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SYNTHESIS OF A NEW ISOXAZOLE COMPOUNDS AS GPR-40 AGONISTS

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Abstract: In this letter, the series of isoxazole triple bond propanoic acid compounds design, synthesis, structure–activity relationship (SAR) and the ability to modulate the activity of GPR40 are described. The Systematic replacement of substituted aryl groups, various substitutions on isoxazole and optimization of chain length led to identification of potent GPR40 agonists. In order to identify candidate suitable for in vivo validation of the target, pharmacokinetic properties were determined and further profiling of the compound is presented. The compound 1 may prove useful for the treatment of Type 2 diabetes.

Keywords: GPR40, diabetes, propanoic acid, SAR

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INTRODUCTION

In the recent past, GPR40, GPR120, GPR41 and GPR43 exemplify a growing number of GPCRs that have been shown to be activated by free fatty acids. GPR40 and GPR120 are activated by medium to long-chain free fatty acids (e.g., linoleic and palmitic acids) whereas short-chain fatty acids (e.g., acetic and propionic acid) activate GPR41 and GPR43 [1-3]. Each GPR displays a characteristic tissue distribution. GPR40 is preferentially expressed in pancreatic beta cells [4] .Its gene is located downstream of CD22 on chromosome 19q13.1 [5], close to a region that has shown linkage to elevated serum triglycerides in families with type 2 diabetes [6]. Two polymorphisms, an Arg211His substitution and a rare Asp175Asn mutation have been identified in the GPR40 gene [7] Lately, GPR40 expression was also seen in omental adipose tissue and pancreatic alpha cells [8]. It is well established that fatty acids function acutely to maintain basal insulin secretion and to 'prime' the islet β-cells to respond to glucose following a prolonged fasting [9] (Gravena C et al., 2002). Furthermore the finding that activation of the receptor resulted in elevation of intracellular Ca2+ via coupling to Gαq/11, leading to activation of PKC suggested a possible role for GPR40 in insulin secretion [10-12] .Down-regulation of GPR40 expression in the mouse insulinoma cell lines resulted in a decrease in the ability of fatty acids to potentiate insulin secretion. [13-14] GPR40 was shown to play a role not only in fatty acid modulation of insulin secretion, but also in GSIS after high-fat feeding. [15] In light of the literature, GPR40 could be construed as a potential target for Type II Diabetes and a small molecule GPR40 ligand could help in regulate the insulin secretion.

In literature, different classes of GPR40 agonists are reported as shown in Figure 1. The substituted carboxylic acid compounds from sanofi-aventis (1, 2)[16-17], Propionic acid compounds from GSK (3, 4)[18-20], J&J (5)[21], Takeda (6)[22] and Amgen Inc (7)[23], bicyclic compound from Merck (8)[24], cyclopropane carboxylic acid compound (9)[25] and TAK-875 (10)[26] from Takeda Pharmaceuticals. Though there are different scaffolds identified as GPR40 agonists, there is still a need to identify novel scaffolds as GPR40 agonists. We have synthesized several phenylpropanoic acids having different linkers that have shown nanomolar potency. The present article describes the synthesis, GPR40 agonist activity and SAR of the synthesized compounds. A review of the literature revealed that only long chain fatty acids exhibited GPR40 agonist activity at nanomolar potency. It has been evaluated, carboxylic acids of varying chain length and found that only phenylpropanoic acids were effective as GPR40 agonists at nanomolar concentration [27, 28]. Prior art searches limited to the incorporation of several linkers, such as ether, aryl amino, sulphonyl groups. Hence it was proposed to design and synthesize novel isoxazole triple bond propanoic acids containing different linker, screen them for GPR40 agonist activity and derive an SAR.

