Supporting Information

Discovery and Optimization of a Novel Triazole Series of GPR142 Agonists for the Potential Treatment of Type 2 Diabetes Mellitus

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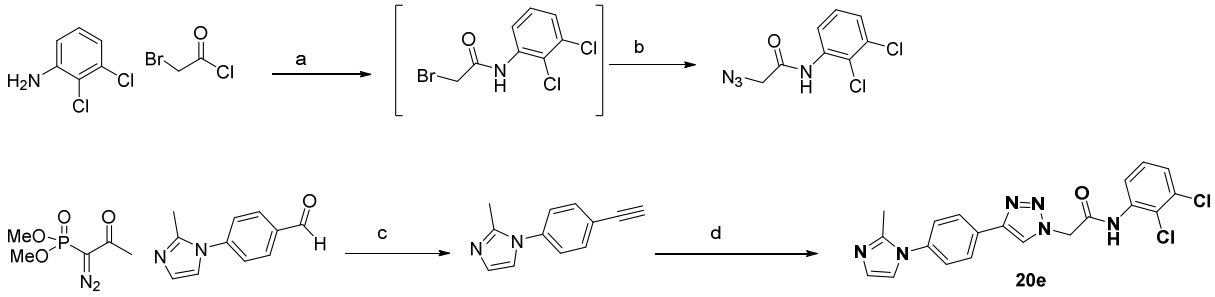
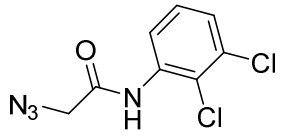
1) Synthesis of compound **20e**

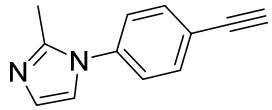


Reagents and conditions: (a) DIEA, DMF, rt, overnight; (b) NaN_3, 70oC, overnight (c) K_2CO_3, MeOH; rt, overnight; d) 2-azido-N-(2,3-dichlorophenyl)acetamide; $CuSO_4$, sodium ascorbate, DMF-H_2O.



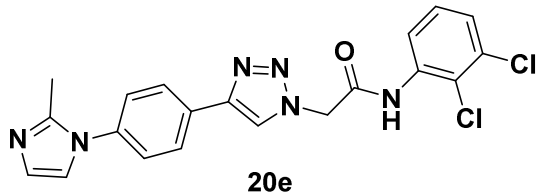
Synthesis of 2-azido-N-(2,3-dichlorophenyl)acetamide

To the stirred solution of bromoacetyl chloride (1.57 g, 10 mmol) in DMF (14 mL) was added DIEA (1.75 ml, 10 mmol) and 2,3-dichloroaniline (1.62 g, 10 mmol). The mixture was stirred at room temperature overnight to afford complete conversion to 2-bromo-N-(2,3-dichlorophenyl)acetamide (Calc. Mass = 281 for C8H6BrCl2NO; found M+3 = 284.) NaN_3 (0.65 g, 10 mmol) and water (0.3 ml) were then added to the above mixture. The mixture was stirred at 70°C for overnight, then cooled to rt to give the product 2-azido-N-(2,3-dichlorophenyl)acetamide in final volume of 16 mL in DMF with calculated concentration as 0.625 M, and will be used as is without further purification. Calc. (M+H) = 244.99 for C8H6Cl2N4O; found M+H = 245.15.



Synthesis of 1-(4-ethynylphenyl)-2-methyl-1H-imidazole

To the suspension of 4-(2-methyl-1H-imidazol-1-yl)benzaldehyde (Cas# 88427-96-7, 1.5 g, 8.06 mmol) and K_2CO_3 (2.23 g, 16.11 mmol) in MeOH (80 mL) under N_2 was added dimethyl (1-diazo-2-oxopropyl)phosphonate (1.7 g, 8.86 mmol) via syringe. The mixture was stirred at room temperatureovernight. The reaction was then quenched by adding satd. $NaHCO_3$ in water (75 mL). The resluting mixture was stirred at room temperature for 3h. Solid was formed. The mixture was filtered and washed with a small amount of MeOH. The filtrate was then extracted with EtOAc for three times. The organic phases were combined, dried over Na_2SO_4, filtered, concentrated and purified using ISCO CombiFlash system (120g silica gel column) and eluting with 0-100% EtOAc in hexane to give desired product (1.06 g, 77% yield). Calc. M+H = 183 for C12H10N2; found M+H = 183. ^{1}HNMR (CD_3OD, 500mHz): δ = 7.65 (d, J = 8.5 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 7.21 (s, 1H), 6.99 (s, 1H), 3.66 (s, 1H), 2.36 (s, 3H).



