies of Compound 6g

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 GLP-1 secretion activity in C57 mice Model

Male C57 mice of 8-12 week age, bred in Zydus research Centre Animal house will be used for this experiment. Animal will be issued and subjected for 3-7 days acclimatization. On first day animal will be grouped based on non fasting serum glucose levels and kept on fasting for overnight. On second day of the experiment, formulation of test compounds will be prepared and fasting body weight of animals will be recorded. Each animal will receive a single dose of vehicle/test compounds administered per orally as per specified group and dose levels. Exactly 15 min post dosing glucose load (3 g/kg/10 mL) will be administered orally to all the groups. Then exactly after 10 min of glucose load animal will bled from

retro orbital plexus. Blood collection will be done in micro centrifuge tube containing 30 µL of 2% EDTA and with or without 5 µL of DPP-IV inhibitor. Blood samples immediately after collection will be centrifuged and plasma will be separated and analyzed for total GLP-1 level using ELISA kit. The percent or fold change Vs Vehicle will be calculated to determine the total GLP-1 secretion activity for the test compound.

Evaluation of pharmacological efficacy of TGR5 agonist compounds

The high fat diet induced obesity (DIO) in mice exhibits various features of metabolic syndrome in humans. The metabolic syndrome is characterized by abdominal obesity, high triglycerides, impaired fasting glucose and hyperinsulinemia.

In this DIO model, C57 mice of 4-6 week of age will be kept on high (60%) fat diet for 4-6 weeks, when they will become glucose intolerant, used for the single dose Oral Glucose Tolerance Test (OGTT) study for antdiabetic activity. On day 0 non fasting body weight of animals will be recorded, grouped based on non-fasting body weights and will be kept on fasting for overnight, on the day of experiment animals will be subjected for OGTT (oral glucose tolerance test). In OGTT, body weight of all animals will be recorded and mice will receive a single dose of vehicle administered per orally on the basis of body weight, 10 min post dosing single dose of vehicle/ test compounds will be administered, 15 min post dosing blood collection will be done (0 min) and glucose load (2 g/kg/10 mL) will be administered per orally. Blood will then be collected at time points corresponding to 10, 30, 60, and 120 min after glucose load administration. At 10 min plasma will also be collected for insulin and GLP-1 level measurement. Serum will be separated for determination of glucose levels at all time points. Glucose AUC and glucose excursion was calculated using MS excel sheet and graph pad software.

Homology Modeling and Ligand Docking

TGR5 protein model was generated from template protein crystal structure (PDB ID: 1F88)[6] with sequence identity 21% using PRIME[7,8] module of Schrödinger. The model was further process for loop modeling and energy minimization. The lowest energy protein structure conformation was used to generate grid file by specifying the model protein structure ligand as a binding site. The model was evaluated for Backbone conformation by the inspection of the Psi/Phi Ramachandran plot obtained from PROCHECK analysis. The lowest energy protein structure conformation was used to generate grid file by specifying the model protein structure ligand as a binding site. The synthesized ligands were minimized by using Ligprep module of Schrödinger and docked in the TGR5 protein model using GLIDE module of Schrödinger.[9,10] The ligand poses resulting from the molecular docking analysis have been subsequently been used in a retrospective analysis to rationalize the structure-activity relationship of the synthesized compounds. In case, the docking protocol showed a sufficient trend with the EC_{50} values, these protein structure were further analyzed to determine which active site features they contained that might be of importance for protein-ligand interactions.

