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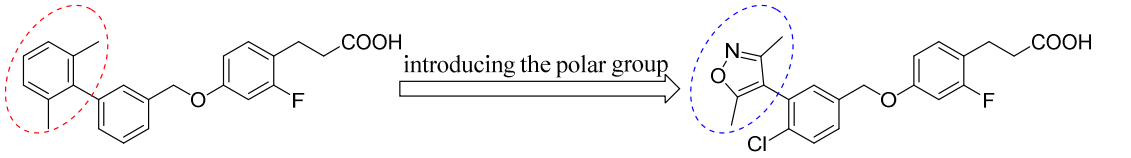
Graphical abstract



1

- EC_{50} = 7.7 nM
- tPSA = 41.6
- cLog P = 6.16
- In vivo efficacy

11k

- EC_{50} = 15.9 nM
- tPSA = 68.1
- cLog P = 4.17
- In vivo efficacy & favorable PK properties

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Synthesis and biological evaluation of GPR40/FFAR1 agonists containing 3,5-dimethylisoxazole

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Abstract

GPR40 is an attractive target due to its glucose-stimulated insulin secretion effect with low risk of causing hypoglycemia, which also can be seen from the clinical studies using TAK-875 (fasiglifam). In the present studies, we discovered a series of analogues containing 3,5-dimethylisoxazole as potent GPR40 agonists, especially compound **11k** with an EC_{50} value of 15.9 nM. Moreover, compound **11k** reduced glucose excursion to 23.1% in ICR mice and 29.5% in type 2 diabetic C57BL/6 mice at 30 mg/kg. It also exhibited satisfactory PK profile. Docking studies were conducted to explain the interaction mode of this series. In summary, compound **11k** with robust efficacy in vitro and in vivo is a promising drug candidate for further investigation.

Keywords

Diabetes; GPR40 agonist; 3,5-dimethylisoxazole; oral glucose tolerance test

1. Introduction

Type 2 diabetes mellitus (T2DM) is mainly characterized by insulin deficiency and insulin resistance, often accompanied by multiple complications such as cardiovascular disease, renal failure and retinopathy. Currently, the most common oral antidiabetic agents include sulfonylureas, metformin, glitazones, all of which are associated with limited efficacy and adverse side effects [1, 2]. There are only a few insulin secretagogues with low risk of hypoglycemia and weight gain, such as DPP-IV inhibitors, SGLT2 inhibitors and GLP-1 agonists [3, 4]. The urgent need for novel oral antidiabetic agents with sustained safety and efficacy stimulates efforts to develop new mechanisms to improve glycemic control.

GPR40 (also known as FFAR1) has, since its deorphanization as a cell surface free fatty acids receptor in 2003, become an attractive and potential target for T2DM therapy [5-7]. GPR40 is a G-protein-coupled receptor, highly expressed in pancreatic β-cells and intestinal enteroendocrine cells [8]. Activation of GPR40 by medium (C6–C12) to long (C14–C24) chain saturated and unsaturated fatty acids enhances glucose-stimulated insulin secretion (GSIS). However, mechanisms of the activation of GPR40 to GSIS are only partially understood and remain to be fully explained. Several studies have pointed that GPR40 is coupled with heterotrimeric G protein Gαq/11, which activates phospholipase C, leading to the formation of diacylglycerol (DAG) and inositol-triphosphate (IP3). IP3 binding to its receptor on endoplasmatic reticulum activates an intracellular Ca^{2+} mobilization. The generation of DAG stimulates protein kinase D1 and promotes F-Actin polymerization. Subsequently, both Ca^{2+} mobilization and F-Actin polymerization enhance insulin secretion [9, 10]. The modulation of insulin secretion via GPR40 is dependent on

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glucose concentration, indicating GPR40 agonists could have a low risk of hypoglycemia.

A variety of synthetic GPR40 agonists containing 3-phenylpropanoic acid and its bioisostere have been reported (Fig. 1) [11-14]. Among these potent molecules, TAK-875, developed from a modification of compound **1**, was the most advanced in clinical phase III. TAK-875 showed significant glycemic control and ideal pharmacokinetics profiles. The dihydrobenzofuran ring of TAK-875 is highly resistant to β-oxidation, thus slowing down its rate of metabolism [15-17]. Unfortunately, the development of TAK-875 was terminated by Takeda due to liver toxicity in December 2013 [18]. The modification of TAK-875 from compound **1** inspired us to choose compound **1** as a starting structure to explore novel GPR40 agonists in a different way.

It has been recognized that GPR40 is expressed in the brain. While the consequences of activation of GPR40 in the brain have not been identified [19, 20]. A recent study found a GPR40 agonist (AM-3189) with high topological polar surface area (tPSA) showed minimal CNS penetration. tPSA is defined as the surface area occupied by nitrogen and oxygen atoms, and polar hydrogens bonded to these heteroatoms [21]. It is a novel parameter associated with incidence of toxicity for highly lipophilic and low polarity compounds. To avoid potential effects in central nervous system, we decided to improve tPSA by introducing functional groups with greater tPSA to compound **1**, which has a high lipophilicity by itself [22, 23]. Compared with compound **1**, the replacement of the 2,6-dimethylphenyl group with 3,5-dimethylisoxazole in compound **11a** led to a noticeable increase in the tPSA [24]. The well-known rule of five describes clog P should less than 5. For GPR40 agonists, strict range of cLog P has not been proposed as far. However, a high cLog P value is related with undesirable ADMET properties [25]. Most of GPR40 agonists present cLog P values between 4 and 5. We considered this range was probably ideal and the value of compound **1** (cLog P = 6.16) was too high. The introduction of 3,5-dimethylisoxazole also decreased cLog P. Based on compound **11a**, we designed and synthesized a series of compounds containing 3,5-dimethylisoxazole. On the one hand, we introduced halogen, alkyl or alkoxyl on the phenyl to obtain compound **11b–l** and **22a–h**. On the other hand, we replaced phenyl with pyridyl to obtain compound **28a–d** [26-28]. TUG-770 is a highly potent GPR40 agonist with favorable physicochemical and pharmacokinetic properties [29]. The introduction of the ethynyl connecting the phenyl rings is very successful. Comparing the size of compound **11a** and TAK-875, we thought 11a was shorter. So we decided to introduce the ethynyl into compound **36** to make the 3,5-dimethylisoxazole closer to the solvent region. Most of these compounds were proved to be novel GPR40 agonist by biological evaluation in vitro and in vivo, along with optimized tPSA and cLog P. Among them, compound **11k** was found to be the most efficacious in vitro and in vivo as a promising lead compound.

Figure 1.

2. Results and discussion

2.1. Chemistry

3,5-dimethylisoxazole derivatives **11a–l**, **22a–h**, **28a–d** and **36** were synthesized as outlined in Schemes 1–4. All compounds were purified by flash chromatography and checked for purity using HPLC before being tested in biological evaluation (purity was > 97%). The structures of 3,5-dimethylisoxazole derivatives were confirmed by ^{1}H NMR and ^{13}C NMR spectrum, mass spectrometry, IR spectrum.

As shown in Scheme 1, the synthesis of (E)-3-(2-fluoro-4-hydroxyphenyl)acrylic acid (**3**) was carried out by Knoevenagel condensation of 2-fluoro-4-hydroxybenzaldehyde (**2**) with propanedioic acid in pyridine. The key intermediate methyl 3-(2-fluoro-4-hydroxyphenyl)propanoate (**5**) was prepared by reduction of intermediate **3** in the presence of Pd/C, followed by methyl esterification. (3,5-dimethylisoxazol-4-yl)benzaldehyde (**7a–l**) were synthesized by Suzuki coupling reaction. The chloride intermediates (**9a–l**) were prepared by the substitution of corresponding benzyl alcohols (**8a–l**) with phosphorus oxychloride, which were derived from reduction of benzaldehyde (**7a–l**) with $NaBH_4$. The target compounds **11a–l** were obtained by condensation of intermediates **9a–l** with intermediate **5**, followed by hydrolysis of the methyl esters **10a–l**. Derivatives **28a–d** were synthesized as outlined in Scheme 3. The synthetic route was similar to that in Scheme 1.

Scheme 1.

As shown in Scheme 2, 3-bromo-5-nitrobenzaldehyde (**13**) was prepared by bromination with 3-nitrobenzaldehyde (**12**). The key intermediate **17** was synthesized by Suzuki coupling reaction with borate ester and 3-bromo-5-hydroxybenzaldehyde (**16**), which was obtained in several steps by the introduction of 1,3-dioxolane as protecting group in intermediate **13**, reduction of intermediate **14** employing iron powder, subsequent Sandmeyer reaction and removal of the protecting group. Intermediate **18a-h** were prepared by condensation of intermediate **17** with the corresponding bromoalkanes. The following procedures were depicted in Scheme 1 to give compounds **22a–h**.

Scheme 2.

Scheme 3.

As shown in Scheme 4, 3-((trimethylsilyl)ethynyl)benzaldehyde (**30**) was synthesized by Sonogashira coupling of 3-iodobenzaldehyde with trimethylsilylacetylene (**29**). Removal of trimethylsilyl by TBAF and second Sonogashira reaction with 4-iodo-3,5-dimethylisoxazole were conducted to give intermediate **32**. The desired compound **36** was prepared as the synthetic route described previously.

Scheme 4.

2.2. GPR40 agonist activity and SAR study

The GPR40 agonist activities of synthesized compounds were evaluated by FLIPR calcium assay in Chinese hamster ovary (CHO) cells stably expressing human GPR40. TAK-875 was used as positive control. The agonist activities were presented in Table 1–4. We initially modified the substituents on the phenyl connecting with 3,5-dimethylisoxazole. As shown in Table 1, compounds **11a-l** containing halogen, alkyl or alkoxyl maintained strong GPR40 agonist activities with improved tPSA and lowered cLog P values compared with compound **1**. It demonstrated 3,5-dimethylisoxazole was highly tolerable as a terminal group in GPR40 agonists. The introduction of nitrogen and oxygen to the heterocycle was beneficial to tPSA and cLog P. It was expected to reduce the risk of the potential effects in central nervous system. In this series, compounds **11b**, **11f**, **11g** and **11k** showed good potency and were selected for in vivo profiling. To understand the binding mode of this series, we performed docking studies with Glide docking in Schrodinger 9.4 (Fig. 2). The first crystal structure of human GPR40 receptor (PDB ID: 4PHU) binding to TAK-875 was reported by Takeda in 2014 [30]. A comparison of TAK-875 to energy minimized conformation of compound **11a** indicated a high percentage of overlap. Both carboxylate moiety in two molecules interacted with arginine residues Arg 183 and Arg 258

(displayed as Arg 2258 in Schrodinger 9.4). The fluorine atom overlapped with the oxygen atom on dihydrobenzofuran of TAK-875. The 3,5-dimethylisoxazole in compound **11a** and the left 2,6-dimethylphenyl in TAK-875 was orthogonal to the middle phenyl ring. The similarities between 3,5-dimethylisoxazole analogues and TAK-875 allowed explanation of the GPR40 agonist activities we evaluated.

Table 1.

Figure 2.

Next, we explored alkoxyl into the *meta*-position of the middle phenyl ring to obtain compounds **18a-h** (Table. 2). The EC_{50} values ranged from 53.1 nM to 143 nM, indicating that *meta*-position of the phenyl could tolerate substituents with differing length with minimal loss of potency. However, the replacement of the middle phenyl ring with a variety of the pyridine rings reduced agonist activity significantly (Table. 3). Although the tPSA value of compound **22a-d** were improved, the pyridine ring was not suitable for the middle ring of GPR40 agonists. The introduction of the ethynyl connecting the 3,5-dimethylisoxazole and the phenyl gave compound **36** an EC_{50} value of 214 nM (Table. 4).

Table 2.

Table 3.

Table 4.

2.3. Antihyperglycemic effect of 11b, 11f, 11g and 11k in ICR mice

According to the results of GPR40 agonist activity in vitro, compound **11b**, **11f**, **11g** and **11k** were selected for in vivo profiling. Acute oral glucose tolerance test (OGTT) was conducted in male ICR mice initially. Mice were dosed orally with single doses of vehicle, TAK-875 (30 mg/kg) and test compounds (30 mg/kg), 30 min prior to oral glucose load (2 g/kg). Among these compounds, compound **11f** and **11k** exhibited robust potency of glycemic control. Compound **11f**, **11k** and TAK-875 reduced area under the curve from 0 to 120 min ($AUC_{0–120\ min}$) by 19.1%, 23.1% and 23.3% (**18f**, 785.9 ± 31.0; **18k**, 746.4 ± 52.3; TAK-875, 744.9 ± 24.9), respectively (Fig. 3).