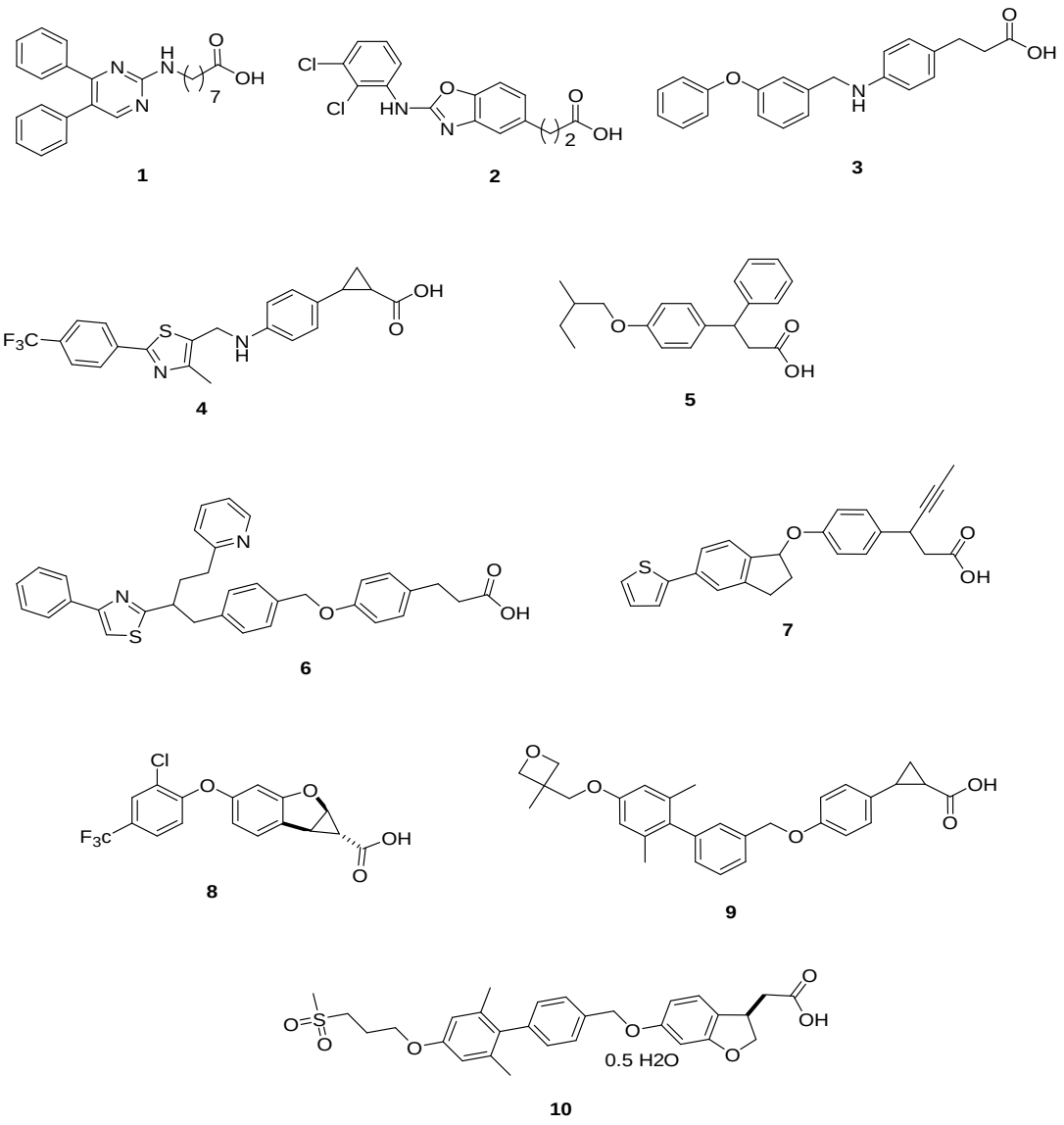
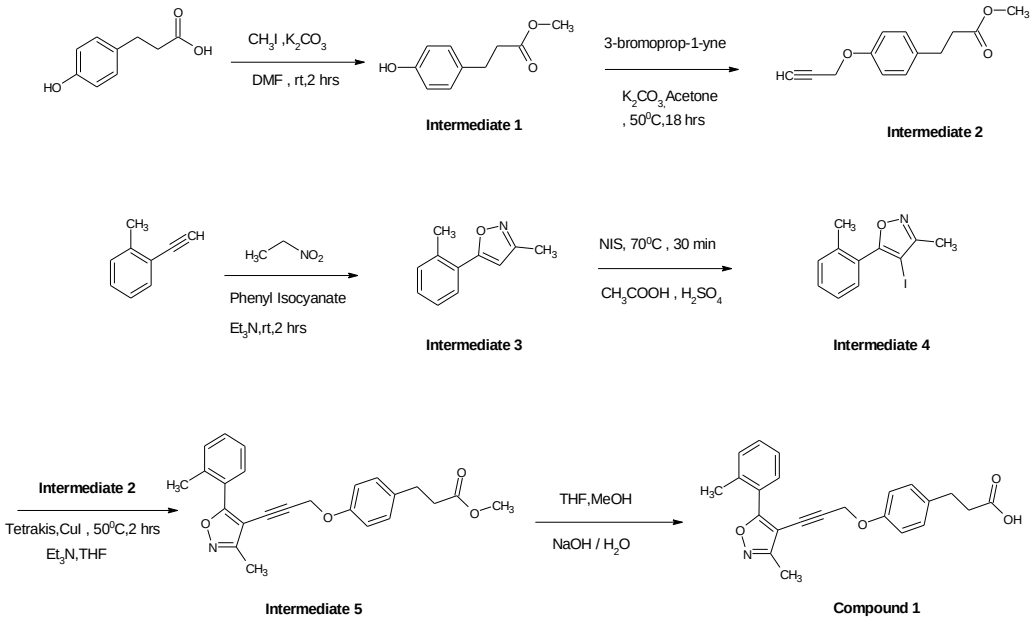


