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# Synthesis, characterization and in vitro biological evaluation of some new 3-substituted 3-(4-aryloxyaryl)-propanoic acids as GPR40 agonists

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## Abstract

In this letter, the series of 3-(4-aryloxyaryl) propanoic acid compounds design, synthesis, structure–activity relationship (SAR) and the ability to modulate the activity of GPR40 is described. The Systematic replacement of aryl, Heteroaryl groups, various substitutions and optimization of chain length led to identification of potent GPR40 agonists. In order to identify candidates suitable for in vivo validation of the target, pharmacokinetic properties were determined for few compounds and further profiling of these compounds are presented. The compound 16 may prove useful for the treatment of Type 2 diabetes**.**

**Keywords:** GPR40, SAR, In-vitro, pharmacokinetics

## Contents



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PAPER-QR CODE



## 1. Introduction

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 $Ca^{2+}$ *via* coupling to $G\alpha_{q/11}$, 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 phenyl propanoic acids having different linkers that have shown nanomolar potency. The present article describes the synthesis, GPR40 agonist activity and SAR of the synthesized compounds.

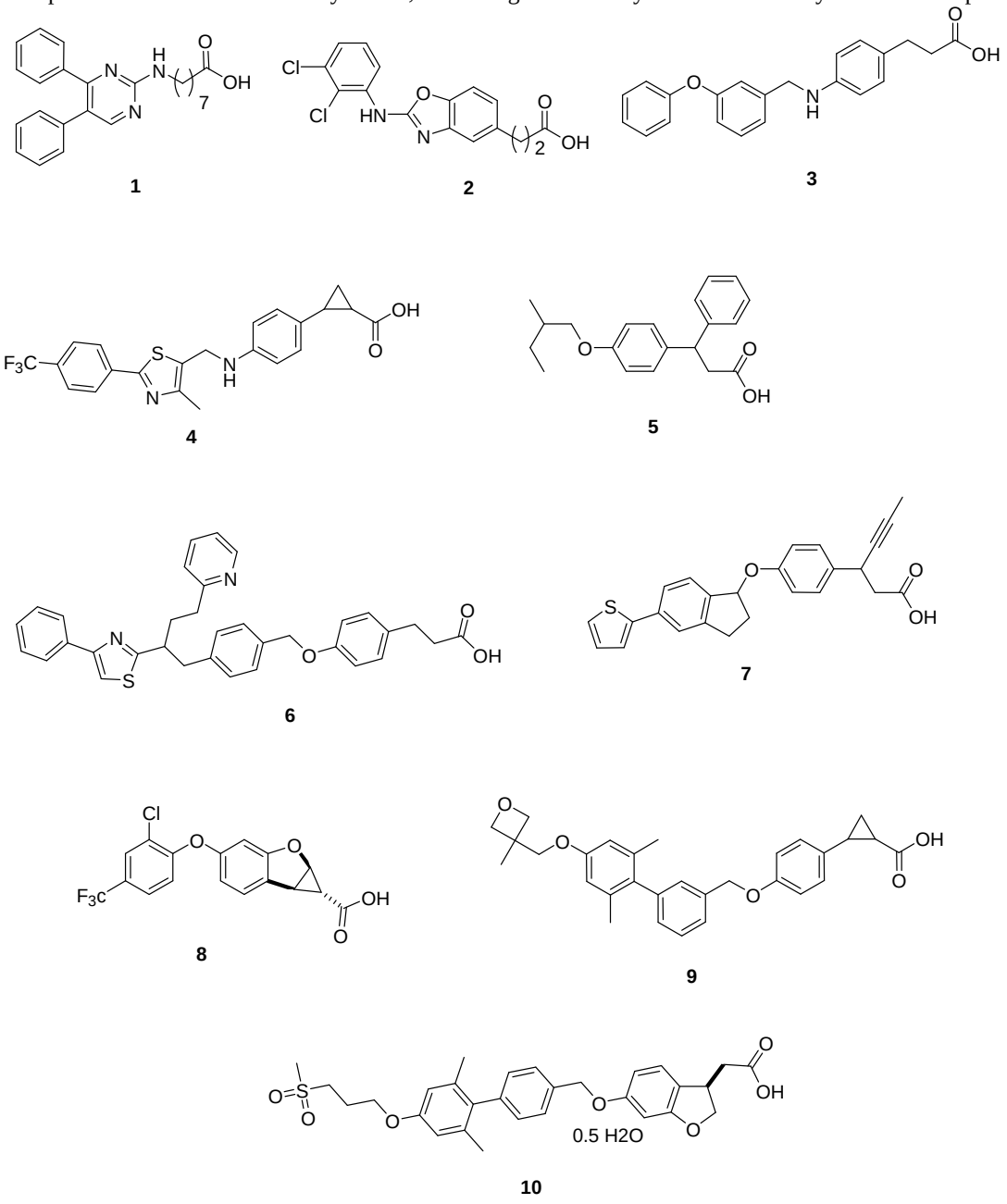



**Figure 1**. Structures of GPR40 agonists reported in literature

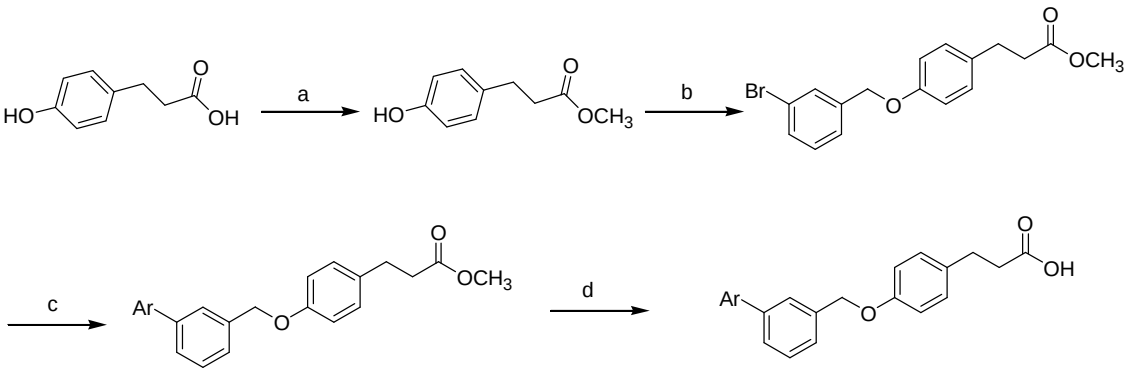
## 2. Materials and Methods

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 phenyl propanoic acids containing different linker, screen them for GPR40 agonist activity and derive an SAR.

**Experimental:**

A general synthesis of propionic acids 11–15 is shown in Scheme 1. Initially, the Methyl 3-(-4-hydroxyphenyl) propionate was coupled to 1-Bromo-3-bromomethyl-benzene, and then Buchwald–Hartwig coupling with different secondary aromatic cyclo amines followed by hydrolysis given the compounds 11-15. The compounds **12, 14 & 15** secondary amines like 4,5,6,7-Tetrahydro-thieno[3,2-c]pyridine, 4-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine and 3,4-Dihydro-2H-benzo[1,4]oxazine compounds have shown good potency, **Table-1**.