Figure 3.

2.4. Antihyperglycemic effect of 11f and 11k in type 2 diabetic C57BL/6 mice

Based on the data in ICR mice OGTT, compound **11f** and **11k** were tested in type 2 diabetic C57BL/6 mice. Mice were dosed orally with single doses of vehicle, TAK-875 (30 mg/kg), compound **11f** and **11k** (30 mg/kg), 30 min prior to oral glucose load (1 g/kg). Fig. 4 depicted that compound **11k** reduced notably $AUC_{0–120\ min}$ by 29.5% and its antihyperglycemic effect was similar to that of TAK-875 (**11k**, 1298.5 ± 47.5; TAK-875, 1216.6 ± 75.0).

Figure 4.

2.5 Pharmacokinetic profiles of 11k in SD rats

Compound 11k was selected to the further evaluation for oral pharmacokinetic profiles in SD rats (Table 5.). It exhibited favorable pharmacokinetic profiles, with lasting half-life (2.87 ± 0.33 h), fast oral absorption (T_{max} = 1.0 h), high maximum plasma concentration (C_{max} = 0.84 ± 0.15 μg/mL) and high plasma drug exposure ($AUC_{0\text{-}8\ h}$ = 4.18 ± 0.71 μg·h/mL).

Table 5.

3. Conclusion

In summary, we designed and discovered a series of novel GPR40 agonists containing 3,5-dimethylisoxazole with improved tPSA and ideal cLog P. Most of these compounds were confirmed as excellent GPR40 agonists in vitro. Especially, compound **11k** exhibited robust

potency of glycemic control in both ICR mice and type 2 diabetic C57BL/6 mice compared with TAK-875. The pharmacokinetic profiles was evaluated and presented desirable results. In addition, docking studies were conducted to explain the potency of 3,5-dimethylisoxazole analogues. Those qualities made compound **11k** a promising lead compound for the treatment of T2DM and worth for further investigation.

4. Experimental section

4.1. Chemistry

All chemical reagents were commercially available and treated with standard methods before use. Solvents were dried and redistilled before use. Column chromatography (CC): silica gel 60 (200–300 mesh). Thin-layer chromatography (TLC): silica gel 60 F254 plates (250 nm; Qingdao Ocean Chemical Company, Qingdao, China). M.p.: capillary tube, RY-1 capillary apparatus (Tianjin Optical Instrument Company, Tianjin, China), uncorrected. %Purity of the target compounds (> 97%) were determined by HPLC analysis (UV detector, wavelength: 272 nm). IR spectra: Nicolet Impact 410 spectrophotometer with KBr disks; in cm^{-1}. ^1H and ^{13}C NMR spectra: Bruker ACF-300Q apparatus (300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR), in DMSO-d_6 or $CDCl_3$ unless otherwise indicated, δ in ppm rel. to Me_4Si, *J* in Hz. Mass spectrometry (MS): Hewlett-Packard 1100 LC/MSD spectrometer; elemental analyses: CHN-O-Rapid instrument.

4.1.1. Preparation of (E)-3-(2-fluoro-4-hydroxyphenyl)acrylic acid (3)

To a solution of **2** (1.0 g, 7.1 mmol) in pyridine (10 mL) was added propanedioic acid (1.1 g, 10 mmol) at room temperature. The reaction mixture was stirred at 80°C for 24 h. After cooling to the room temperature, the mixture was acidified with HCl (1 N) and filtered. The filter cake was washed with water and dried to give **3** (1.1 g, 84%) as white solid. ^1H NMR (300 MHz, DMSO-d_6) δ: 12.29 (s, 1H), 10.47 (s, 1H), 7.64 (t, *J* = 8.8 Hz, 1H), 7.56 (d, *J* = 16.1 Hz, 1H), 6.72–6.56 (m, 2H), 6.36 (d, *J* = 16.1 Hz, 1H).

4.1.2. Preparation of 3-(2-fluoro-4-hydroxyphenyl)propanoic acid (4)

To a solution of **3** (0.80 g, 4.4 mmol) in THF (25 mL) was hydrogenated on 10% Pd/C under hydrogen atmosphere (balloon pressure) at room temperature for 24 h. The mixture was filtered through a celite pad, and the filtrate was concentrated to afford **4** (0.79 g, 99%) as a colorless solid without further purification. ^1H NMR (300 MHz, DMSO-d_6) δ: 11.80 (br s, 1H), 9.93 (br s, 1H), 7.06 (t, *J* = 8.8 Hz, 1H), 6.60–6.38 (m, 2H), 2.72 (t, *J* = 7.4 Hz, 2H), 2.44 (t, *J* = 7.5 Hz, 2H).

4.1.3. Preparation of methyl 3-(2-fluoro-4-hydroxyphenyl)propanoate (5)

To a solution of **4** (0.70 g, 3.8 mmol) in methanol (20 mL) was added concentrated sulfuric acid (0.50 mL) and then refluxed for 12 h. After cooling to the room temperature, the solvent was concentrated in vacuo. The residue was quenched with saturated $NaHCO_3$ solution and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and evaporated in vacuo to afford **5** (0.69 g, 92%) as a brown solid. ^1H NMR (300 MHz, DMSO-d_6) δ: 9.65 (s, 1H), 7.04 (t, *J* = 8.9 Hz, 1H), 6.57–6.42 (m, 2H), 3.56 (s, 3H), 2.74 (t, *J* = 7.5 Hz, 2H), 2.53 (t, *J* = 7.5 Hz, 2H).

4.1.3. The following procedures described the synthesis of compound (11a)

These procedures can also be applied to the synthesis of compounds **11b–l** and **28a–d**.

4.1.3.1 Preparation of 3-(3,5-dimethylisoxazol-4-yl)benzaldehyde (7a)

To a mixture of 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole (0.50 g, 2.2 mmol), 3-bromobenzaldehyde (0.50 g, 2.7 mmol), $Pd(PPh_3)_4$ (0.13 g, 0.12 mmol) and

sodium carbonate (0.71 g, 6.7 mmol) in toluene/ethanol/H_2O (10 mL, 3/1/3) was refluxed under nitrogen atmosphere for 24 h. Then the mixture was diluted with saturated ammonium chloride solution and EtOAc, and the insoluble material was filtered through Celite. The organic layer of the filtrate was washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (EtOAc/petroleum ether, 1/15 to 1/5) to give **7a** (0.37 g, 77%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.08 (s, 1H), 7.99–7.85 (m, 2H), 7.81–7.63 (m, 2H), 2.43 (s, 3H), 2.25 (s, 3H).

4.1.3.2 Preparation of (3-(3,5-dimethylisoxazol-4-yl)phenyl)methanol (8a)

To a solution of **7a** (0.37 g, 1.8 mmol) in THF/methanol (20 mL, 5/1) was added $NaBH_4$ (0.14 g, 3.7 mmol) in small portions at 0°C. The reaction mixture was stirred at room temperature for 15 min, and then quenched with HCl (1 N). The mixture was extracted with EtOAc (3 × 20 mL) and the combined organic layers were washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo to afford **8a** (0.35 g, 94%) as a colorless solid without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.42 (t, J = 7.5 Hz, 1H), 7.37–7.28 (m, 2H), 7.28–7.15 (m, 1H), 5.24 (t, J = 5.8 Hz, 1H), 4.54 (d, J = 5.8 Hz, 2H), 2.39 (s, 3H), 2.22 (s, 3H).

4.1.3.3 Preparation of 4-(3-(chloromethyl)phenyl)-3,5-dimethylisoxazole (9a)

To a solution of **8a** (0.35 g, 1.7 mmol) in N-methyl-2-pyrrolidone (3.0 mL) was added phosphorus oxychloride (0.43 mL, 5.2 mmol) dropwise at 0°C. The reaction mixture was stirred at room temperature for 2 h, then quenched with saturated sodium bicarbonate solution. The product was prepiticated, filtered and washed with water to afford **9a** (0.34 g, 89%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.57–7.43 (m, 3H), 7.36 (dt, J = 6.9, 1.8 Hz, 1H), 4.82 (s, 2H), 2.41 (s, 3H), 2.23 (s, 3H).

4.1.3.4 Preparation of methyl 3-(4-((3-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluorophenyl) propanoate (10a)

To a solution of **9a** (0.20 g, 0.89 mmol), **5** (0.16 g, 0.81 mmol) and potassium carbonate (0.22 g, 1.6 mmol) in DMF (3.0 mL) was stirred at 60°C for 5 h. Then the mixture was quenched with saturated ammonium chloride solution and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/petroleum ether, 1/9 to 1/3) to give **10a** (0.23 g, 71%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.53–7.40 (m, 3H), 7.35 (d, J = 7.2 Hz, 1H), 7.20 (t, J = 8.8 Hz, 1H), 6.89 (dd, J = 12.2, 2.4 Hz, 1H), 6.80 (dd, J = 8.5, 2.5 Hz, 1H), 5.15 (s, 2H), 3.57 (s, 3H), 2.79 (t, J = 7.5 Hz, 2H), 2.56 (t, J = 7.5 Hz, 2H), 2.38 (s, 3H), 2.21 (s, 3H).

4.1.3.5 Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluorophenyl)propanoic acid (11a)

To a solution of **10a** (0.23 g, 0.60 mmol) and LiOH (60 mg, 2.4 mmol) in methanol/H_2O (10 mL, 5/1) was stirred at 40°C for 6 h. The solvent was evaporated in vacuo and the residue was acidified with HCl (1 N). The mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/petroleum ether, 1/5 to 1/2) to give compound **11a** (0.14 g, 63%) as a white solid. m.p.: 106–108°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.71 (br s, 1H), 7.63–7.27 (m, 4H), 7.27–7.07 (m, 1H), 6.98–6.69 (m, 2H), 5.16 (s, 2H), 2.75 (t, J = 7.1 Hz, 2H), 2.47 (t, J = 7.1 Hz, 2H), 2.39 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.63 (s), 165.22 (s), 160.96 (d, J = 224.2 Hz), 158.07 (d, J = 5.8

Hz), 157.88 (s), 137.48 (s), 130.96 (d, *J* = 6.8 Hz), 130.02 (s), 129.06 (s), 128.38 (s), 128.19 (s), 126.88 (s), 119.36 (d, *J* = 16.3 Hz), 115.72 (s), 110.96 (d, *J* = 2.8 Hz), 102.34 (d, *J* = 26.0 Hz), 69.23 (s), 34.16 (s), 23.18 (s), 11.31 (s), 10.45 (s); IR (cm^{-1}): 3427.73, 2926.25, 1731.75, 1628.47, 1585.31, 1509.12, 1456.27, 1433.66, 1412.74, 1364.38, 1286.93, 1189.05, 1143.95, 1107.57, 1036.02, 844.93, 805.09, 757.31, 710.14; MS (ESI), *m/z*: 368.2 $(M–H)^-$. Anal. calcd. for $C_{21}H_{20}FNO_4$: C, 68.28; H, 5.46; N, 3.79. Found: C, 68.31; H, 5.47; N, 3.74.

4.1.4. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl) -5-methylbenzyl)oxy)-2-fluorophenyl)propanoic acid (11b)

Compound **11b** (0.12 g, 54%) as a white solid. m.p.: 120–122°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.15 (br s, 1H), 7.34–7.11 (m, 4H), 6.95–6.74 (m, 2H), 5.10 (s, 2H), 2.75 (t, *J* = 7.1 Hz, 2H), 2.46 (t, *J* = 7.1 Hz, 2H), 2.37 (s, 6H), 2.19 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.55 (s), 165.11 (s), 162.44 (s), 159.21 (s), 157.99 (d, *J* = 11.8 Hz), 138.44 (s), 137.35 (s), 130.94 (d, *J* = 6.7 Hz), 129.94 (s), 128.97 (s), 127.50 (s), 125.39 (s), 119.27 (d, *J* = 16.1 Hz), 115.74 (s), 110.94 (d, *J* = 2.8 Hz), 102.31 (d, *J* = 26.1 Hz), 69.26 (s), 34.08 (s), 23.13 (s), 20.94 (s), 11.29 (s), 10.44 (s); IR (cm^{-1}): 3440.82, 2930.61, 1705.68, 1627.51, 1587.51, 1511.23, 1427.25, 1319.48, 1287.01, 1251.36, 1164.35, 1105.05, 859.94, 846.44; MS (ESI), *m/z*: 382.2 $(M–H)^-$. Anal. calcd. for $C_{22}H_{22}FNO_4$: C, 68.92; H, 5.78; N, 3.65. Found: C, 68.88; H, 5.73; N, 3.64.