The TGR5 agonist **5**, was docked into the putative binding pocket in the TGR5 homology model. The ligand binding site in TGR5 is hydrophobic and Tyr 240 appears to be an important amino acid that stabilizes the ligand through H-bond interactions and aromatic stacking interactions. 3,4–Dimethoxyphenyl group of ligand has OH•••O interaction between the oxygen of the meta methoxy and the hydroxy of Tyr 240. This group also forms π-π stacking interaction with Phe 96. The fluoro-phenyl group also shows π-π stacking interaction with Trp 237, which nicely fits in the bottom pocket and perhaps adds to the stability. Modeling studies show that the Van der Waals surface of the active

site of TGR5 receptor has a narrow channel extending from the terminal aryl group of **5** to a hollow opening near the extracellular loop between TM4 and TM5 (Figure 4). Thus, it was hypothesized that, a linker, perhaps with a hydrogen bond acceptor such as oxygen atom, that fits suitably in the narrow channel and provides conformational flexibility and positional adaptability, would be beneficial. Keeping this in view a linker was attached to **5** which provided **6**. Moreover, further substitution on the terminal phenyl ring of **6** may also provide an opportunity for potential hydrogen bonds or π-π interactions.

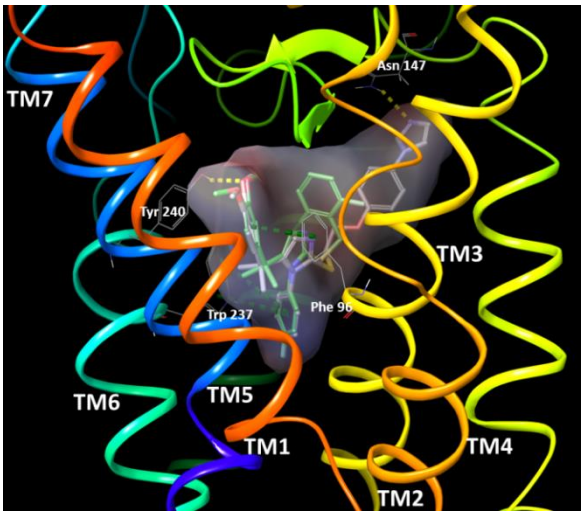


Figure 4. Binding mode of compound **5** (Green) and **6g** (Grey) in homology model of TGR5 receptor.

In addition to the hydrogen-bonds and hydrophobic bonds interactions observed in compound **5**, it was found that the N-3 nitrogen atom of terminal C-4 imidazole group of **6g** involved hydrogen-bonding interactions with Asn 147 of extra cellular loop between TM4 and TM5 which stabilizes the conformations and may be crucial in the activation of TGR5. However, such interaction was not observed with compounds **6c**, **6d** and **6e**. (Figure 5)

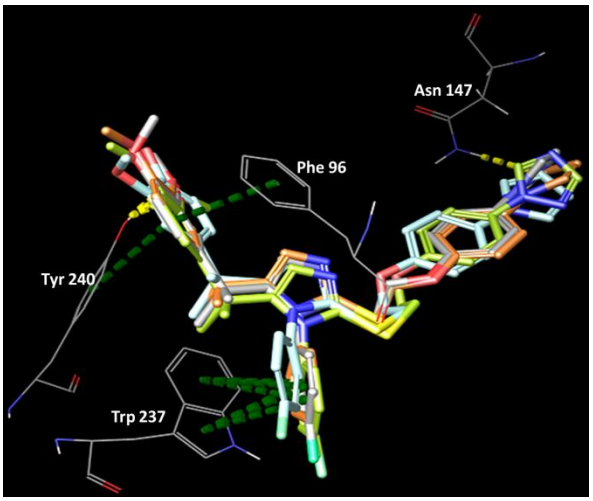


Figure 5. Binding mode of compounds **6c** (Turquoise), **6d** (Orange), **6e** (Yellow green) and **6g** (Grey) in homology model of TGR5 receptor.

Further, in addition to docking, MM GBSA[11] calculations were carried out for the above mentioned molecules to estimate the relative binding affinity and are tabulated below.

Table 4. Glide score and MM-GBSA dG Energy of compounds **6c**, **6d**, **6e** and **6g.**

Compound	Glide Score	MMGBSA dG Bind
6c	-11.49	-118.49
6d	-8.15	-116.84
6e	-8.30	-114.56
6g	-12.53	-121.03

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