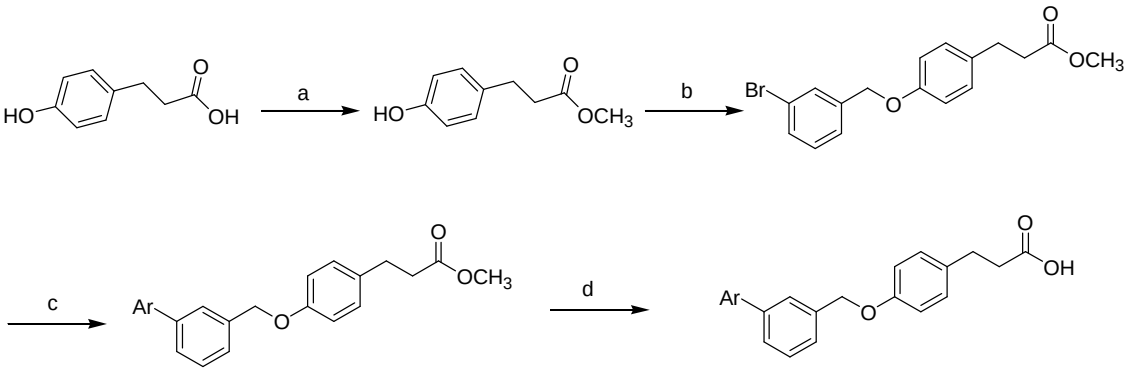
**Scheme – 1:**




**Scheme 1 :** (a) $K_2CO_3$, MeI, DMF, rt, 1 h; (b)1-Bromo-3-bromomethyl-benzene, $K_2CO_3$, DMF, 60 $^0$C, 2 h: (c) Ar, $Pd(OAc)_2$, BINAP, $Cs_2CO_3$, Toluene, 12 h (d) NaOH / $H_2O$, MeOH , THF.

**Synthesis procedures:**

**Scheme – 1:**




**Scheme 1 :** (a) $K_2CO_3$, MeI, DMF, rt, 1 h; (b)1-Bromo-3-bromomethyl-benzene, $K_2CO_3$, DMF, 60 $^0$C, 2 h: (c) Ar, $Pd(OAc)_2$, BINAP, $Cs_2CO_3$, Toluene, 12 h (d) NaOH / $H_2O$, MeOH , THF

**Step-A:**

3-(4-Hydroxyphenyl) propionic acid (15.00 g, 0.09 moles) and methyl iodide (6.77 ml, 0.11 moles) were dissolved in N, N-dimethylformamide (60 ml) and potassium carbonate (37.42 g, 0.27 moles) was added. The mixture was stirred at room temperature for 1 hr. The reaction mixture was filtrated and the filtrate was added to water and extracted with ethyl acetate. The Ethyl acetate layer was back washed with water and saturated brine solution and dried over anhydrous sodium sulfate and evaporated under reduced pressure and the residue was purified by silica gel column chromatography using Hexane and Ethyl acetate were eluents to give 10.2 g of Methyl 3-(4-Hydroxy-phenyl)propionic acid.

**Step-B:**

Methyl 3-(4-Hydroxy-phenyl) propionic acid (10.00 g, 0.05 moles) and 1-Bromo-3-bromomethyl-benzene (16.52 g, 0.07 moles) were dissolved in N, N-dimethylformamide (50 ml) and potassium carbonate (15.32 g, 0.11 moles) was

added. The mixture was stirred at 60 $^0$C temperature for 2 hr. The reaction mixture was cooled to RT and filtrated, the filtrate was evaporated under reduced pressure and the residue was diluted with ethyl acetate and washed with water and saturated brine solution. It was dried over anhydrous sodium sulfate, evaporated and purified by silica gel column chromatography using Hexane and Ethyl acetate were eluents to give 9.8 g of 3-[4-(3-Bromo-benzyloxy)-phenyl]-propionic acid methyl ester.