4.1.5. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-5-fluorobenzyl)oxy)-2-fluorophenyl) propanoic acid (11c)

Compound **11c** (0.15 g, 68%) as a white solid. m.p.: 160–161°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.20 (br s, 1H), 7.49 (d, *J* = 10.2 Hz, 1H), 7.32 (d, *J* = 7.4 Hz, 2H), 7.17 (t, *J* = 8.8 Hz, 1H), 6.74 (d, *J* = 12.1 Hz, 1H), 6.65 (d, *J* = 8.4 Hz, 1H), 4.79 (s, 2H), 2.74 (t, *J* = 7.5 Hz, 2H), 2.45 (t, *J* = 7.5 Hz, 2H), 2.19 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.54 (s), 165.80 (s), 162.37 (s), 162.17 (d, *J* = 243.0 Hz), 159.00 (d, *J* = 22.5 Hz), 157.63 (d, *J* = 11.1 Hz), 138.80 (d, *J* = 7.7 Hz), 133.44 (d, *J* = 8.5 Hz), 130.97 (d, *J* = 6.8 Hz), 125.44 (d, *J* = 3.1 Hz), 119.64 (d, *J* = 2.4 Hz), 119.44 (s), 116.26 (d, *J* = 22.2 Hz), 115.55 (d, *J* = 21.2 Hz), 110.62 (d, *J* = 3.0 Hz), 102.06 (d, *J* = 26.0 Hz), 67.32 (s), 34.02 (s), 23.11 (s), 11.01 (s), 9.99 (s); IR (cm^{-1}): 3351.03, 2938.05, 1746.94, 1714.40, 1625.61, 1586.89, 1507.90, 1409.29, 1278.97, 1264.92, 1243.20, 1179.46, 1162.99, 1148.78, 1096.45, 1043.51, 871.73, 826.81; MS (ESI), *m/z*: 386.1 $(M–H)^-$. Anal. calcd. for $C_{21}H_{19}F_2NO_4$: C, 65.11; H, 4.94; N, 3.62. Found: C, 65.06; H, 4.94; N, 3.60.

4.1.6. Preparation of 3-(4-((3-chloro-5-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluorophenyl)propanoic acid (11d)

Compound **11d** (0.16 g, 64%) as a white solid. m.p.: 116–118°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.14 (br s, 1H), 7.53 (s, 1H), 7.45 (s, 1H), 7.41 (s, 1H), 7.23 (t, *J* = 8.8 Hz, 1H), 6.90 (dd, *J* = 12.1, 2.2 Hz, 1H), 6.82 (dd, *J* = 8.5, 2.0 Hz, 1H), 5.18 (s, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 2.47 (t, *J* = 7.5 Hz, 2H), 2.40 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.54 (s), 165.85 (s), 160.82 (d, *J* = 243.2 Hz), 158.02 (s), 157.69 (d, *J* = 11.2 Hz), 139.86 (s), 133.64 (s), 132.17 (s), 131.00 (d, *J* = 6.8 Hz), 127.87 (s), 126.76 (s), 126.45 (s), 119.56 (d, *J* = 15.8 Hz), 114.68 (s), 110.92 (d, *J* = 2.6 Hz), 102.41 (d, *J* = 25.9 Hz), 68.36 (s), 34.07 (s), 23.15 (s), 11.31 (s), 10.33 (s); IR (cm^{-1}): 3439.53, 2926.25, 1704.01, 1625.66, 1588.05, 1576.71, 1510.35, 1428.02, 1408.71, 1315.85, 1293.73, 1278.87, 1248.75, 1162.90, 1148.62, 1104.29, 858.36, 782.15; MS (ESI), *m/z*: 402.1 $(M–H)^-$. Anal. calcd. for $C_{21}H_{19}ClFNO_4$: C, 62.46; H, 4.74; N, 3.47. Found: C, 62.44; H, 4.71; N, 3.53.

4.1.7. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-5-(trifluoromethyl)benzyl)oxy)-2-

fluorophenyl)propanoic acid (11e)

Compound **11e** (0.12 g, 48%) as a white solid. m.p.: 153–156°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.15 (br s, 1H), 7.82 (s, 1H), 7.75 (s, 1H), 7.71 (s, 1H), 7.22 (t, *J* = 8.8 Hz, 1H), 6.96–6.87 (m, 1H), 6.87–6.79 (m, 1H), 5.26 (s, 2H), 2.77 (t, *J* = 7.5 Hz, 2H), 2.46 (t, *J* = 7.5 Hz, 2H), 2.40 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.53 (s), 166.07 (s), 160.83 (d, *J* = 243.2 Hz), 158.04 (s), 157.69 (d, *J* = 11.9 Hz), 139.14 (s), 132.03 (s), 131.42 (s), 131.02 (d, *J* = 6.7 Hz), 129.76 (s), 125.14–124.35 (m), 123.72–122.67 (m), 122.15 (s), 119.63 (d, *J* = 16.4 Hz), 114.68 (s), 110.91 (d, *J* = 2.7 Hz), 102.42 (d, *J* = 25.8 Hz), 68.40 (s), 34.07 (s), 23.13 (s), 11.27 (s), 10.27 (s); IR (cm^{-1}): 3056.05, 2932.15, 2678.47, 1712.24, 1628.25, 1587.19, 1508.86, 1452.92, 1438.66, 1419.48, 1348.83, 1275.62, 1238.36, 1165.87, 1119.43, 878.08, 698.54; MS (ESI), *m/z*: 436.1 (M–H)$^{-}$. Anal. calcd. for $C_{22}H_{19}F_4NO_4$: C, 60.41; H, 4.38; N, 3.20. Found: C, 60.47; H, 4.46; N, 3.17.

4.1.8. Preparation of 3-(4-((5-(3,5-dimethylisoxazol-4-yl)-2-fluorobenzyl)oxy)-2-fluorophenyl) propanoic acid (11f)

Compound **11f** (0.15 g, 60%) as a white solid. m.p.: 139–142°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.16 (br s, 1H), 7.55 (d, *J* = 7.0 Hz, 1H), 7.49–7.29 (m, 2H), 7.22 (t, *J* = 8.8 Hz, 1H), 6.92 (d, *J* = 12.2 Hz, 1H), 6.82 (d, *J* = 8.7 Hz, 1H), 5.18 (s, 2H), 2.76 (t, *J* = 7.6 Hz, 2H), 2.46 (t, *J* = 7.6 Hz, 2H), 2.36 (s, 3H), 2.19 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.63 (s), 165.28 (s), 161.48 (s), 160.83 (d, *J* = 243.5 Hz), 158.14 (d, *J* = 8.5 Hz), 157.74 (d, *J* = 10.9 Hz), 131.31 (d, *J* = 4.0 Hz), 130.99 (d, *J* = 7.3 Hz), 126.24 (d, *J* = 3.5 Hz), 124.08 (d, *J* = 15.2 Hz), 119.61 (d, *J* = 16.2 Hz), 116.19 (t, *J* = 3.2 Hz), 115.91 (s), 114.91 (s), 110.87 (d, *J* = 2.7 Hz), 102.35 (d, *J* = 26.0 Hz), 63.81 (s), 34.12 (s), 23.16 (s), 11.21 (s), 10.31 (s); IR (cm^{-1}): 3439.53, 2943.95, 1709.60, 1627.59, 1586.03, 1508.26, 1424.83, 1306.05, 1281.20, 1258.41, 1249.56, 1220.61, 1149.83, 1108.46, 1098.59, 1013.71, 836.12, 645.40; MS (ESI), *m/z*: 386.1 (M–H)$^{-}$. Anal. calcd. for $C_{21}H_{19}F_2NO_4$: C, 65.11; H, 4.94; N, 3.62. Found: C, 65.20; H, 4.92; N, 3.61.

4.1.9. Preparation of 3-(4-((2-chloro-5-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluoro-phenyl)propanoic acid (11g)

Compound **11g** (0.14 g, 63%) as a white solid. m.p.: 131–132°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.18 (br s, 1H), 7.64–7.46 (m, 2H), 7.45–7.30 (m, 1H), 7.20 (t, *J* = 8.2 Hz, 1H), 6.97–6.67 (m, 2H), 5.11 (s, 2H), 2.75 (t, *J* = 7.3 Hz, 2H), 2.47 (t, *J* = 7.3 Hz, 2H), 2.31 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.55 (s), 166.51 (s), 160.82 (d, *J* = 243.0 Hz), 160.60 (s), 158.19 (d, *J* = 60.9 Hz), 157.69 (d, *J* = 43.6 Hz), 133.49 (d, *J* = 3.5 Hz), 131.30 (d, *J* = 3.3 Hz), 130.95 (d, *J* = 6.9 Hz), 129.94 (d, *J* = 8.4 Hz), 119.42 (d, *J* = 16.2 Hz), 117.12 (d, *J* = 16.2 Hz), 116.17 (d, *J* = 22.4 Hz), 110.96 (d, *J* = 2.8 Hz), 110.17 (s), 102.37 (d, *J* = 25.9 Hz), 68.58 (s), 34.10 (s), 23.16 (s), 11.22 (s), 10.11 (s); IR (cm^{-1}): 3433.63, 3050.15, 3002.95, 1726.28, 1627.56, 1508.85, 1464.03, 1365.70, 1296.13, 1152.40, 1130.43, 1110.93, 838.37; MS (ESI), *m/z*: 402.1 (M–H)$^{-}$. Anal. calcd. for $C_{21}H_{19}ClFNO_4$: C, 62.46; H, 4.74; N, 3.47. Found: C, 62.43; H, 4.78; N, 3.50.

4.1.9. Preparation of 3-(4-((5-(3,5-dimethylisoxazol-4-yl)-2-methoxybenzyl)oxy)-2-fluoro-phenyl)propanoic acid (11h)

Compound **11h** (0.13 g, 54%) as a white solid. m.p.: 125–127°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.43–7.27 (m, 2H), 7.25–7.09 (m, 2H), 6.92–6.73 (m, 2H), 5.08 (s, 2H), 3.85 (s, 3H), 2.75 (t, *J* = 7.2 Hz, 2H), 2.45 (t, *J* = 7.2 Hz, 2H), 2.31 (s, 3H), 2.14 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.54 (s), 164.65 (s), 160.85 (d, *J* = 243.1 Hz), 158.15 (s), 158.03 (s), 156.46 (s),

130.94 (d, *J* = 6.8 Hz), 129.92 (d, *J* = 10.3 Hz), 124.78 (s), 121.71 (s), 119.22 (d, *J* = 15.9 Hz), 115.46 (s), 111.47 (s), 110.75 (d, *J* = 2.6 Hz), 102.24 (d, *J* = 25.7 Hz), 64.80 (s), 55.65 (s), 34.12 (s), 23.15 (s), 11.16 (s), 10.35 (s); IR (cm^{-1}): 3091.45, 2979.35, 2843.66, 1740.33, 1628.35, 1609.89, 1585.33, 1509.33, 1491.55, 1464.35, 1405.91, 1292.77, 1252.61, 1155.33, 1103.87, 1037.17, 827.05; MS (ESI), *m/z*: 398.2 $(M–H)^-$. Anal. calcd. for $C_{22}H_{22}FNO_5$: C, 66.16; H, 5.55; N, 3.51. Found: C, 66.13; H, 5.62; N, 3.47.