Figure 1. Structures of GPR40 agonists reported in literature

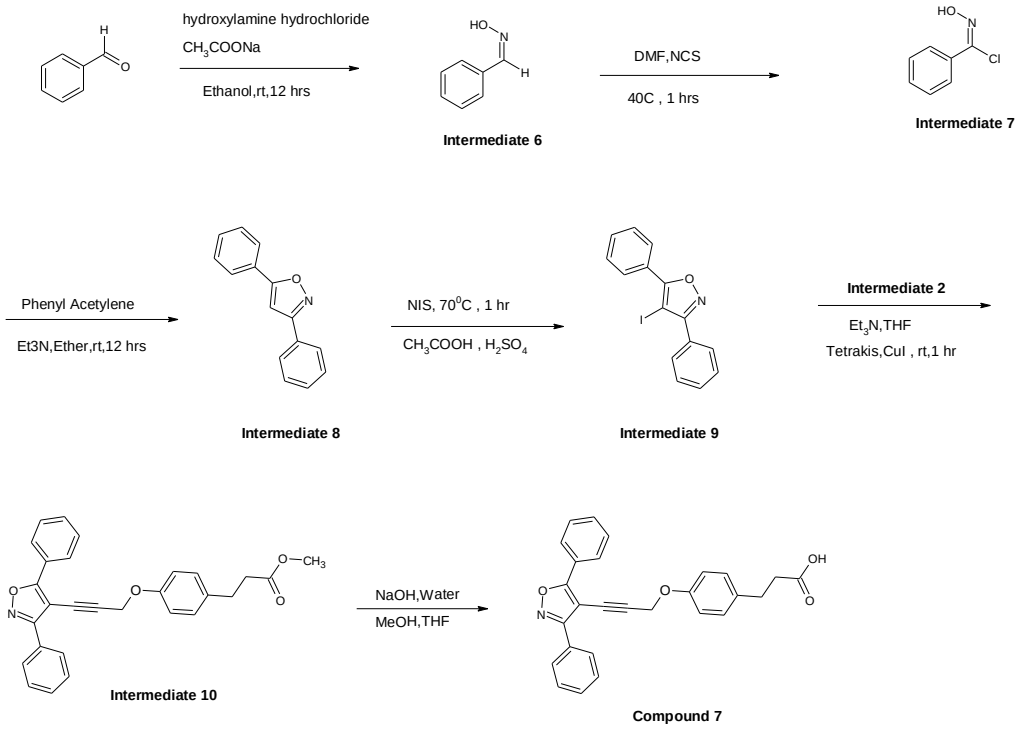
Materials and Methods

In an effort to evaluate the isoxazole substituent activity, a series of substituted 3-{4-[3-(5-Phenyl-isoxazol-4-yl)-prop-2-ynyloxy]-phenyl}-propionic acid compounds were examined (1-8).The synthesis of compounds 1-6, in which different substituted aryl acetylene compounds were cyclised with 2-nitroethane and phenyl isocyanate to give isoxazole intermediates which upon iodination gave different substituted Iodo phenyl isoxazole compounds. The coupling reaction with Methyl 3-[4-(prop-2-yn-1-yloxy) phenyl] propanoate, followed by hydrolysis gives the compounds 1-6. A general synthesis of compounds 1-6 is shown in Scheme 1.

Scheme 1



The synthesis of compounds 7-8, in which different aldehyde compounds were converted to hydroxyl amine then to N-Hydroxy carboximidoyl chloride and cyclised with phenyl acetylene to give diphenyl oxazole which further halogenated and reacted with with Methyl 3-[4-(prop-2-yn-1-yloxy) phenyl] propanoate, followed by hydrolysis gives compounds 7-8. A general synthesis of compounds 7-8 is shown in **Scheme 2**.



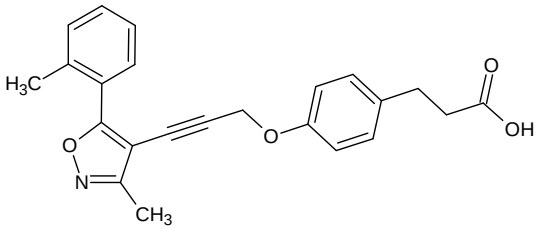
The compounds 1- 8 were screened against human GPR40 through calcium flux Assay. Calium Flux Assay to detect GPR40 activation: CHO-K cells stably expressing hGPR40 were selected in a media containing Neomycin/G418. The cells were plated at a concentration of 20,000 cells per well in black 96-well clear bottom tissue culture treated plates. The cells were cultured for 24 h at 37°C in a humidified 5% CO_2 air environment to allow for protein expression.

The next day, after removal of media from wells, Fluo-4 NW (Invitrogen) is added at 100 ul/well and the cells were incubated for 30 min at 37°C and for a further 30 min at RT. All test compounds were diluted to appropriate concentrations in HEPES buffer. Compounds were added to the cells and the wells were read in the BioTEK synergy reader and readings were taken for 4 min with an interval of 5 s, the results were shown in table 1 & 2.

EXPERIMENTAL:

Compound 1

3-[4-({3-[3-Methyl-5-(2-methylphenyl)-1, 2-oxazol-4-yl] prop-2-yn-1-yl} oxy) phenyl] propanoic acid (1)



1

Intermediate 1: Methyl 3-(4-hydroxyphenyl) propanoate

To a 1000 mL RB flask fitted with magnetic stirrer was charged 250 mL of DMF, 3-(4-Hydroxy-phenyl)-propionic acid (25.0 g, 150.43 mmol) and K_2CO_3 (41.58 g, 300.87 mmol). The resulting mixture was stirred at RT for 30 minutes. Methyl Iodide (25.627 g, 180.51 mmol) was added to the resulting mixture which was precooled to 0 °C. The resulting mixture was stirred at RT for 3 h. After completion of the reaction (reaction monitored by TLC), the solvent was removed under reduced pressure and the crude mass was dissolved in ethyl acetate (250 mL). The solution was washed with water (250 mL), saturated sodium bicarbonate solution (250 mL X 2), saturated brine solution (250 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The product was obtained as yellow color oil (26 g, Yield: 95.9 %): MS (ESI, 120 eV) : m/z = 178.9 $(M-H)^+$; ^{1}H NMR (300MHz, $CDCl_3$): δ 6.97-7.00(d, 2H), 6.66-6.69(d, 2H), 4.94(s, 1H), 3.59(s, 3H), 2.78-2.83(t, 2H), 2.50-2.55(t, 2H).