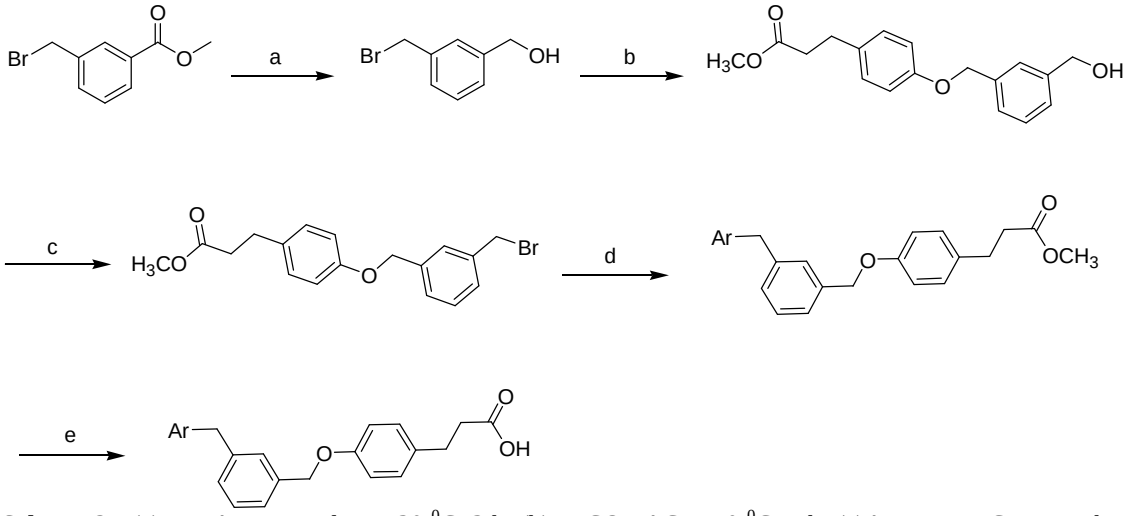
**Step-C:**

3-[4-(3-Bromo-benzyloxy)-phenyl]-propionic acid methyl ester ( 0.82 g, 0.002 moles ) was dissolved in 10 ml of toluene, I4,5,6,7-Tetrahydro-thieno[3,2-c]pyridine ( 0.28 g, 0.002 moles), cesium carbonate ( 1.53 g, 0.0047 moles) , palladium acetate ( 0.026 g, 0.0001 moles) and (R)-(+)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl ( 0.072 g, 0.0001 moles) were added thereto, and then the mixture was heated at reflux temperature for 24 hours. The reaction solution was cooled, added water and extracted with ethyl acetate. The organic layer was washed with water, saturated brine solution, dried over anhydrous sodium sulfate and evaporated on rotavapor under reduced pressure. After evaporating the solvent, the residue was purified by basic alumina chromatography by using hexane and ethyl acetate as eluents to obtain 30 mg of 3-{4-[3-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-yl)-benzyloxy]-phenyl}-propionic acid methyl ester

**Step-D:**

To a 25 mL RB flask fitted with magnetic stirrer was charged 1 mL of THF: 0.5 mL MeOH: 0.5 mL $H_2O$. To the stirred solvent, were added 3-{4-[3-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-yl)-benzyloxy]-phenyl}-propionic acid methyl ester (0.030 g, 0.0001moles) and Sodium hydroxide (0.012 g, 0.0003 moles), the resulting solution was stirred at room temperature for Overnight. After completion of the reaction (reaction monitored by TLC), Solvents were evaporated under reduced pressure, the reaction mass was washed with ethyl acetate to remove the nonpolar impurities and it was acidified by 1N HCl to (PH=4), the obtained solid filtered and dried. The product was obtained as an off white colour solid. Yield: 0.016 g (55%).

**Scheme–2:**




**Scheme 2:** (a) DIBAL-H, Toulene, -30 $^0$C, 3 h; (b) $K_2CO_3$, ACN, 70 $^0$C, 5 h; (c)Ar, $PBr_3$, DCM, rt, 2 h; (d) $Et_3N$, THF, rt, 5 h; (e) NaOH / $H_2O$, MeOH , THF.

**Step-A:**

To a solution of Diisobutyl aluminium hydride (3.80 mL, 2eq) in Toluene at -30 $^0$C was added 3-Bromomethyl-benzoic acid methyl ester (2.65 g, 0.012 moles) in dry toluene. The RM stirred at -30 $^0$C for 3 hrs. After completion of the reaction (reaction monitored by TLC), RM quenched with cold MeOH. Then it was filtered through celite and evaporated to dryness. Yield: 0.495 g.

**Step-B:**

To a 250 mL RB flask fitted with magnetic stirrer was charged 25 mL of Acetonitrile. To the stirred solvent was added 3-(4-Hydroxy-phenyl)-propionic acid methyl ester (0.425 g, 0.0024 moles) and $K_2CO_3$ (0.978 g, 0.007 moles), it was stirred for 5 min then (3-Bromomethyl-phenyl)-methanol (0.475 g, 0.0024 moles) was added. The RM stirred at 80°C for 5hrs. After completion of the reaction (reaction monitored by TLC), RM evaporated to remove the acetonitrile, the crude material was diluted with water extracted with ethyl acetate. The organic layer was back washed with saturated brine solution and dried over anhydrous sodium sulphate, evaporated to dryness to give the title compound. Yield: 0.205 g.