4.1.10. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-2-fluorobenzyl)oxy)-2-fluoro-phenyl)propanoic acid (11i)

Compound **11i** (0.12 g, 52%) as a white solid. m.p.: 132–134°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.20 (br s, 1H), 7.65–7.56 (m, 1H), 7.44 (td, *J* = 7.5, 1.9 Hz, 1H), 7.34 (t, *J* = 7.6 Hz, 1H), 7.22 (t, *J* = 8.8 Hz, 1H), 6.92 (dd, *J* = 12.1, 2.5 Hz, 1H), 6.82 (dd, *J* = 8.5, 2.4 Hz, 1H), 5.18 (s, 2H), 2.77 (t, *J* = 7.5 Hz, 2H), 2.46 (t, *J* = 7.5 Hz, 2H), 2.33 (s, 3H), 2.15 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.62 (s), 166.55 (s), 160.86 (d, *J* = 243.3 Hz), 158.67 (s), 157.86 (d, *J* = 11.1 Hz), 155.93 (s), 131.89 (d, *J* = 2.6 Hz), 131.03 (d, *J* = 6.7 Hz), 130.67 (d, *J* = 4.0 Hz), 124.78 (d, *J* = 4.3 Hz), 124.41 (d, *J* = 15.1 Hz), 119.64 (d, *J* = 15.8 Hz), 117.47 (s), 110.86 (d, *J* = 2.7 Hz), 102.32 (d, *J* = 25.8 Hz), 63.96 (s), 34.12 (s), 23.17 (s), 11.32 (s), 10.20 (s); IR (cm^{-1}): 2991.15, 1706.73, 1627.14, 1581.58, 1508.62, 1457.43, 1420.08, 1290.99, 1255.86, 1148.86, 1096.61, 1007.33, 966.47; MS (ESI), *m/z*: 386.1 $(M–H)^-$. Anal. calcd. for $C_{21}H_{19}F_2NO_4$: C, 65.11; H, 4.94; N, 3.62. Found: C, 65.12; H, 4.96; N, 3.65.

4.1.11. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-4-fluorobenzyl)oxy)-2-fluoro-phenyl)propanoic acid (11j)

Compound **11j** (0.15 g, 60%) as a white solid. m.p.: 111–113°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.15 (br s, 1H), 7.66– .55 (m, 2H), 7.42 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.22 (t, *J* = 8.8 Hz, 1H), 6.93 (dd, *J* = 12.1, 2.4 Hz, 1H), 6.82 (dd, *J* = 8.4, 2.3 Hz, 1H), 5.19 (s, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.46 (t, *J* = 7.5 Hz, 2H), 2.35 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.57 (s), 165.58 (s), 160.86 (d, J = 243.3 Hz), 158.05 (s), 157.75 (d, J = 11.0 Hz), 134.55 (s), 132.07 (s), 131.06 (d, J = 6.9 Hz), 130.69 (s), 130.28 (s), 129.97 (s), 129.10 (s), 119.66 (d, J = 15.8 Hz), 114.80 (d, J = 1.7 Hz), 110.83 (s), 102.42 (d, J = 26.0 Hz), 67.06 (s), 34.09 (s), 23.15 (s), 11.27 (s), 10.35 (s); IR (cm^{-1}): 2985.25, 1709.06, 1627.47, 1588.01, 1509.58, 1479.08, 1450.46, 1423.33, 1288.56, 1241.81, 1194.47, 1161.44, 1153.56, 1109.62, 1096.28, 1062.48, 1031.96, 968.22, 836.24; MS (ESI), *m/z*: 386.1 $(M–H)^-$. Anal. calcd. for $C_{21}H_{19}F_2NO_4$: C, 65.11; H, 4.94; N, 3.62. Found: C, 65.15; H, 4.88; N, 3.57.

4.1.12. Preparation of 3-(4-((4-chloro-3-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluoro-phenyl)propanoic acid (11k)

Compound **11k** (0.13 g, 54%) as a white solid. m.p.: 143–145°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.14 (br s, 1H), 7.64 (d, *J* = 8.3 Hz, 1H), 7.52 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.46 (d, *J* = 1.9 Hz, 1H), 7.21 (t, *J* = 8.8 Hz, 1H), 6.87 (dd, *J* = 12.2, 2.4 Hz, 1H), 6.79 (dd, *J* = 8.4, 2.4 Hz, 1H), 5.14 (s, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.46 (t, *J* = 7.5 Hz, 2H), 2.25 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.56 (s), 166.23 (s), 160.83 (d, *J* = 243.2 Hz), 158.61 (s), 157.77 (d, *J* = 11.6 Hz), 136.47 (s), 132.77 (s), 131.64 (s), 130.98 (d, *J* = 6.7 Hz), 129.69 (d, *J* = 32.4 Hz), 128.44 (s), 119.49 (d, *J* = 15.6 Hz), 113.79 (d, *J* = 19.9 Hz), 110.99 (d, *J* = 3.0 Hz), 102.42 (d, *J* = 25.8 Hz), 68.44 (s), 34.07 (s), 23.13 (s), 11.34 (s), 10.18 (s); IR (cm^{-1}): 3398.23, 2973.45, 1736.09, 1628.70, 1588.09, 1509.08, 1460.68, 1357.83, 1295.35, 1189.95, 1160.55, 1149.09, 1114.39, 1097.53, 1085.66, 818.17; MS (ESI), *m/z*: 402.1 $(M–H)^-$. Anal. calcd. for $C_{21}H_{19}ClFNO_4$: C,

62.46; H, 4.74; N, 3.47. Found: C, 62.52; H, 4.69; N, 3.48.

4.1.13. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-4-methoxybenzyl)oxy)-2-fluoro-phenyl)propanoic acid (11l)

Compound **11l** (0.15 g, 58%) as a white solid. m.p.: 140–142°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.16 (s, 1H), 7.46 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.28 (d, *J* = 2.1 Hz, 1H), 7.19 (t, *J* = 8.8 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 6.86 (dd, *J* = 12.2, 2.5 Hz, 1H), 6.78 (dd, *J* = 8.4, 2.4 Hz, 1H), 5.05 (s, 2H), 3.78 (s, 3H), 2.75 (t, *J* = 7.4 Hz, 2H), 2.45 (t, *J* = 7.4 Hz, 2H), 2.23 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.57 (s), 165.58 (s), 160.83 (d, *J* = 243.2 Hz), 159.04 (s), 158.04 (d, *J* = 11.2 Hz), 156.59 (s), 131.22 (s), 130.90 (d, *J* = 6.7 Hz), 129.64 (s), 128.75 (s), 119.18 (d, *J* = 15.8 Hz), 118.01 (s), 111.56 (s), 111.01 (d, *J* = 2.7 Hz), 102.34 (d, *J* = 25.8 Hz), 69.06 (s), 55.44 (s), 34.10 (s), 23.15 (s), 11.30 (s), 10.32 (s); IR (cm^{-1}): 3445.43, 2932.15, 1732.65, 1625.59, 1508.45, 1458.87, 1441.34, 1363.13, 1297.39, 1253.68, 1191.98, 1146.87, 1098.23, 1027.57, 813.30; MS (ESI), *m/z*: 398.2 $(M–H)^-$. Anal. calcd. for $C_{22}H_{22}FNO_5$: C, 66.16; H, 5.55; N, 3.51. Found: C, 66.21; H, 5.56; N, 3.43.

4.1.14. Preparation of 3-bromo-5-nitrobenzaldehyde (13)

To a solution of 3-nitrobenzaldehyde (1.0 g, 6.6 mmol) in concentrated sulfuric acid (4.0 mL) was added N-bromosuccinimide (1.4 g, 7.9 mmol) in small portions at room temperature and then heated to 65°C for 1 h. After cooling to the room temperature, the solution was poured into ice water and the precipitate was filtered. The crude product was dried over Na_2SO_4 and recrystallized from EtOAc/petroleum ether (1/10) to obtain **13** (1.3 g, 82%) as white crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.09 (s, 1H), 8.79–8.55 (m, 2H), 8.51 (s, 1H).

4.1.15. Preparation of 2-(3-bromo-5-nitrophenyl)-1,3-dioxolane (14)

To a solution of 3-bromo-5-nitrobenzaldehyde **13** (0.90 g, 3.9 mmol), ethylene glycol (2.2 mL, 39 mmol) and p-TsOH (70 mg, 0.39 mmol) in toluene (25 mL) was refluxed for 5 h. After cooling to the room temperature, the solution was diluted with EtOAc (25 mL) and washed with brine (20 mL). The organic layers were dried over Na_2SO_4 and concentrated to afford a crude product **14** (0.99 g, 92%) as a pale yellow solid without further purification.

4.1.16. Preparation of 3-bromo-5-(1,3-dioxolan-2-yl)aniline (15)

To a solution of **14** (0.99 g, 3.6 mmol) and ammonium chloride (0.29 g, 5.4 mmol) in ethanol/H_2O (30 mL, 5/2) was added iron powders (1.0 g, 18 mmol) at room temperature. The reaction mixture was refluxed for 4 h and then filtered. The filtrate was diluted with EtOAc (30 mL) and washed with brine (20 mL). The organic layers were dried over Na_2SO_4 and concentrated to afford **15** (0.72 g, 82%) as a yellow oily product. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.71 (t, *J* = 1.8 Hz, 1H), 6.65 (s, 1H), 6.60 (s, 1H), 5.56 (s, 1H), 5.46 (s, 2H), 4.03–3.88 (m, 4H).

4.1.17. Preparation of 3-bromo-5-hydroxybenzaldehyde (16)

To a solution of **15** (0.71 g, 6.0 mmol) in AcOH/H_2SO_4/H_2O (5.0 mL, 8/1/1) was added sodium nitrite aqueous solution (0.24 g, 3.5 mmol, 1 mL) dropwise and stirred for 1 h at 0°C. The reaction was added 10% sulphuric acid (20 mL) and refluxed for 2 h. After cooling to the room temperature, the reaction mixture was extracted with EtOAc (3 × 30 mL), washed with saturated sodium bicarbonate solution (3 × 30 mL) and brine (30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc/petroleum ether, 1/8) to give **16** (0.47 g, 80%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.43 (s, 1H), 9.88 (s, 1H), 7.51 (s, 1H), 7.31–7.18 (m, 2H).

4.1.18. Preparation of 3-(3,5-dimethylisoxazol-4-yl)-5-hydroxybenzaldehyde (17)

To a mixture of 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole (0.50 g, 2.0 mmol), **16** (0.41 g, 2.0 mmol), $Pd(PPh_3)_4$ (0.12 g, 0.10 mmol) and sodium carbonate (0.43 g, 4.0 mmol) in toluene/ethanol/H_2O (15 mL, 3/1/3) was refluxed under nitrogen atmosphere for 24 h. Then the mixture was diluted with saturated ammonium chloride solution and EtOAc, and the insoluble material was filtered through Celite. The organic layer of the filtrate was washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (EtOAc/petroleum ether, 1/5 to 1/3) to give **17** (0.30 g, 69%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 9.96 (s, 1H), 7.37 (s, 1H), 7.25 (s, 1H), 7.08 (s, 1H), 2.42 (s, 3H), 2.24 (s, 3H).

4.1.19. Preparation of 3-(3,5-dimethylisoxazol-4-yl)-5-methoxybenzaldehyde (18a)

To a solution of **17** (0.14 g, 0.64 mmol), iodomethane (0.11 g, 0.77 mmol) and potassium carbonate (0.18 g, 1.3 mmol) in DMF (3.0 mL) was stirred at 60°C for 5 h. Then the mixture was quenched with saturated ammonium chloride solution and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/petroleum ether, 1/9 to 1/5) to give **10a** (0.12 g, 81%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.03 (s, 1H), 7.52 (s, 1H), 7.43 (s, 1H), 7.29 (s, 1H), 3.88 (s, 3H), 2.43 (s, 3H), 2.25 (s, 3H).