20e

Synthesis of N-(2,3-dichlorophenyl)-2-(4-(4-(2-methyl-1H-imidazol-1-yl)phenyl)-1H-1,2,3-triazol-1-yl)acetamide (**20e**)

A mixture of 2-azido-N-(2,3-dichlorophenyl)acetamide (0.625M in DMF, 1.49 mL, 0.933 mmol) and 1-(4-ethynylphenyl)-2-methyl-1H-imidazole (100 mg, 0.55 mmol) was added DMF (0.3 mL) and water (0.3 mL), followed by addition of sodium ascorbate (43.5 mg, 0.22 mmol), and $CuSO_4$ (1.0M in water, 0.11 mL, 0.11 mmol). The mixture was stirred at room temperature overnight. The solid was filtered and washed with DMF. The filtrate was purified by reversed HPLC Gilson system (column: YMC HPLC column, 250x20 mm I.D.,eluting solvent: 0-80% acetonitrile (0.05%TFA) in water (0.05% TFA) over 30 min). The fractions were lyophilized to give product 20e as white solid (TFA salt, 160 mg, 54% yield). Calc. M+H = 427.08 for C20H16Cl2N6O; found M+H = 427.08; 429. ^{1}HNMR (CD_3OD, 500mHz): δ = 8.60 (s, 1H), 8.15 (d, J = 8.5 Hz, 2H), 7.84 (d, J = 8.0 Hz, 1H), 7.74 (s, 1H), 7.68-7.64 (m, 3H), 7.43-7.41 (m, 1H), 7.34-7.30 (m, 1H), 5.58 (s, 2H), 2.64 (s, 3H).

2) In Vitro Assays

Human and Mouse GPR142 FLIPR Assay

CHO cells expressing human GPR142 under control of an Mfp-inducible promoter were cultured in Ham's F-12 containing 10% heat-inactiviated FBS, 2 mM glutamate, 100 units/ml each penicillin and streptomycin, 100 ug/ml Hygromycin B and 250 ug/ml Zeocin. For the assay, cells were seeded in 384-well tissue culture plates, at 10,000 cells/well, in 20 ul of culture media containing 1 nM mifepristone and incubated for 2-18 hrs at 37C/5% CO2. Assay buffer consisting of 1X Hanks buffer saline solution, 20 mM HEPES and 0.1% BSA (final concentrations) along with 1.6 mM TR40 and 2.5 mM Probenecid was used to prepare compound titration plates. The Fluo-4AM dye was loaded into the cells by adding 20 ul assay buffer containing Fluo-4AM (2X), 5 mM Probenecid and 1.6 mM TR40. The cells were incubated 1 hr at 37C/5% CO2. The titrated compounds were then added to the cells (13.3 ul at 4X concentrations to give 4000 to 0.244 nM final concentrations) and the fluorescence was measured at 535nm on a FLIPR instrument for 3-5 min. A standard GPR142 agonist was included in each assay and the FLIPR data for this compound is as follows: hGPR142 EC50 = 37.3 nM (n = 52, St. Dev. = 36.3); mGPR142 EC50 = 30.0 nM (n= 57, St. Dev. = 41.8).

Human and Mouse GPR142 IP-One Assay (Cisbio IP-One Tb Kit catalogue #62IPAPEC)

CHO cells expressing human GPR142 under control of an Mfp-inducible promoter were cultured in Ham's F-12 containing 10% heat-inactiviated FBS, 2 mM glutamate, 100 units/ml each penicillin and streptomycin, 100 ug/ml Hygromycin B and 250 ug/ml Zeocin. For the assay, cells were seeded in 384-well tissue culture plates, at 20,000 cells/well, in 50 ul of culture media containing 2 nM mifepristone and incubated for 2-18 hrs at 37C/5% CO2. The media was removed and 10 ul of IP1 stimulation assay buffer (1X) containing titrated compound (4000 to 0.244 nM final concentrations) was added to the cells followed by incubation at 37C/5% CO2 for 30-60 min. The IP1-d2 reagent (5 ul) and anti-IP1-Tb reagent (5 ul) were added to the cells and incubated at room

temperature in the dark for 60 min. The Fluorescence was measured on an Envision plate reader (Excitation at 340nm; Emission at 615nm). A standard GPR142 agonist was included in each assay and the IP data for this compound is as follows: hGPR142 EC50 = 235 nM (n = 42, St. Dev. = 19.5); mGPR142 EC50 = 6.3 nM (n= 44, St. Dev. = 6.3).