**Step-C:**

To a 250mL RB flask fitted with magnetic stirrer was charged 25 mL of DCM. To the stirred solvent was added 3-[4-(3-Hydroxymethyl-benzyloxy)-phenyl]-propionic acid methyl ester (0.2 g, 0.0007 moles), cooled to 0 °C then added $PBr_3$ (0.069 mL, 0.0008 moles) drop wise. The reaction mixture was stirred for 2 hrs at RT, reaction

completed, the RM quenched with water at 0°C and extracted with ethyl acetate. The organic layer washed with saturated brine solution and dried over anhydrous sodium sulphate and evaporated to dryness on rotavapor. Yield: 0.280 g.

**Step-D:**

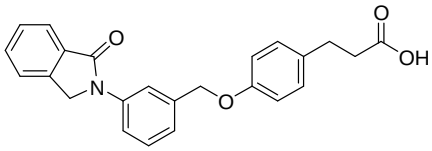
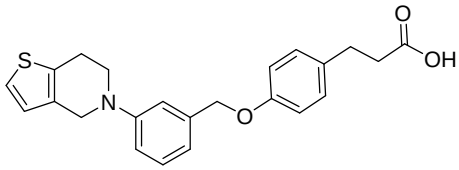
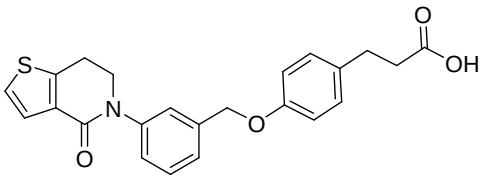
To a 25 mL RB flask fitted with magnetic stirrer was charged 5 mL of tetrahydrofuran. To the stirred solvent, was added 1, 2, 3, 4-Tetrahydro-isoquinoline (0.121g, 0.0009 moles) followed by the addition of triethylamine (0.462 mL, 0.004 moles) under nitrogen atmosphere. The reaction mixture was cooled to 0 $^0$C and stirred for 5 minutes. To the stirred solution, 3-[4-(3-Bromomethyl-benzyloxy)-phenyl]-propionic acid methyl ester (0.3 g, 0.0008 moles) was added. The resulting solution was stirred at room temperature for 5 hours. After completion of the reaction (reaction monitored by TLC), solvent was evaporated on Rotavapor under reduced pressure. The crude was diluted with water and extracted with ethyl acetate. The organic layer was washed with saturated brine solution and dried over anhydrous sodium sulfate and evaporated to give the compound. Yield: 0.27 g

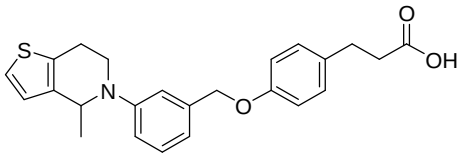
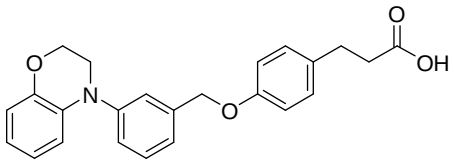
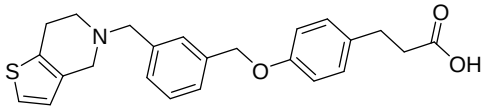
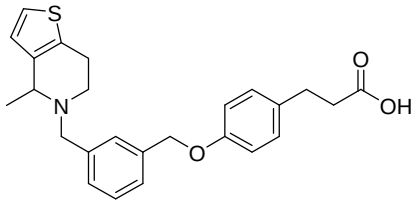
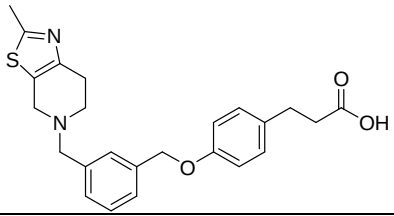
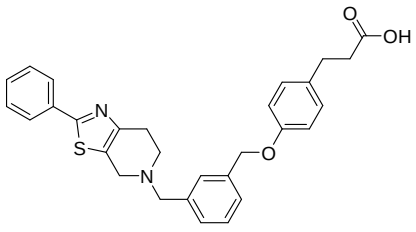
**Step-E:**

To a 25 mL RB flask fitted with magnetic stirrer was charged with methanol (2 mL) and THF (2 mL). To the stirred solvent 3-{4-[3-(3, 4-Dihydro-1H-isoquinolin-2-ylmethyl)-benzyloxy]-phenyl}-propionic acid methyl ester (0.27 g, 0.0006 moles) and NaOH (0.104 gm, 0.0024 moles) in water were added. The resulting solution was stirred at room temperature for 3 hours. After completion of the reaction (reaction monitored by TLC), solvents were evaporated under reduced pressure. The reaction mass was diluted with water, cooled to 0 $^0$C, then acidified with saturated citric acid to pH-5. The resulting solid was filtered and dried under vacuum. Yield: 0.249 g.