4.1.20. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-5-methoxybenzyl)oxy)-2-fluoro-phenyl)propanoic acid (22a)

Compound **22a** was prepared from **18a** according to the general procedures of compound **11a**. These procedures can also be applied to the synthesis of compounds **22b-h**. Compound **22a** (80 mg, 64%) as a white solid. m.p.: 142–144°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.19 (t, J = 8.7 Hz, 1H), 7.01 (s, 1H), 6.99 (s, 1H), 6.93–6.72 (m, 3H), 5.11 (s, 2H), 3.79 (s, 3H), 2.72 (t, J = 7.6 Hz, 2H), 2.44–2.30 (m, 5H), 2.20 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 174.36 (s), 165.28 (s), 160.41 (d, J = 204.8 Hz), 159.66 (s), 157.94 (d, J = 22.7 Hz), 138.97 (s), 131.26 (s), 130.90 (d, J = 7.2 Hz), 120.36 (s), 115.71 (s), 113.88 (s), 112.30 (s), 110.87 (d, J = 2.5 Hz), 102.29 (d, J = 25.8 Hz), 69.13 (s), 55.29 (s), 35.47 (s), 23.67 (s), 11.31 (s), 10.43 (s); IR (cm^{-1}): 3457.23, 2938.05, 1711.15, 1628.62, 1593.95, 1508.72, 1457.13, 1417.74, 1334.09, 1287.19, 1253.87, 1156.05, 1114.12, 859.39, 830.45; MS (ESI), *m/z*: 398.2 $(M–H)^-$. Anal. calcd. for $C_{22}H_{22}FNO_5$: C, 66.16; H, 5.55; N, 3.51. Found: C, 66.21; H, 5.54; N, 3.53.

4.1.21. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-5-ethoxybenzyl)oxy)-2-fluoro-phenyl)propanoic acid (22b)

Compound **22b** (0.10 g, 66%) as a white solid. m.p.: 166–168°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.17 (s, 1H), 7.20 (t, J = 8.8 Hz, 1H), 6.98 (d, J = 6.3 Hz, 2H), 6.91–6.76 (m, 3H), 5.11 (s, 2H), 4.07 (q, J = 6.9 Hz, 2H), 2.75 (t, J = 7.3 Hz, 2H), 2.47 (t, J = 7.4 Hz, 2H), 2.37 (s, 3H), 2.20 (s, 3H), 1.33 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.53 (s), 165.24 (s), 160.82 (d, J = 243.0 Hz), 158.92 (s), 158.08 (s), 157.91 (d, J = 11.0 Hz), 138.91 (s), 131.26 (s), 130.92 (d, J = 6.9 Hz), 120.21 (s), 119.32 (d, J = 16.0 Hz), 115.72 (s), 114.25 (s), 112.74 (s), 110.95 (d, J = 2.6 Hz), 102.35 (d, J = 25.8 Hz), 69.12 (s), 63.23 (s), 34.07 (s), 23.15 (s), 14.59 (s), 11.30 (s), 10.43 (s); IR (cm^{-1}): 3445.43, 2991.15, 1709.11, 1629.33, 1589.76, 1511.65, 1284.11, 1267.68, 1252.88, 1155.03, 1116.18, 1045.04, 860.63; MS (ESI), *m/z*: 412.2 $(M–H)^-$. Anal. calcd. for $C_{23}H_{24}FNO_5$: C, 66.82; H, 5.85; N, 3.39. Found: C, 66.86; H, 5.81; N, 3.41.

4.1.22. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-5-propoxybenzyl)oxy)-2-fluoro-

phenyl)propanoic acid (22c)

Compound **22c** (80 mg, 57%) as a pale yellow solid. m.p.: 126–128°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.16 (br s, 1H), 7.20 (t, J = 8.8 Hz, 1H), 7.00 (s, 1H), 6.97 (s, 1H), 6.91–6.83 (m, 2H), 6.83–6.76 (m, 1H), 5.11 (s, 2H), 3.97 (t, J = 6.5 Hz, 2H), 2.75 (t, J = 7.6 Hz, 2H), 2.45 (d, J = 7.6 Hz, 2H), 2.37 (s, 3H), 2.20 (s, 3H), 1.80–1.65 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.51 (s), 165.23 (s), 160.80 (d, J = 243.3 Hz), 159.07 (s), 158.07 (s), 157.90 (d, J = 11.0 Hz), 138.90 (s), 131.25 (s), 130.92 (d, J = 6.8 Hz), 120.22 (s), 119.31 (d, J = 16.1 Hz), 115.72 (s), 114.27 (s), 112.82 (s), 110.95 (d, J = 2.8 Hz), 102.35 (d, J = 25.8 Hz), 69.11 (s), 69.03 (s), 34.07 (s), 23.12 (s), 22.00 (s), 11.30 (s), 10.42 (s), 10.39 (s); IR (cm^{-1}): 2938.05, 1713.15, 1629.71, 1589.66, 1509.86, 1420.92, 1337.64, 1282.99, 1264.25, 1251.46, 1154.94, 1116.18, 859.26; MS (ESI), *m/z*: 426.2 $(M–H)^-$. Anal. calcd. for $C_{24}H_{26}FNO_5$: C, 67.43; H, 6.13; N, 3.28. Found: C, 67.41; H, 6.10; N, 3.35.

4.1.23. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-5-isopropoxybenzyl)oxy)-2-fluoro-phenyl)propanoic acid (22d)

Compound **22d** (80 mg, 56%) as a clear oily product. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.21 (t, J = 9.0 Hz, 1H), 7.01–6.71 (m, 5H), 5.11 (s, 2H), 4.74–4.60 (m, 1H), 2.76 (t, J = 7.0 Hz, 2H), 2.46 (t, J = 7.0 Hz, 2H), 2.38 (s, 3H), 2.20 (s, 3H), 1.34–1.13 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.57 (s), 165.24 (s), 160.82 (d, J = 243.1 Hz), 158.11 (s), 157.99 (s), 157.85 (d, J = 5.1 Hz), 138.97 (s), 131.29 (s), 130.95 (d, J = 6.9 Hz), 120.10 (s), 119.33 (d, J = 15.8 Hz), 115.74 (s), 115.18 (s), 113.90 (s), 110.97 (d, J = 3.0 Hz), 102.36 (d, J = 25.7 Hz), 69.29 (s), 69.11 (s), 34.11 (s), 23.15 (s), 21.80 (s), 11.36 (s), 10.48 (s); IR (cm^{-1}): 3439.53, 2979.35, 1710.75, 1627.51, 1589.22, 1508.42, 1421.24, 1323.76, 1285.66, 1258.54, 1207.13, 1152.89, 1110.11, 1015.84, 847.88; MS (ESI), *m/z*: 426.2 $(M–H)^-$. Anal. calcd. for $C_{24}H_{26}FNO_5$: C, 67.43; H, 6.13; N, 3.28. Found: C, 67.28; H, 6.15; N, 3.29.

4.1.24. Preparation of 3-(4-((3-butoxy-5-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluoro-phenyl)propanoic acid (22e)

Compound **22e** (80 mg, 52%) as a clear oily product. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.21 (br s, 1H), 7.20 (t, J = 8.8 Hz, 1H), 7.00 (s, 1H), 6.97 (s, 1H), 6.91–6.83 (m, 2H), 6.83–6.74 (m, 1H), 5.11 (s, 2H), 4.01 (t, J = 6.5 Hz, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.45 (t, J = 7.5 Hz, 2H), 2.37 (s, 3H), 2.20 (s, 3H), 1.76–1.64 (m, 2H), 1.50–1.36 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.55 (s), 165.26 (s), 160.81 (d, J = 242.8 Hz), 159.09 (s), 158.09 (s), 157.91 (d, J = 11.7 Hz), 138.90 (s), 131.25 (s), 130.94 (d, J = 6.9 Hz), 120.23 (s), 119.99 (d, J = 66.0 Hz), 115.73 (s), 114.27 (s), 112.82 (s), 110.96 (d, J = 3.0 Hz), 102.35 (d, J = 25.9 Hz), 69.12 (s), 67.33 (s), 34.09 (s), 30.73 (s), 23.13 (s), 18.75 (s), 13.72 (s), 11.33 (s), 10.45 (s); IR (cm^{-1}): 2967.55, 1710.48, 1627.67, 1592.05, 1508.38, 1421.66, 1328.41, 1304.07, 1285.79, 1259.00, 1207.44, 1152.19, 1109.42, 1098.47, 1024.68, 851.01; MS (ESI), *m/z*: 440.2 $(M–H)^-$. Anal. calcd. for $C_{25}H_{28}FNO_5$: C, 68.01; H, 6.39; N, 3.17. Found: C, 67.99; H, 6.35; N, 3.21.

4.1.25. Preparation of 3-(4-((3-(3,5-dimethylisoxazol-4-yl)-5-isobutoxybenzyl)oxy)-2-fluoro-phenyl)propanoic acid (22f)

Compound **22f** (90 mg, 62%) as a pale yellow solid. m.p.: 122–124°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.10 (br s, 1H), 7.20 (t, J = 8.8 Hz, 1H), 7.01 (s, 1H), 6.97 (s, 1H), 6.90–6.76 (m, 3H), 5.11 (s, 2H), 3.79 (d, J = 6.5 Hz, 2H), 2.76 (t, J = 7.4 Hz, 2H), 2.45 (t, J = 7.3 Hz, 2H), 2.37 (s, 3H), 2.19 (s, 3H), 2.07–1.96 (m, 1H), 0.99 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.57 (s), 165.26 (s), 160.82 (d, J = 243.7 Hz), 159.04 (s), 158.11 (s), 157.90 (d, J = 11.2 Hz),

138.89 (s), 131.25 (s), 130.94 (d, *J* = 6.8 Hz), 120.23 (s), 119.34 (d, *J* = 15.6 Hz), 115.73 (s), 114.38 (s), 112.79 (s), 110.97 (d, *J* = 2.7 Hz), 102.37 (d, *J* = 25.9 Hz), 72.23 (s), 69.12 (s), 34.11 (s), 23.17 (s), 11.33 (s), 10.46 (s), 10.13 (s), 3.15 (s); IR (cm^{-1}): 3463.13, 2908.55, 1716.10, 1628.88, 1588.68, 1508.91, 1423.01, 1335.26, 1305.24, 1282.11, 1263.62, 1248.12, 1202.33, 1157.26, 1115.32, 1100.72, 1028.72, 965.75, 856.72, 830.20; MS (ESI), *m/z*: 440.2 $(M–H)^-$. Anal. calcd. for $C_{25}H_{28}FNO_5$: C, 68.01; H, 6.39; N, 3.17. Found: C, 68.00; H, 6.37; N, 3.22.

4.1.26. Preparation of 3-(4-((3-(cyclopropylmethoxy)-5-(3,5-dimethylisoxazol-4-yl)benzyl) oxy)-2-fluorophenyl)propanoic acid (22g)

Compound **22g** (0.10 g, 69%) as a clear oily product. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.07 (br s, 1H), 7.20 (t, *J* = 8.8 Hz, 1H), 7.05–6.93 (m, 2H), 6.91–6.74 (m, 3H), 5.11 (s, 2H), 3.86 (d, *J* = 7.0 Hz, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.45 (d, *J* = 7.5 Hz, 2H), 2.37 (s, 3H), 2.19 (s, 3H), 1.29–1.18 (m, 1H), 0.65 – 0.49 (m, 2H), 0.39–0.25 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.55 (s), 165.25 (s), 160.81 (d, *J* = 244.9 Hz), 158.09 (s), 157.97 (d, *J* = 2.5 Hz), 157.84 (s), 138.91 (s), 131.27 (s), 130.94 (d, *J* = 7.0 Hz), 120.28 (s), 119.33 (d, *J* = 16.4 Hz), 115.73 (s), 114.30 (s), 112.91 (s), 110.95 (d, *J* = 2.8 Hz), 102.36 (d, *J* = 25.7 Hz), 73.89 (s), 69.12 (s), 34.10 (s), 27.71 (s), 23.15 (s), 19.06 (s), 11.32 (s), 10.44 (s); IR (cm^{-1}): 2960.05, 1711.11, 1627.59,1592.24, 1508.28, 1469.64, 1421.73, 1327.62, 1285.32, 1259.37, 1208.02, 1152.09, 1109.38, 1098.30, 1031.56, 846.61; MS (ESI), *m/z*: 438.2 $(M–H)^-$. Anal. calcd. for $C_{25}H_{26}FNO_5$: C, 68.32; H, 5.96; N, 3.19. Found: C, 68.35; H, 6.01; N, 3.15.