Intermediate 2: Methyl 3-[4-(prop-2-yn-1-yloxy) phenyl] propanoate

To a 100 mL RB flask fitted with magnetic stirrer was charged 50 mL of acetone, methyl 3-(4-hydroxyphenyl) propanoate (2.5 g, 13.9 mmol), K_2CO_3 (5.75 g, 41.7 mmol). The RM was cooled to 0 ^{0}C, 3-bromoprop-1-yne (2.48 g, 20.8 mmol) was added drop wise. The resulting mixture was stirred at 50 °C for 18 h. After completion of the reaction (reaction monitored by TLC), the RM was filtered through sintered funnel and the filtrate was concentrated. The residue was dissolved in ethyl acetate (100 mL). The organic layer was washed with cold 10 % sodium hydroxide solution (25 mL), water (50 mL X 2) and saturated brine solution (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The product was obtained as pale yellow liquid (2.5 g, yield: 82.6 %). ^{1}H NMR (300MHz, $CDCl_3$): δ 7.06(d, 2H), 6.82 – 6.85 (m, 2H), 4.60(d, 2H), 3.60(s, 3H), 2.83(t 2H), 2.53(t, 2H), 2.44(t, 1H).

Intermediate 3: 3-Methyl-5-(2-methylphenyl)-1, 2-oxazole

To a 50 mL RB flask fitted with magnetic stirrer were charged with 15 mL of toluene, 1-ethynyl-2-methyl-benzene (1.5 g, 12.9 mmol), 2-nitroethane (1.16 g, 1.1 mL, 15.5 mmol) and phenyl isocyanate (1.84 g, 15.5 mmol). The RM was cooled to 0 °C and triethylamine (3.91 g, 38.7

mmol) was added drop wise. The RM was stirred at RT for 2 h. The RM was diluted with ethyl acetate (50 mL). The organic layer was washed with water (50 mL X 2), saturated brine solution (50mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude was triturated with diethyl ether (25 mL) and the solid was removed by filtration. The ether layer was evaporated and crude was purified by column chromatography on silica gel (100/200 mesh), using ethyl acetate and petroleum ether (60-80) as eluant. The product was obtained as colorless oil (0.31 g, yield: 13.9 %). MS (ESI, 120 eV): m/z =174 $(M+H)^+$

Intermediate 4: 4-Iodo-3-methyl-5-(2-methylphenyl)-1, 2-oxazole

To a 10 mL RB flask fitted with magnetic stirrer was charged 5 mL of acetic acid. To the stirred solvent was added 3-methyl-5-o-tolyl-isoxazole (0.31 g, 1.79 mmol), followed by N-iodosuccinimide (0.44 g, 1.971 mmol) and concentrated sulfuric acid (0.5 mL) under nitrogen atmosphere. The RM was stirred at 70 °C for 30 minutes. The RM was poured into ice cooled water (10 mL). The aqueous layer was extracted with ethyl acetate (25 mL). Then the organic layer was washed with saturated $NaHCO_3$ solution (20 mL X 2), saturated brine solution (25 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product was obtained as pale yellow oil (0.5 g, yield: 96.6 %). ^{1}H NMR (300MHz, $CDCl_3$): δ 7.31 - 7.38 (m, 2H), 7.23 - 7.26 (m, 2H), 2.30(s, 3H), 2.26(s, 3H).

Intermediate 5: Methyl 3-[4-({3-[3-methyl-5-(2-methylphenyl)-1, 2-oxazol-4-yl] prop-2-yn-1-yl} oxy) phenyl] propanoate

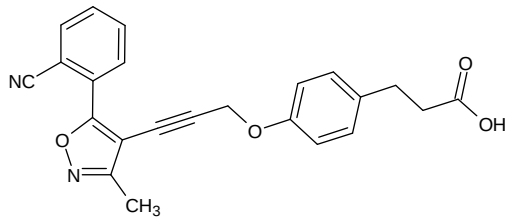
To a 25 mL two neck RB flask fitted with magnetic stirrer were charged 2 mL of triethylamine and 5 mL of tetrahydrofuran. To the stirred solvent was added 4-iodo-3-methyl-5-o-tolyl-isoxazole (0.5 g, 1.66 mmol) and 3-(4-prop-2-ynyloxy-phenyl)-propionic acid methyl ester (0.36 g, 1.66 mmol) under argon atmosphere. The RM was purged with argon gas for 30 minutes, tetrakis (triphenylphosphine) palladium (0) (0.19 g, 0.166 mmol) and copper (I) iodide (0.03 g, 0.166 mmol) were added and the RM was purged with argon gas for another10 minutes. The reaction was stirred at 50 °C for 1.5 h. After 1.5 h, the RM was filtered through celite and the residue was washed with ethyl acetate (75 mL). The organic layer was washed with saturated ammonium chloride solution (50 mL), water (50 mL), saturated brine solution (10 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (100/200 mesh), using ethyl acetate and petroleum ether (60-80) as eluent. The product was obtained as colorless oil (0.21 g, yield : 32.4 %): ^{1}H NMR (300MHz, $CDCl_3$): δ 7.57(d, 1H), 7.31(t 1H), 7.23(s, 1H), 7.14(t, 1H), 7.05(d, 2H), 6.83(d, 2H), 4.21(s, 2H), 3.68(s, 3H), 2.84(t, 2H), 2.54(t, 2H), 2.37(s, 3H), 2.26(s, 3H).