**NMR:**

**Table 1. NMR Spectral Analysis**

| S.No | Structure | $^1$H NMR | m/z $(M+H)^+$ |
|---|---|---|---|
| **11** |  | (300 MHz, DMSO): δ 12.18(s,1H), 8.04(s,1H), 7.85-7.87(d, 1H),7.78-7.81(d,1H) 7.67-7.72(t.2H),7.55-7.57(d, 1H), 7.43-7.48(t, 1H), 7.24-7.26(d,1H),7.13-7.16(d.2H),6.93-6.96(d,2H),5.11(s,2H),5.05(s,2H),2.72-2.77(t.2H),2.45-2.50(t,2H) | 388.1 |
| **12** |  | (300MHz,DMSO-d6) δ12.12(s,1H),7.31-7.33(d,1H),7.19-7.24(t,1H),7.08-7.14(t,3H),6.89-6.98(m,4H),6.81-6.83(d,1H),4.99(s,2H),4.29(s,2H),3.59-3.63(t,2H),2.88-2.91(t,2H),2.72-2.77(t,2H),2.45-2.48(d,2H) | 393.8 |
| **13** |  | (300 MHz, DMSO-d6): δ 12.09 (s,1H), 7.64 (s, 1H), 7.51-7.54(d,1H),7.43-7.46(d,1H),7.33-7.38(t,1H),7.13-7.16(d,2H),7.03-7.07(m,2H),6.90-6.93(d,2H),5.08(s,2H),2.72-2.77(t,2H),2.46-2.51(m,6H). | 407.8 |

| S.No | Structure | $^1$H NMR | m/z $(M+H)^+$ |
|---|---|---|---|
| **14** |  | (300MHz,DMSO-d6) δ 12.14(s,1H),7.29-7.31(d,1H),7.17-7.22(t,1H),7.11-7.14(d,2H),7.01(s,1H),6.89-6.93(t,4H),6.74-6.77(d,1H), 4.99(s,2H), 3.8( m, 1H), 3.62-3.68( t, 2H), 2.72-2.77(m, 4H), 2.45-2.50(t, 2H), 1.28-1.30(d,3H) | 408.2 |
| **15** |  | (300 MHz, CDCl3): δ 7.24-7.27(d, 1H), 7.07-7.09(d,2H), 7.01-7.03(t, 3H),6.76-6.84(m,5H) 6.62-6.71(m.2H),4.88(s, 2H), 4.17-4.20(t, 2H), 3.60-3.36(t, 2H), 2.78(s, 2H), 2.51(s, 2H), | 389.8 |
| **16** |  | (300MHz,DMSO-d6) δ12.09(s,1H), 7.42(s,1H),7.36-7.25(m, 4H),7.14-7.11(d, 2H),6.92-6.89(d, 2H),6.76-6.75(d,1H), 5.06(s, 2H),3.69(s, 2H),3.45(s,2H),2.76-2.71(m,6H),2.50(t,2H) | 407.9 |
| **17** |  | (300MHz, DMSO): δ 7.31(s, 1H), 7.24-7.26 (m, 3H), 7.08-7.10 (d, 1H), 6.86 - 6..88(d, 2H), 6.84 - 6.85 (d, 3H), 5.05(s, 2H), 3.82-3.87 (d, 1H),3.65-3.71(m,2H),2.56-2.79(m,6H),2.15-2.20(t,2H),1.28-1.30(d,3H) | 421.9 |
| **18** |  | (300 MHz, DMSO): δ 12.09(s,1H), 7.28-7.41(m, 4H), 7.11-7.14 (d, 2H), 6.89-6.91(d,2H), 5.06(s,2H), 3.69(s, 2H),3.56(s, 2H), 2.69-2.76(m,6H),2.57(s, 3H), 2.45-2.47(d, 2H), | 422.9 |
| **19** |  | (300MHz,DMSO-d6) δ 12.04(s,1H), 7.85-7.88(m, 2H), 7.44-7.48(m, 4H), 7.33-7.37(t, 3H), 7.12-7.14(d, 2H), 6.90-6.93(d, 2H), 5.07(s, 2H), 3.69-3.74(d, 4H), 2.83(bro s, 4H), 2.72-2.77(t, 2H), 2.45-2.48(d, 2H) | 484.8 |