4.1.27. Preparation of 3-(4-((3-(cyclopentyloxy)-5-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluorophenyl)propanoic acid (22h)

Compound **22h** (90 mg, 62%) as a clear oily product. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.18 (br s, 1H), 7.20 (t, *J* = 8.8 Hz, 1H), 6.95 (s, 2H), 6.91–6.74 (m, 3H), 5.10 (s, 2H), 4.86 (t, *J* = 5.8 Hz, 1H), 2.75 (t, *J* = 7.5 Hz, 2H), 2.45 (t, *J* = 7.5 Hz, 2H), 2.38 (s, 3H), 2.20 (s, 3H), 2.00–1.84 (m, 2H), 1.77–1.51 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.56 (s), 165.24 (s), 160.83 (d, *J* = 242.9 Hz), 159.71 (s), 158.05 (d, *J* = 6.2 Hz), 157.86 (s), 138.92 (s), 131.22 (s), 130.95 (d, *J* = 6.9 Hz), 119.97 (s), 119.33 (d, *J* = 15.8 Hz), 115.75 (s), 114.90 (s), 113.88 (s), 110.97 (d, *J* = 2.8 Hz), 102.36 (d, *J* = 25.7 Hz), 78.82 (s), 69.11 (s), 34.11 (s), 32.26 (s), 23.66 (s), 23.15 (s), 11.35 (s), 10.48 (s); IR (cm^{-1}): 3463.13, 2960.75, 1710.08, 1627.41, 1589.73, 1508.18, 1421.09, 1327.49, 1302.92, 1285.50, 1258.64, 1206.58, 1152.32, 1109.13, 1098.16, 1016.96, 849.80; MS (ESI), *m/z*: 452.2 $(M–H)^-$. Anal. calcd. for $C_{26}H_{28}FNO_5$: C, 68.86; H, 6.22; N, 3.09. Found: C, 68.89; H, 6.17; N, 3.11.

4.1.28. Preparation of 3-(4-((6-(3,5-dimethylisoxazol-4-yl)pyridin-2-yl)methoxy)-2-fluoro-phenyl)propanoic acid (28a)

Compound **28a** (0.11 g, 76%) as a white solid. m.p.: 97–100°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.18 (br s, 1H), 7.92 (t, *J* = 7.8 Hz, 1H), 7.56–7.40 (m, 2H), 7.21 (t, *J* = 8.9 Hz, 1H), 6.96–6.75 (m, 2H), 5.22 (s, 2H), 2.76 (t, *J* = 7.4 Hz, 2H), 2.56 (s, 3H), 2.46 (t, *J* = 7.4 Hz, 2H), 2.37 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.57 (s), 167.50 (s), 160.83 (d, *J* = 243.2 Hz), 158.38 (s), 157.87 (d, *J* = 11.3 Hz), 156.54 (s), 149.50 (s), 137.95 (s), 131.00 (d, *J* = 6.9 Hz), 121.93 (s), 119.83 (s), 119.49 (d, *J* = 16.0 Hz), 115.16 (s), 110.86 (d, *J* = 2.8 Hz), 102.36 (d, *J* = 25.9 Hz), 70.46 (s), 34.05 (s), 23.13 (s), 12.20 (s), 11.26 (s); IR (cm^{-1}): 3415.93, 2926.25, 1739.94, 1624.57, 1584.38, 1571.98, 1510.54, 1474.32, 1449.04, 1439.21, 1296.20, 1185.13, 1166.36, 1102.90, 963.15; MS (ESI), *m/z*: 369.2 $(M–H)^-$. Anal. calcd. for $C_{20}H_{19}FN_2O_4$: C, 64.86; H, 5.17; N, 7.56. Found: C, 64.89; H, 5.15; N, 7.55.

4.1.29. Preparation of 3-(4-((5-(3,5-dimethylisoxazol-4-yl)pyridin-3-yl)methoxy)-2-fluoro-phenyl)propanoic acid (28b)

Compound **28b** (0.12 g, 83%) as a white solid. m.p.: 162–164°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.13 (br s, 1H), 8.68 (s, 1H), 8.60 (s, 1H), 7.91 (s, 1H), 7.24 (t, *J* = 8.8 Hz, 1H), 6.92 (d, *J* = 12.1 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 5.22 (s, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 2.48 (d, *J* = 7.5 Hz, 2H), 2.43 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.59 (s), 166.25 (s), 160.36 (d, *J* = 315.7 Hz), 158.05 (d, *J* = 32.9 Hz), 148.93 (s), 148.09 (s), 135.83 (s), 132.50 (s), 131.04 (d, *J* = 6.6 Hz), 125.84 (s), 119.63 (d, *J* = 16.1 Hz), 112.74 (s), 110.98 (d, *J* = 2.4 Hz), 102.46 (d, *J* = 25.9 Hz), 67.04 (s), 34.10 (s), 23.16 (s), 11.33 (s), 10.34 (s); IR (cm^{-1}): 3439.53, 2938.05, 1711.52, 1628.40, 1586.42, 1510.45, 1433.59, 1387.10, 1284.10, 1233.01, 1148.92, 1111.69, 841.96, 714.98; MS (ESI), *m/z*: 369.1 $(M–H)^-$. Anal. calcd. for $C_{20}H_{19}FN_2O_4$: C, 64.86; H, 5.17; N, 7.56. Found: C, 64.83; H, 5.18; N, 7.45.

4.1.30. Preparation of 3-(4-((2-(3,5-dimethylisoxazol-4-yl)pyridin-4-yl)methoxy)-2-fluoro-phenyl)propanoic acid (28c)

Compound **28c** (0.11 g, 73%) as a white solid. m.p.: 162–164°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.15 (s, 1H), 8.64 (d, *J* = 5.6 Hz, 1H), 7.51 (s, 1H), 7.35 (d, *J* = 9.7 Hz, 1H), 7.20 (t, *J* = 8.8 Hz, 1H), 6.94–6.71 (m, 2H), 5.23 (s, 2H), 2.74 (t, *J* = 7.6 Hz, 2H), 2.51 (s, 3H), 2.44 (d, *J* = 7.4 Hz, 2H), 2.32 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.59 (s), 167.43 (s), 160.87 (d, *J* = 243.3 Hz), 158.34 (s), 157.57 (d, *J* = 11.2 Hz), 150.14 (s), 149.88 (s), 146.91 (s), 131.09 (d, *J* = 6.8 Hz), 120.83 (s), 120.07 (s), 119.74 (d, *J* = 16.2 Hz), 115.36 (s), 110.90 (d, *J* = 2.9 Hz), 102.45 (d, *J* = 25.9 Hz), 67.74 (s), 34.06 (s), 23.15 (s), 12.18 (s), 11.27 (s); IR (cm^{-1}): 3433.63, 2926.25, 1719.39, 1627.41, 1585.40, 1511.83, 1449.93, 1423.81, 1296.09, 1194.35, 1182.19, 1153.18, 965.96, 842.49, 824.80, 618.05; MS (ESI), *m/z*: 369.1 $(M–H)^-$. Anal. calcd. for $C_{20}H_{19}FN_2O_4$: C, 64.86; H, 5.17; N, 7.56. Found: C, 64.92; H, 5.23; N, 7.51.

4.1.31. Preparation of 3-(4-((4-(3,5-dimethylisoxazol-4-yl)pyridin-2-yl)methoxy)-2-fluoro-phenyl)propanoic acid (28d)

Compound **28d** (0.12 g, 81%) as a white solid. m.p.: 176–179°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.72 (d, *J* = 5.2 Hz, 1H), 7.65 (s, 1H), 7.55 (d, *J* = 5.1 Hz, 1H), 7.23 (t, *J* = 8.8 Hz, 1H), 6.93 (d, *J* = 12.0 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 5.29 (s, 2H), 2.77 (t, *J* = 7.4 Hz, 2H), 2.49–2.39 (m, 5H), 2.27 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.57 (s), 168.11 (s), 160.85 (d, *J* = 243.3 Hz), 158.04 (s), 157.44 (d, *J* = 11.1 Hz), 154.64 (s), 146.87 (s), 142.35 (s), 131.14 (d, *J* = 6.8 Hz), 123.61 (s), 122.64 (s), 120.02 (d, *J* = 15.9 Hz), 113.40 (s), 110.95 (d, *J* = 2.6 Hz), 102.63 (d, *J* = 25.9 Hz), 68.49 (s), 34.10 (s), 23.19 (s), 11.79 (s), 10.57 (s); IR (cm^{-1}): 3362.83, 3079.65, 1730.02, 1612.11, 1506.15, 1424.41, 1403.28, 1285.17, 1261.58, 1174.88, 1157.83, 1103.49, 964.52, 843.18; MS (ESI), *m/z*: 369.1 $(M–H)^-$. Anal. calcd. for $C_{20}H_{19}FN_2O_4$: C, 64.86; H, 5.17; N, 7.56. Found: C, 64.86; H, 5.24; N, 7.53.

4.1.32. Preparation of 3-((trimethylsilyl)ethynyl)benzaldehyde (30)

To a mixture of 3-iodobenzaldehyde (0.50 g, 2.2 mmol), trimethylsilylacetylene (0.24 g, 2.4 mmol), $Pd(PPh_3)_4$ (70 mg, 0.060 mmol) and CuI (40 mg, 0.19 mmol) in triethylamine (15 mL) was refluxed under nitrogen atmosphere for 24 h. Then the mixture was filtered through a celite pad and the filtrate concentrated in vacuo. The residue was purified by column chromatography (EtOAc/petroleum ether, 1/20 to 1/15) to give **30** (0.31 g, 71%) as a yellow oily product. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.00 (s, 1H), 7.98 (s, 1H), 7.91 (d, *J* = 7.7 Hz, 1H), 7.77 (d, *J* = 7.7 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 0.25 (s, 9H).

4.1.33. Preparation of 3-ethynylbenzaldehyde (31)

To a solution of **30** (0.30 g, 1.5 mmol) in THF (20 mL) was added tetrabutylammonium fluoride (0.78 g, 3.0 mmol) in small portions at room temperature. The reaction mixture was stirred for 3 h at room temperature and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/petroleum ether, 1/20 to 1/10) to give **31** (0.17 g, 88%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.01 (s, 1H), 8.00 (s, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.63 (t, J = 7.7 Hz, 1H), 4.38 (s, 1H).

4.1.34. Preparation of 3-((3,5-dimethylisoxazol-4-yl)ethynyl)benzaldehyde (32)

To a mixture of 4-iodo-3,5-dimethylisoxazole (0.26 g, 1.2 mmol), 31 (0.15 g, 1.2 mmol), $Pd(PPh_3)_4$ (40 g, 0.030 mmol) and CuI (20 mg, 0.10 mmol) in triethylamine (10 mL) was refluxed under nitrogemn atmosphere for 24 h. Then the mixture was filtered through a celite pad and the filtrate concentrated in vacuo. The residue was purified by column chromatography (EtOAc/petroleum ether, 1/20) to give **32** (0.22 g, 85%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.03 (s, 1H), 8.08 (s, 1H), 7.94 (d, J = 7.7 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.67 (t, J = 7.7 Hz, 1H), 2.54 (s, 3H), 2.31 (s, 3H).

4.1.35. Preparation of 3-(4-((3-((3,5-dimethylisoxazol-4-yl)ethynyl)benzyl)oxy)-2-fluoro-phenyl)propanoic acid (36)

Compound **36** was prepared from **32** according to the general procedures of compound **11a**. Compound **36** (90 mg, 92%) as a white solid. m.p.: 104–106°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.18 (s, 1H), 7.63 (s, 1H), 7.56–7.42 (m, 3H), 7.21 (t, J = 8.8 Hz, 1H), 6.91–6.76 (m, 2H), 5.11 (s, 2H), 2.76 (t, J = 7.6 Hz, 2H), 2.51 (s, 3H), 2.46 (t, J = 7.4 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.62 (s), 171.70 (s), 161.29 (d, J = 178.5 Hz), 159.25 (s), 157.92 (d, J = 11.3 Hz), 137.57 (s), 130.99 (d, J = 6.8 Hz), 130.83 (s), 130.42 (s), 129.02 (s), 128.24 (s), 122.13 (s), 119.45 (d, J = 16.1 Hz), 111.34 (s), 110.89 (d, J = 2.7 Hz), 102.33 (d, J = 25.8 Hz), 93.93 (s), 77.60 (s), 68.95 (s), 34.12 (s), 23.20 (s), 11.72 (s), 10.13 (s); IR (cm^{-1}): 3421.83, 2949.85, 1717.33, 1629.28, 1583.29, 1508.97, 1309.06, 1293.68, 1263.90, 1228.85, 1163.39, 1098.05, 1018.39, 830.69; MS (ESI), *m/z*: 392.2 $(M–H)^-$. Anal. calcd. for $C_{23}H_{20}FNO_4$: C, 70.22; H, 5.12; N, 3.56. Found: C, 70.26; H, 5.18; N, 3.49.