Compound 1: 3-[4-({3-[3-Methyl-5-(2-methylphenyl)-1, 2-oxazol-4-yl] prop-2-yn-1-yl} oxy) phenyl] propanoic acid

To a 25 mL RB flask fitted with magnetic stirrer was charged 5 mL of tetrahydrofuran. To the stirred solvent was added 3-{4-[3-(3-methyl-5-o-tolyl-isoxazol-4-yl)-prop-2-ynyloxy]-phenyl}-propionic acid methyl ester (0.21 g, 0.539 mmol), followed by methanol (2 mL). The RM was cooled to 0 °C and sodium hydroxide (0.55 g) in water (2 mL) was added drop wise. The RM was stirred at RT for 1 h. After 1 h, the solvents were concentrated and the crude was dissolved in minimum amount of water (2 mL) and the aqueous layer was washed with ether (5 mL). The aqueous layer was acidified to pH 3 with 1N HCl and extracted with ethyl acetate (10 mL). The organic layer was washed with saturated brine solution (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure and the solid was triturated with n-hexane and dried thoroughly. The product was obtained as white solid (0.049 g, yield: 24.3 %): Purity: 95.07 %.

Compound 2

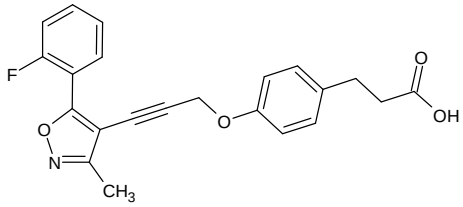
3-[4-({3-[5-(2-Cyanophenyl)-3-methyl-1, 2-oxazol-4-yl] prop-2-yn-1-yl} oxy) phenyl] propanoic acid (2)



2

Compound 3

3-[4-({3-[5-(2-Fluorophenyl)-3-methyl-1, 2-oxazol-4-yl] prop-2-yn-1-yl} oxy) phenyl] propanoic acid (3)



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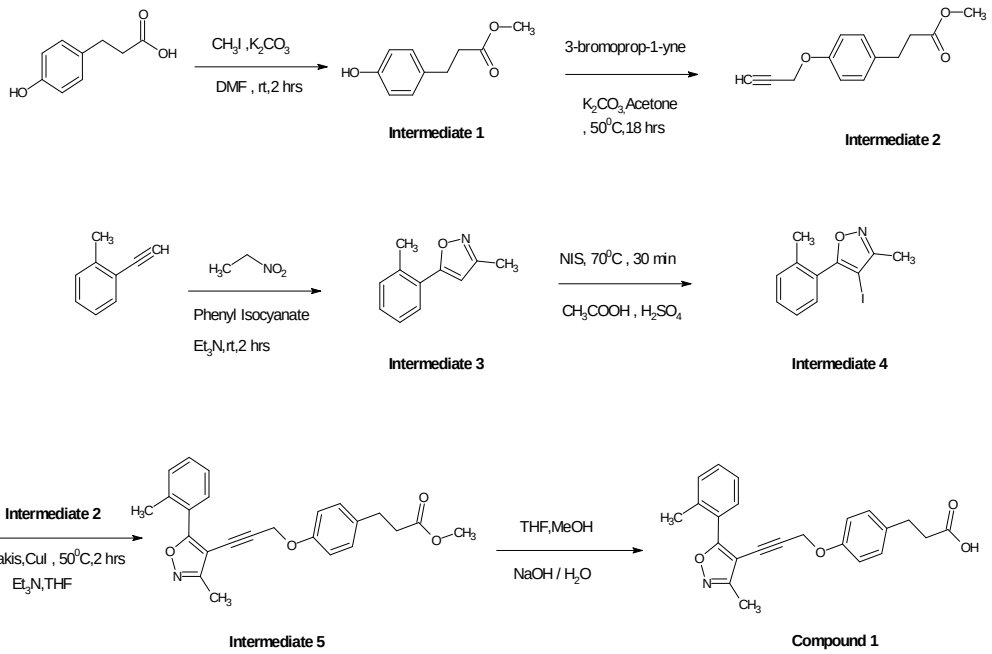
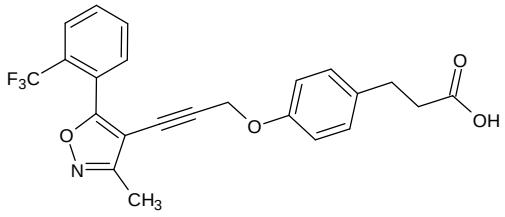


Compound 4

3-{4-[(3-{3-Methyl-5-[2-(trifluoromethyl) phenyl]-1, 2-oxazol-4-yl} prop-2-yn-1-yl) oxy] phenyl} propanoic acid (4)



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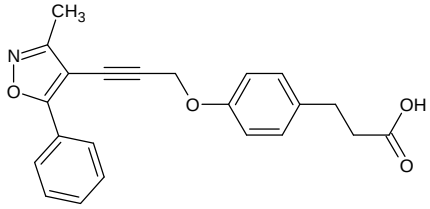
Compound 5

3-(4-{[3-(3-Methyl-5-phenyl-1, 2-oxazol-4-yl) prop-2-yn-1-yl] oxy} phenyl) propanoic acid (6)



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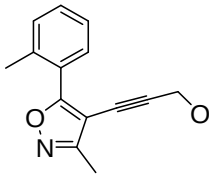
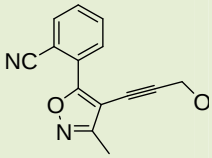
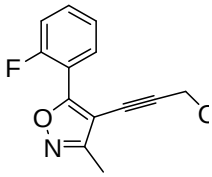
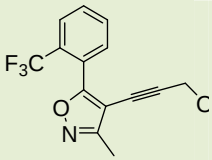
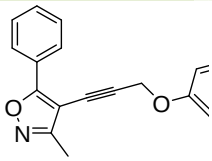
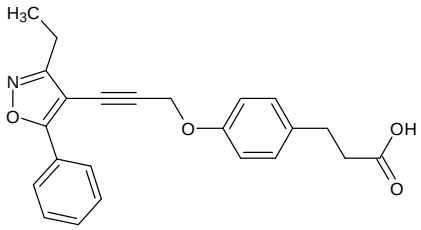
NMR FOR SAR TABLE-1 COMPOUNDS