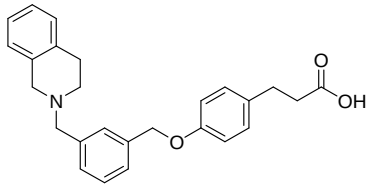
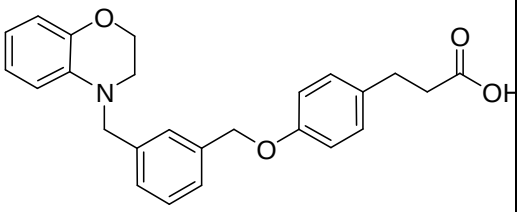
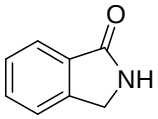
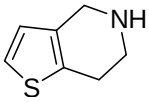
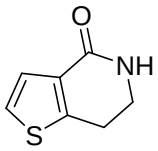
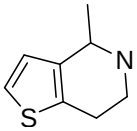
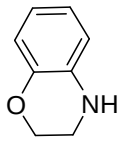
| S.No | Structure | $^1$H NMR | m/z $(M+H)^+$ |
|---|---|---|---|
| **20** |  | 1H-NMR (300MHz DMSO) : 7.42(s,1H), 7.38-7.31(m, 3H),7.13-7.04(m, 5H), 6.98-6.97(d, 1H), 6.91-6.88(d, 2H), 5.05(s, 2H), 3.64(s, 2H), 3.52 (s, 2H), 2.79-2.71(m, 4H), 2.67-2.63(t, 2H), 2.46-2.43(t, 2H). | 401.9 |
| **21** |  | (300 MHz, CDCl3-d6): δ 7.26-7.28(d, 3H), 7.19(s, 1H), 7.04-7.06(d, 2H),6.80-6.83(d,2H) 6.71-6.76(m, 2H), 6.57-6.59(d, 2H), 4.95(s, 2H), 4.38(s, 2H), 4.18-4.21(t, 2H), 3.27-3.30(t, 2H),2.81-2.86(t,2H),2.56-2.61(t,2H) | 401.9 |