3) Glucose Tolerance Test

Mouse oGTT procedure
C57BL/6 mice (Taconic), 12 weeks old were fasted 6 hrs. Blood glucose was measured at -60 min with Glucometer prior oral dosing with compound or vehicle. Mice were oral challenge with 5 g/kg dextrose at time 0, Tail blood glucose was measured at time 0 and 20, 40, 60, and 90 min post dextrose. Mice challenge with water as a background. Calculation: % inhibition is determined as difference from vehicle group AUC with water Group (ref: Tan, C. P.; Feng, Y.; Zhou, Y-P.; Eiermann, G. J.; Petrov, A.; Zhou, C.; Lin, S.; Salituro, G.; Meinke, P.; Mosley, R.; Akiyama, T. E.; Einstein, M.; Kumar, S.; Berger, J. P.; Mills, S. G.; Thornberry, N. A.; Yang, L.; Howard, A. D. Selective small-molecule agonists of G protein-coupled receptor 40 promote glucose-dependent insulin secretion and reduce blood glucose in mice. Diabetes 2008, 57(8), 2211-2219.)

4) Computational modeling

DFT calculations were performed using Gaussian09.[1] Conformational space was exhaustively sampled using three conformer generators (rules-based and random displacement) followed by molecular mechanics minimization using MMFF94, a workflow that has been previously published.[2] DFT with the B3LYP functional[3] and the 6-31G** basis set[5-7] was used to identify the lowest energy conformers contributing to the Boltzmann distributions for each structure at 298.15 K. All stationary points were confirmed with frequency calculations.

The parent compound **20d** is not fully planar, but has a dihedral angle of 37.7° between the imidazole and phenyl. The effect of methylation of the imidazole induces an increase of 10° in the dihedral between the imidazole and phenyl rings. There is little effect on conformation moving from **20e** to **21b**. The largest distortion of the preferred conformation of **20d** is when the amide is methylated moving the preferred conformer of **21a** to a cis amide and reducing the profile of the overall shape of the molecule. Rotamers of the phenyl-imidazole (2 dominant conformers with opposite dihedrals) are equi-energetic and only one conformer for each molecule is depicted. It is possible that methylation at any point may interrupt crystal packing.

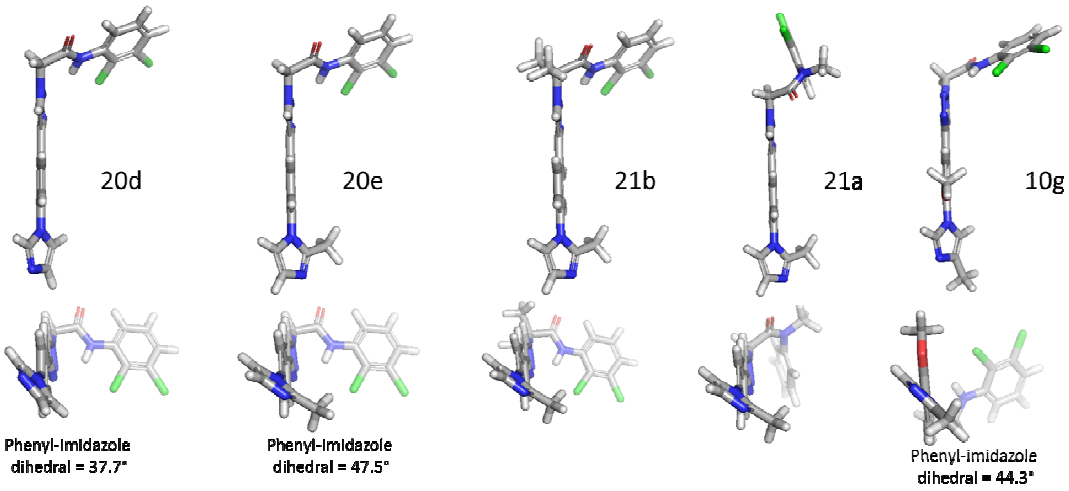


Figure S1: Global minima conformations for relevant compounds.

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