No	Structure	^{1}H NMR	m/z $(M+H)^+$
1		**δ 12.1 (br s, 1H), 7.58(d, 1H), 7.45 - 7.50 (m, 1H), 7.39 (d, 1H), 7.30(t, 1H), 7.15(d, 2H), 6.91(d, 2H), 5.01(s, 2H), 2.76(t, 2H), 2.47(s, 2H), 2.34(s, 3H), 2.27(s, 3H).**	**376.1**
2		δ 12.10 (br s, 1H), 8.07 - 8.10 (m, 1H), 8.01 - 8.04 (m, 1H), 7.76 - 7.79 (m, 2H), 7.17(d, 2H), 6.95(d, 2H), 5.08(s, 2H), 2.77(t, 2H), 2.47(s, 2H), 2.31(s, 3H).	387.1
3		δ 7.87(t, 1H), 7.62 - 7.69 (m, 1H), 7.45(t, 1H), 7.32(t, 1H), 7.12(d, 2H), 6.90(d, 2H), 5.04(s, 2H), 2.68(t, 2H), 2.28(s, 3H), 2.09(t, 2H)	380.1
4		δ 7.99(d, 1H), 7.81 - 7.87 (m, 2H), 7.73 - 7.79 (m, 1H), 7.08(d, 2H), 6.82(d, 2H), 4.94(s, 2H), 2.67(t, 2H), 2.3(s, 3H), 2.08(t, 2H)	430.1
5		δ 7.93 - 7.96(dd, 2H), 7.32 - 7.38 (m, 3H), 7.11(d, 2H), 6.92(d, 2H), 4.92(s, 2H), 2.86(t, 7.8 Hz, 2H), 2.59(t, 7.8 Hz, 2H), 2.25(s, 3H).	362.1

Compound 6

3-(4-{[3-(3-Ethyl-5-phenyl-1, 2-oxazol-4-yl) prop-2-yn-1-yl] oxy} phenyl) propanoic acid



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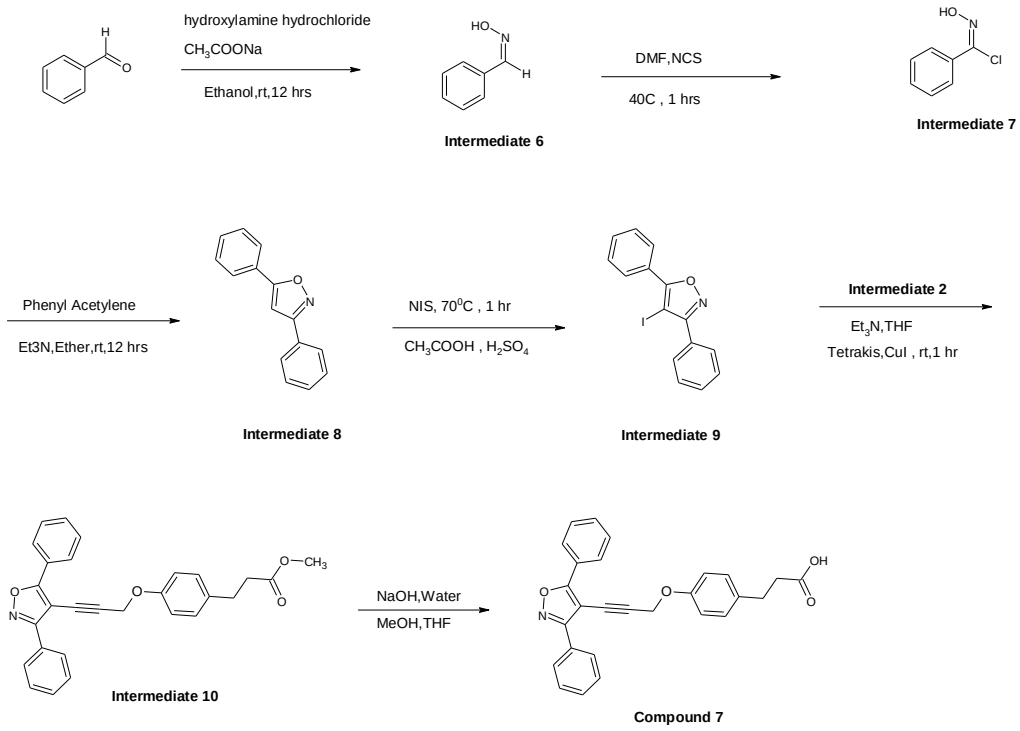
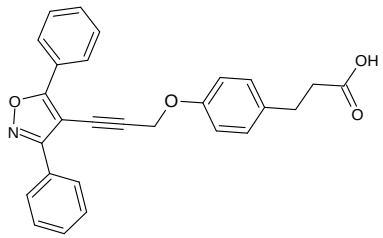
Scheme 2



Compound 7

3-(4-{[3-(3, 5-Diphenyl-1, 2-oxazol-4-yl) prop-2-yn-1-yl] oxy} phenyl) propanoic acid



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Intermediate 6: (E, Z)-N-Hydroxy-1-phenylmethanimine

To a 100 mL RB flask fitted with magnetic stirrer was charged 15 mL of ethanol. To the stirred solvent was added benzaldehyde (5 g, 47.1 mmol), followed by addition of hydroxylamine hydrochloride (9.82 g, 141.3 mmol) and sodium acetate (11.59 g, 141.3 mmol). The RM was stirred at RT for 12 h. The RM was concentrated by removal of solvent; water (100 mL) was added. The aqueous layer was extracted with ethyl acetate (100 mL). The organic layer was washed with saturated brine solution (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The product was obtained as colorless liquid (6.0 g, yield: 94.3 %), without further purification it was taken for next step.