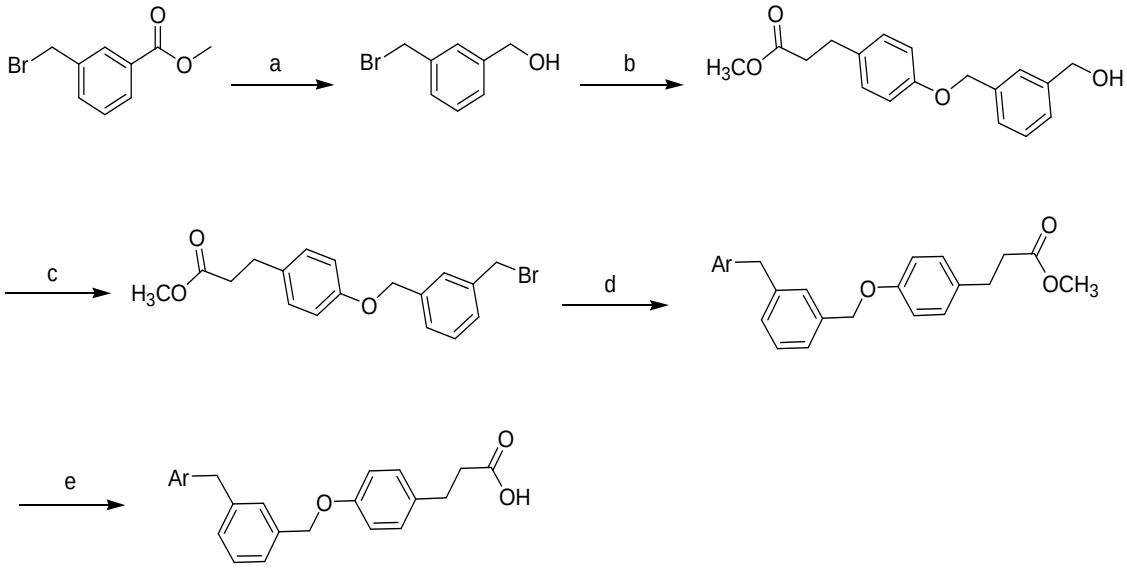
**Table 2. GPR40 agonistic activity**

| Compound Code | Ar | hGPR40 $EC_{50}$ (nM) |
|---|---|---|
| 11 |  | 2000 |
| 12 |  | 120 |
| 13 |  | 791 |
| 14 |  | 128 |
| 15 |  | 36 |

The same active compounds were tested out with an increased chain length; they also turned out to be showing similar potency, **Table-2.**The synthesis of these compounds (1**6-21)** is shown in Scheme 2. The reduction of Methyl 3-(bromomethyl) benzoate and then coupled with Methyl 3-(-4-hydroxyphenyl) propionate gives aromatic primary

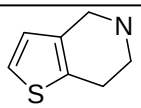
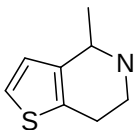
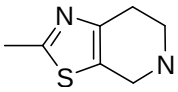
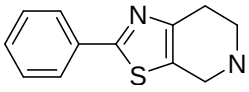
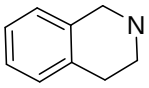
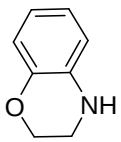
alcohol. The alcohol converted as reactive bromine, in turn coupled with different secondary aromatic cyclo amines followed by hydrolysis given the compounds 1**6-21**.

**Scheme–2:**




**Scheme 2 :** (a) DIBAL-H, Toulene, -30 $^0$C, 3 h; (b) $K_2CO_3$, ACN, 70 $^0$C, 5 h; (c)Ar, $PBr_3$, DCM, rt, 2 h; (d) $Et_3N$, THF, rt, 5 h; (e) NaOH / $H_2O$, MeOH , THF.

**Table 3. GPR40 agonistic activity**

| Compound | Ar | hGPR40 $EC_{50}$ (nM) |
|---|---|---|
| 16 |  | 391 |
| 17 |  | 49 |
| 18 |  | 669 |
| 19 |  | 64 |
| 20 |  | 585 |
| 21 |  | 24 |

## 3. Results and Discussion

From the results a set of three compounds were selected for pharmacokinetic profiling in Wister rats, **Table 4.**

**Table 4. Pharmacokinetic properties of selected GPR40 agonists**

| S.No | 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) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 16 | 7-12 | 1 | 0.929 & 5.8 at 10 & 25 | 0.25 | 0.79 | 13.5 at 20 | 0.819 & 3.8 at 10 & 25 | 0.8 & 3.8 at 10 & 25 |
| **2** | 21 | 6 | 1 | 1317 | 0.58 | 0.75 | 14.5 at 21 | 1908 | 1933 |

Compounds 16 and 21 were selected for further profiling in vivo (Table-3). The compound 21 shown genotoxicity liability where as the compound 16 turned out to be safe. So the compound 16 was taken further for both human and mouse S9 stability where it has shown a good stability in both mouse and human by more than 65%. The molecule 16 was taken for OGTT and IPGTT studies [29-30] in *Swiss albino mice* per oral administration at 200mg/Kg, which exhibited 44% decrease in glucose AUC when compared to the vehicle control indicating good glucose clearance activity. The molecule has shown a good delay in gastric emptying rate as well [31-32]. The molecule has no Genotoxic liability that was tested in-vitro human lymphocyte assay up to a concentration of 100µM with and without metabolic activation [33-34].

## 4. Conclusion

In conclusion, a series of compounds were prepared and their activities as GPR40 agonists were evaluated. Following SAR optimization, compound 16 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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