4.2. Biological evaluation

4.2.1. Calcium flux assay in the GPR40 FLIPER assay

The culture medium was removed carefully and CHO cells stably expressing human GPR40 were washed with PBS (5 mL). Trypsin (3 mL) was added and cells were incubated in 37°C for 2–5 min. Then plate medium (10 mL) was added to suspend cells. The cells suspension (50 μL) was seeded into 96-well plates at a density of 1.5×10^4 cells/well, and the assay plates were placed in 37°C, 5% CO_2 incubator for 16–24 hours. After incubation, the plate medium was removed and dye solution (30 μL) was added to each well. The assay plates were placed in 37°C, 5% CO_2 to incubator for 1 hour. Various concentrations of test compounds were added into the cells, and calcium flux signal after addition was monitored by FlexStation3 Molecular Devices. EC_{50} of each compound was calculated using GraphPad Prism V5.0 software.

4.2.2. Animals

Male ICR mice (20 ± 2 g), male C57BL/6 mice (20 ± 2 g) and male SD rats (200 ± 20 g) were obtained from Comparative Medicine Centre of Yangzhou University and left to acclimatize for 1 week before the experimental period. The animal were selected for the study and maintained at a controlled temperature of 25 ± 2 °C and constant humidity (50–70%) under a 12-h light–dark

cycle, with free access to diet and water. The animal study was performed accord the international rules considering animal experiments and the internationaly accepted ethical principles for laboratoryanimal use and care.

4.2.2.1. Oral glucose tolerance test in male ICR mice

Male ICR mice aged 10 weeks were fasted for 12 h, weighted, randomized into groups (n = 6). Mice were dosed orally with single doses of vehicle (0.5% methylcellulose aqueous solution), TAK-875 (suspended in vehicle; 10 mL/kg; 30 mg/kg) or test compounds (suspended in vehicle; 10 mL/kg; 30 mg/kg), 30 min prior to oral glucose load (20% aqueous glucose solution, 2 g/kg). The blood glucose levels were measured by blood glucose test strips (SanNuo GA-3 type, ChangSha, China) before administration of vehicle, TAK-875 or test compounds (–30 min). After glucose oral adminstration, blood glucose were measured at 0, 15, 30, 45, 60 and 120 min.

4.2.2.2. Oral glucose tolerance test in male type 2 diabetic C57BL/6 mice

Male C57BL/6 mice left to acclimatize for 1 week were fed with high-fat diet (MD 12032, rodent diet with 45 kcal% fat, from Mediscience Ltd., Yangzhou, China) for 12 weeks to induce insulin resistance. The mice with fasting blood glucose level 11.1 mmol/L or higher were considered as type 2 diabetic model and selected for acute oral glucose tolerance test[31, 32]. Type 2 diabetic C57BL/6 mice were fasted for 12 h, weighted, randomized into groups (n = 6). Mice were dosed orally with single doses of vehicle (0.5% methylcellulose aqueous solution), TAK-875 (suspended in vehicle; 10 mL/kg; 30 mg/kg) or test compounds (suspended in vehicle; 10 mL/kg; 30 mg/kg), 30 min prior to oral glucose load (10% aqueous glucose solution, 1 g/kg). The blood glucose levels were measured by blood glucose test strips (SanNuo GA-3 type, ChangSha, China) before administration of vehicle, TAK-875 or test compounds (–30 min). After glucose oral adminstration, blood glucose were measured at 0, 15, 30, 45, 60 and 120 min.

4.2.2.3. Pharmacokinetic profiles of 11k in SD rats

Male SD rats were fasted for 12 h and were administered orally with compound **11k** (suspended in 0.5% methylcellulose aqueous solution; 1 mL/kg; 1 mg/kg). Blood samples were collected at 0, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 24 h after the dose via orbital sinus puncture into microcentrifuge tubes containing heparin sodium. Then blood samples were centrifuged at 10000 rpm for 10 min to separate plasma. 300 μL of acetonitrile and 5 μL of internal standard (diazepam) were added to100 μL of plasma sample to precipitate plasma proteins by vortexing and centrifugation at 14000 rpm for 10 min. The supernatant was concentrated and dissolved in 100 μL of methanol again. The mixture was vortexed and centrifuged at 10000 rpm for 5 min. 20 μL of the supernatant was injected into the LC-MS/MS system for analysis. Pharmacokinetic profiles were performed using DAS 2.0 statistical software program.

4.3. Docking studies

Docking studies were performed with Glide docking in Schrodinger 9.4. The crystal structure of GPR40 with TAK-875 (PDB ID: 4PHU) was downloaded from the Protein Data Bank (PDB). Before ligand docking, the protein was treated with removal of crystallized ligand, deletion of water, automatical optimization with ProtAssign. Subsequently, OPLS-2005 force field was added to constrain and the binding site was defined precisely by grid calculation. Glide docking was carried out in standard precision mode to ontput one conformation for every molecule and then these conformations were ranked with the scoring function.

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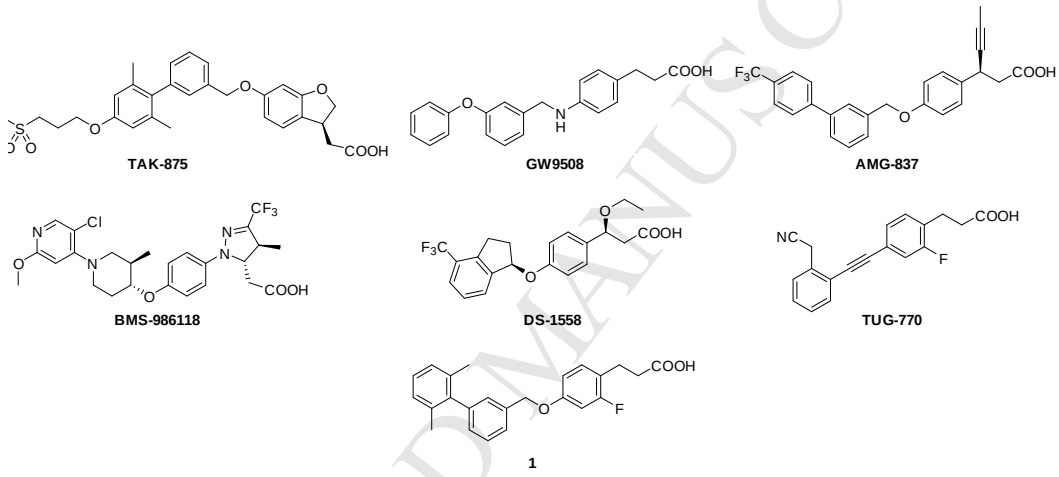


Figure 1. Representative GPR40 agonists.

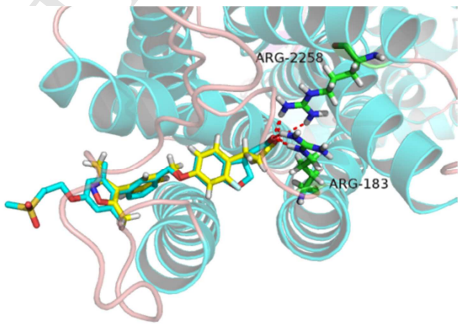


Figure 2. The interaction mode of compound **11a** and TAK-875 within the binding site of hGPR40 and their overlapped conformations. GPR40 is represented as blue ribbon. The carbons of Arg 183 and Arg 258 are shown in green. The carbons of compound **11a** are shown in yellow. The carbons of TAK-875 are shown in blue. Hydrogen bonds are represented by red dashes.

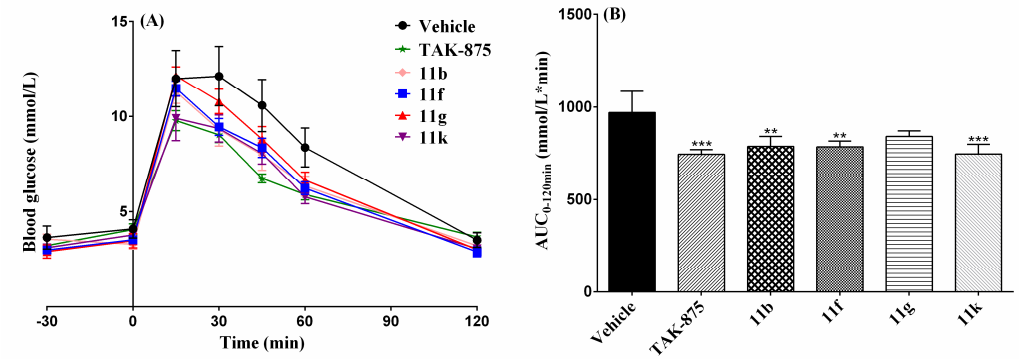


Figure 3. Effect of compounds (30 mg/kg) on oral glucose tolerance test in male ICR mice. (A) show time-dependent changes of blood glucose after oral administration of compounds, followed by 2 g/kg oral glucose challenge. Date in (B) represent $AUC_{0–120\ min}$ of blood glucose levels. Values are mean ± SEM (n = 6). ** $P < 0.01$ compared to vehicle-treated ICR mice by Student's t test; *** $P < 0.001$ compared to vehicle-treated ICR mice by Student's t test.

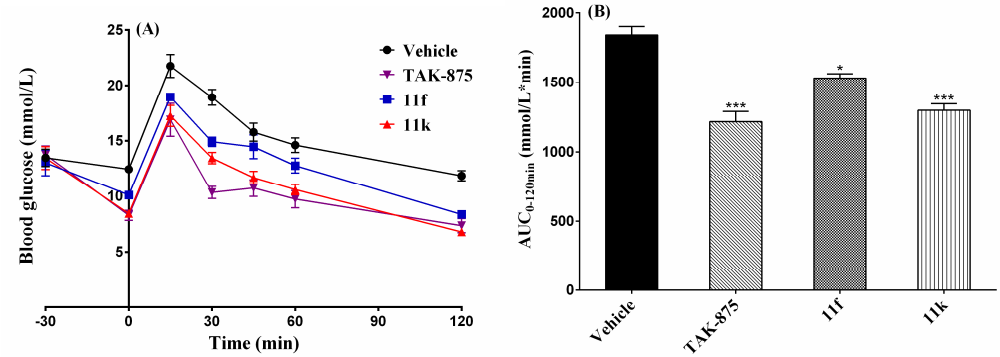
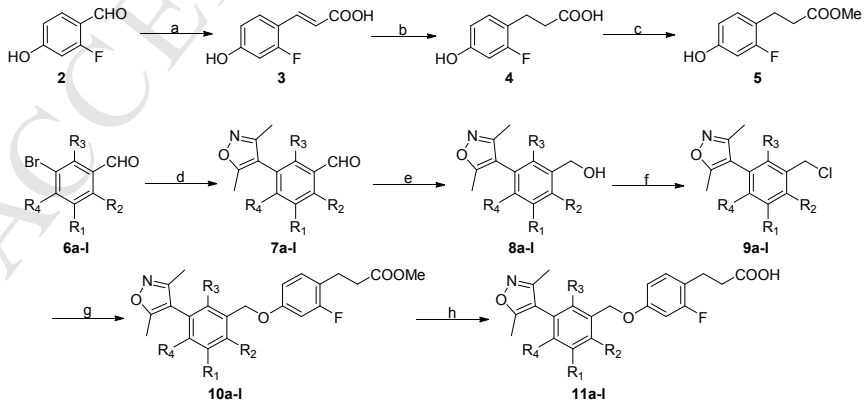
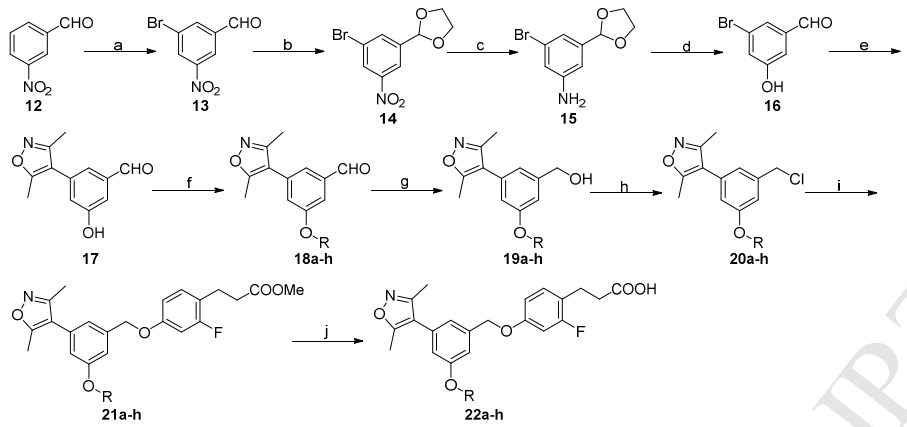


Figure 4. Effect of compounds (30 mg/kg) on oral glucose tolerance test in type 2 diabetic C57BL/6 mice. (A) show time-dependent changes of blood glucose after oral administration of compounds, followed by 1 g/kg oral glucose challenge. Date in (B) represent $AUC_{0–120\ min}$ of blood glucose levels. Values are mean ± SEM (n = 6). * $P < 0.05$ compared to vehicle-treated type 2 diabetic C57BL/6 mice by Student's t test; *** $P < 0.001$ compared to vehicle-treated type 2 diabetic C57BL/6 mice by Student's t test.