Intermediate 7: *N*-Hydroxybenzenecarboximidoyl chloride

To a 100 mL RB flask fitted with magnetic stirrer was charged 30 mL of DMF. To the stirred solvent were added (E, Z)-N-hydroxy-1-phenylmethanimine (6 g, 49.5 mmol) followed by addition of N-chlorosuccinimide (6.94 g, 52 mmol). The RM was stirred at 40 °C for 1 h. The RM was diluted with ethyl acetate (100 mL) and the organic layer was washed with saturated ammonium chloride solution (50 mL X 3), saturated brine solution (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The product was obtained as pale yellow oil (6 g, Yield: 94.3 %,) without further purification it was taken for next step.

Intermediate 8: 3, 5-Diphenyl-1, 2-oxazole

To a 100 mL RB flask fitted with magnetic stirrer was charged 20 mL of diethyl ether. To the stirred solvent were added phenyl acetylene (1.31 g, 12.9 mmol) and N-Hydroxybenzenecarboximidoyl chloride (2 g, 12.9 mmol). The RM was cooled to 0 ^{0}C and triethylamine (1.95 g, 19.4 mmol) was added drop wise. After addition, the RM was stirred at RT for 12 h. The RM was diluted with ethyl acetate (50 mL). The organic layer was washed with water (50 mL), saturated brine solution (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude was purified by column chromatography on silica gel (100/200 mesh), using ethyl acetate and petroleum ether (60-80) as eluent. The product was obtained as a pale yellow liquid: ^{1}H NMR (300MHz, $CDCl_3$): δ 7.75-7.81(m, 4H), 7.35-7.45(m, 6H), 6.76(s, 1H).

Intermediate 9: 4-Iodo-3, 5-diphenyl-1, 2-oxazole

To a 100 mL RB flask fitted with magnetic stirrer was charged 6 mL of acetic acid. To the stirred solvent was added 3, 5-diphenyl-1, 2-oxazole (1.2 g, 5.4 mmol), followed by addition of N-iodosuccinimide (1.22 g, 5.4 mmol) and concentrated sulfuric acid (2 mL) under nitrogen atmosphere. The RM was stirred at 70 °C for 1 h. The RM was poured in to ice cooled water (25

mL) and the aqueous layer was extracted with ethyl acetate (25 mL X 2). The organic layer was washed with saturated $NaHCO_3$ solution (25 mL), saturated brine solution (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The product was obtained as pale yellow oil MS (ESI, 120 eV): m/z = 348 $(M+H)^+$.

Intermediate 10: Methyl 3-(4-{[3-(3, 5-diphenyl-1, 2-oxazol-4-yl) prop-2-yn-1-yl] oxy} phenyl) propanoate

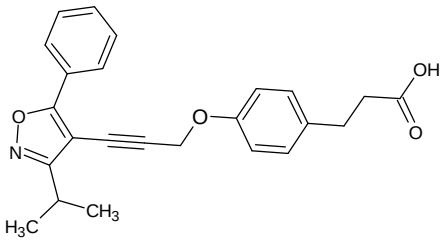
To a 50 mL two neck RB flask fitted with magnetic stirrer were charged 5 mL of triethylamine and 10 mL of tetrahydrofuran. To the stirred solvent was added 4-iodo-3, 5-diphenyl-1, 2-oxazole (0.5 g, 1.4 mmol) and methyl 3-[4-(prop-2-yn-1-yloxy) phenyl] propanoate (0.31 g, 1.4 mmol) under argon atmosphere. The RM was purged with argon gas for 30 minutes then tetrakis (triphenylphosphine) palladium (0) (0.16 g, 0.14 mmol) and copper (I) iodide (0.05 g, 0.28 mmol) were added, the RM was purged with argon gas for another 10 minutes. The RM was stirred at RT for 1 h. The RM was filtered through celite pad and the residue was washed with ethyl acetate (100 mL). The organic layer was washed with water (50 mL), saturated ammonium chloride solution (50 mL) and saturated brine solution (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (100/200 mesh), using ethyl acetate and petroleum ether (60-80) as eluent. The product was obtained as colorless oil (0.1 g, Yield: 15.8 %): : ^{1}H NMR (300MHz, $CDCl_3$) : δ 8.02-8.05(d, 2H), 7.89-7.91(d, 2H), 7.34-7.46(m, 6H), 7.06-7.09(d, 2H), 6.88-6.91(d, 2H), 4.91(s, 2H), 3.58(s, 3H), 2.82-2.87(t, 2H), 2.51-2.56(t, 2H).

Compound 7: 3-(4-{[3-(3, 5-Diphenyl-1, 2-oxazol-4-yl) prop-2-yn-1-yl] oxy} phenyl) propanoic acid

To a 10 mL RB flask fitted with magnetic stirrer was charged 3 mL of tetrahydrofuran. To the stirred solvent was added methyl 3-(4-{[3-(3, 5-diphenyl-1, 2-oxazol-4-yl) prop-2-yn-1-yl] oxy} phenyl) propanoate (0.1 g, 0.16 mmol), followed by methanol (3 mL). The RM was cooled to 0 ^{0}C and sodium hydroxide (0.03 g, 0.32 mmol) in water (2 mL) was added drop wise. The RM was stirred at RT for 1 h. The RM was concentrated by removal of solvent and the crude was dissolved in minimum amount of water (2 mL). The aqueous layer was washed with ether (5 mL). The aqueous layer was acidified to pH 3 with 1N hydrochloric acid and extracted with ethyl acetate (5 mL X 2). The organic layer was washed with saturated brine solution (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The solid was triturated with n-hexane and dried thoroughly. The product was obtained as white color solid. (0.022 g, Yield: 22.73 %) Purity: 95.18 %;:

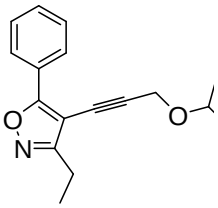
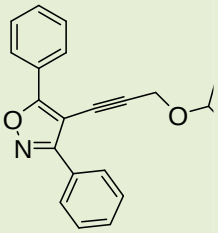
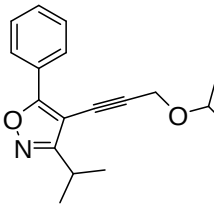
Compound 8

3-[4-({3-[5-Phenyl-3-(propan-2-yl)-1, 2-oxazol-4-yl] prop-2-yn-1-yl} oxy) phenyl] propanoic acid



8

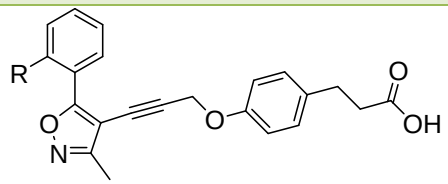
NMR FOR SAR TABLE-2 COMPOUNDS

6		**δ 12.10 (br s, 1H), 7.97 - 7.99 (m, 2H), 7.48 - 7.56 (m, 3H), 7.21(d, 2H), 7.01(d, 2H), 5.15(s, 2H), 2.78(t, 2H), 2.66(q 2H), 2.51(s, 2H), 1.20(t, 3H)**	**376.1**
7		δ 12.11 (br s, 1H), 8.09(d, 2H), 7.93(d, 2H), 7.48 - 7.63 (m, 6H), 7.20(d, 2H), 7.01(d, 2H), 5.18(s, 2H), 2.78(t, 2H), 2.53(s, 2H).	424.1
8		δ 12.11 (br s, 1H), 7.98(d, 2H), 7.48 - 7.56 (m, 3H), 7.20(d, 2H), 7.00(d 2H), 5.16(s, 2H), 2.98 - 3.10 (m, 1H), 2.78(t, 2H), 2.47(s, 2H), 1.26(d, 6H)	390.2

Results and Discussion:

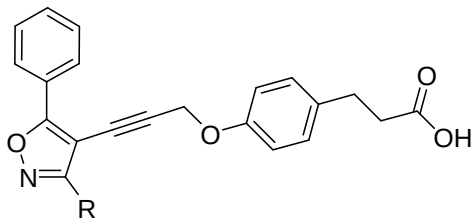
Table -1:

S.No	R	hGPR40 EC_{50} (nM)
1	CH3	108
2	CN	NA
3	F	NA
4	CF3	296
5	H	286



From the results of table-1, the compound 1 has shown very good potency, to evaluate further the methyl group of phenyl ring was replaced with other functional groups such as H, F, CF3 and CN and tested but all the compounds shown less potency.

Table -2:



S.No	R	hGPR40 EC_{50} (nM)
6	Ethyl	**1500**
7	Phenyl	**NA**
8	**Isopropyl**	**NA**

Further the methyl group of isoxazole compound 1 was replaced with ethyl, iso propyl and simple phenyl group but those substituent's not enhanced the potency, with the increase of the size of the substituent the potency was reduced. Compound 1 was selected for further profiling; the compound was tested for S-9 stability in species like rat, mouse and human. It has

shown 100%, 85 % and 80% in rat, mouse and human respectively. The compound is very highly stable across all species. The pharmacokinetic profiling of compound 1 in Wister rats showed good promise, Table 3.

Table 3: Pharmacokinetic properties of selected GPR40 agonist

Compound code	Oral v/s IV (%)	T ½ (hr)	Cmax (µg/mL at mg/kg . bwt)	Tmax (hr)	Kel (l/h at mg/kg bwt)	Plasma Clearance (Ml/min/kg at %QH)	AUC(0-t) (hr*µg/mL at mg/kg bwt)	AUC(0-infinity) ((hr*µg/mL) at mg/kg bwt)	Volume of Distribution (Vd)	Mean resident time (MRT i.v)
1	30-70	1	46.594 at 25 mg/kg . bwt)	0.5	0.73	10 at 14 (Ml/min/kg at %QH)	27.366 at 25 (hr*µg/mL at mg/kg bwt)	27.515 at 25 ((hr*µg/mL) at mg/kg bwt)	250 (mL/kg)	0.37 (hr)

The compound 1 was taken for OGTT and IPGTT studies[29-30].

In Swiss albino mice per oral administration at 200mg/Kg, which exhibited 22% decrease in glucose AUC when compared to the vehicle control indicating good glucose clearance activity.

CONCLUSION

In conclusion, a series of compounds were prepared and their activities as GPR40 agonists were evaluated. Following SAR optimization, compound 1 was selected for in vivo evaluation, where it demonstrated the potential of this class of small molecule GPR40 agonist as glucose lowering agents.

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