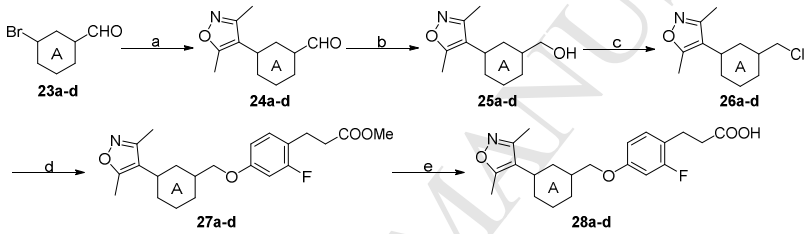
Scheme



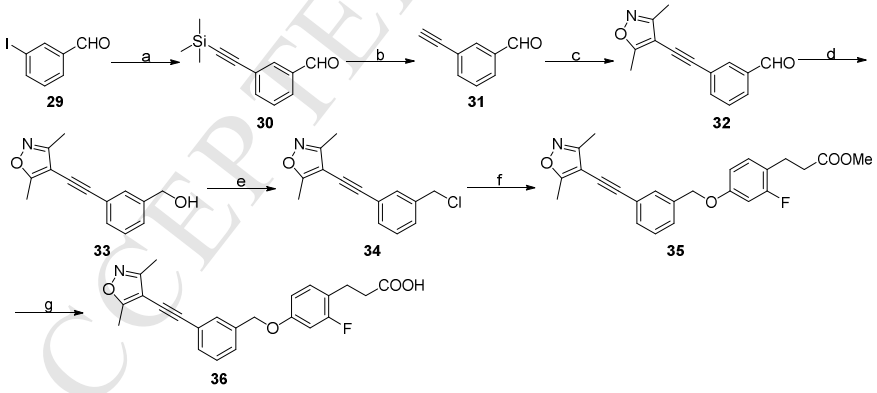
Scheme 1. General synthesis of **11a-l**. Reagents and conditions: (a) propanedioic acid, pyridine, 80°C; (b) Pd/C, H_2, THF, rt; (c) conc. H_2SO_4, methanol, reflux; (d) 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) isoxazole, $Pd(PPh_3)_4$, Na_2CO_3, toluene, ethanol, H_2O, 80°C; (e) $NaBH_4$, methanol, THF, 0°C to rt; (f) $POCl_3$, N-methyl-2-pyrrolidone, 0°C to rt; (g) K_2CO_3, DMF, 60°C; (h) LiOH, methanol, H_2O, 40°C.



Scheme 2. General synthesis of **12a-h**. Reagents and conditions: (a) NBS, H_2SO_4, 65°C; (b) ethylene glycol, p-TsOH, toluene, reflux; (c) Fe, NH_4Cl, ethanol, H_2O, reflux; (d) $NaNO_2$, 10% H_2SO_4, AcOH, conc. H_2SO_4, H_2O, 0°C to reflux; (e) 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole, $Pd(PPh_3)_4$, Na_2CO_3, toluene, ethanol, H_2O, 80°C; (f) CH_3I or brominated alkanes, K_2CO_3, DMF, 60°C; (g) $NaBH_4$, methanol, THF, 0°C to rt; (h) $POCl_3$, N-methyl-2-pyrrolidone, 0°C to rt; (i) K_2CO_3, DMF, 60°C; (j) LiOH, methanol, H_2O, 40°C.



Scheme 3. General synthesis of **28a-d**. Reagents and conditions: (a) 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole, $Pd(PPh_3)_4$, Na_2CO_3, toluene, ethanol, H_2O, 80°C; (b) $NaBH_4$, methanol, THF, 0°C to rt; (c) $POCl_3$, N-methyl-2-pyrrolidone, 0°C to rt; (d) K_2CO_3, DMF, 60°C; (e) LiOH, methanol, H_2O, 40°C.

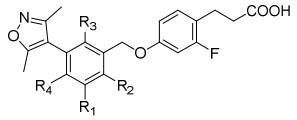


Scheme 4. The synthesis of compound **36**. Reagents and conditions: (a) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, triethylamine, reflux; (b) TBAF, THF, rt; (c) 4-iodo-3,5-dimethylisoxazole, $Pd(PPh_3)_4$, CuI, triethylamine, reflux; (d) $NaBH_4$, methanol, THF, 0°C to rt; (e) $POCl_3$, N-methyl-2-pyrrolidone, 0°C to rt; (f) K_2CO_3, DMF, 60°C; (g) LiOH, methanol, H_2O, 40°C.

Table

Table 1

Structures and GPR40 agonistic activities of compounds **11a–l**



Compound	R_1	R_2	R_3	R_4	hGPR40 EC_{50} (nM)[a]	E_{max} (%)[b]	tPSA[c]	cLog P[d]
TAK-875	-	-	-	-	27.5 ± 0.6	100.0 ± 3.3	99.1	4.70
1	-	-	-	-	7.7[e]	-	46.5	6.16
11a	H	H	H	H	24.8 ± 1.4	103.7 ± 0.3	68.1	3.68
11b	Me	H	H	H	12.0 ± 3.2	107.0 ± 1.9	68.1	4.18
11c	F	H	H	H	33.2 ± 1.9	82.7 ± 1.8	68.1	3.85
11d	Cl	H	H	H	38.9 ± 0.4	98.5 ± 0.8	68.1	4.42
11e	CF_3	H	H	H	114 ± 16	100.6 ± 0.5	68.1	4.62
11f	H	F	H	H	11.1 ± 0.8	102.4 ± 0.1	68.1	3.85
11g	H	Cl	H	H	9.3 ± 1.8	101.5 ± 0	68.1	4.42
11h	H	OMe	H	H	24.3 ± 4.1	98.7 ± 1.3	77.4	3.70
11i	H	H	F	H	25.9 ± 3.1	88.8 ± 1.4	68.1	3.85
11j	H	H	H	F	25.2 ± 7.3	101.5 ± 1.2	68.1	3.85
11k	H	H	H	Cl	15.9 ± 2.6	105.9 ± 1.0	68.1	4.17
11l	H	H	H	OMe	22.9 ± 0.1	108.4 ± 1.3	77.4	3.14

[a] Calcium flux assay in GPR40-transfected CHO cells, values are mean ± SEM (n = 3).

[b] % Compared to reference TAK-875, values are mean ± SEM (n = 3).

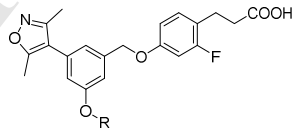
[c] Topological polar surface area values were calculated by ChemBioDraw Ultra 14.0.

[d] cLog P values were calculated by the BioByte's algorithm as implemented in ChemBioDraw Ultra 14.0.

[e] The EC_{50} value of compound **1** was presented in reference[17].

Table 2

Structures and GPR40 agonistic activities of compounds **22a–h**



Compound	R	hGPR40 EC_{50} (nM)[a]	E_{max} (%)[b]	tPSA[c]	cLog P[d]
22a	Me	53.1 ± 5.3	98.7 ± 1.5	77.4	3.70
22b	Et	69.1 ± 1.4	101.4 ± 1.6	77.4	4.23
22c	n-Pr	96.7 ± 13.9	102.0 ± 0.2	77.4	4.76
22d	i-Pr	79.2 ± 5.6	105.3 ± 0	77.4	4.54
22e	n-Bu	89.3 ± 5.7	101.3 ± 0.8	77.4	5.29
22f	i-Bu	143 ± 10	102.0 ± 0	77.4	5.16
22g	cyclopropyl methyl	90.9 ± 0.7	104.3 ± 0.9	77.4	4.68
22h	cyclopentyl	102 ± 3	106.2 ± 0.1	77.4	5.18

[a] Calcium flux assay in GPR40-transfected CHO cells, values are mean ± SEM (n = 3).

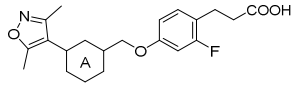
[b] % Compared to reference TAK-875, values are mean ± SEM (n = 3).

[c] Topological polar surface area values were calculated by ChemBioDraw Ultra 14.0.

[d] cLog P values were calculated by the BioByte's algorithm as implemented in ChemBioDraw Ultra 14.0.

Table 3

Structures and GPR40 agonistic activities of compounds **28a–d**



Compound	R	hGPR40 EC_{50} (nM)[a]	E_{max} (%)[b]	tPSA[c]	cLog P[d]
28a		506 ± 49	98.1 ± 0	80.5	2.57
28b		4980 ± 1379	80.5 ± 5.1	80.5	2.36
28c		862 ± 27	107.6 ± 3.4	80.5	2.57
28d		1930 ± 107	85.0 ± 1.0	80.5	2.36

[a] Calcium flux assay in GPR40-transfected CHO cells, values are mean ± SEM (n = 3).

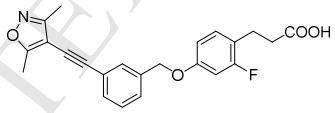
[b] % Compared to reference TAK-875, values are mean ± SEM (n = 3).

[c] Topological polar surface area values were calculated by ChemBioDraw Ultra 14.0.

[d] cLog P values were calculated by the BioByte's algorithm as implemented in ChemBioDraw Ultra 14.0.

Table 4

Structure and GPR40 agonistic activity of compound **36**



Compound	hGPR40 EC_{50} (nM)[a]	E_{max} (%)[b]	tPSA[c]	cLog P[d]
36	214 ± 2	99.5 ± 1.0	68.1	5.03

[a] Calcium flux assay in GPR40-transfected CHO cells, values are mean ± SEM (n = 3).

[b] % Compared to reference TAK-875, values are mean ± SEM (n = 3).

[c] Topological polar surface area values were calculated by ChemBioDraw Ultra 14.0.

[d] cLog P values were calculated by the BioByte's algorithm as implemented in ChemBioDraw Ultra 14.0.

Table 5.

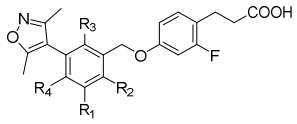
Pharmacokinetic profiles of **11k** in SD rats[a]

Route	Dose (mg/kg)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/mL)	$AUC_{0\text{-}8\ h}$ (μg·h/mL)
po	1.0	2.87 ± 0.33	1.0	0.84 ± 0.15	4.18 ± 0.71

[a] Data are expressed as mean ± SEM (n = 4).

Table 1

Structures and GPR40 agonistic activities of compounds **11a–l**



Compound	R_1	R_2	R_3	R_4	hGPR40 EC_{50} (nM)[a]	E_{max} (%)[b]	tPSA[c]	cLog P[d]
TAK-875	-	-	-	-	27.5	100	99.1	4.70
1	-	-	-	-	7.7[e]	-	46.5	6.16
11a	H	H	H	H	24.8	104	68.1	3.68
11b	Me	H	H	H	12.0	99	68.1	4.18
11c	F	H	H	H	33.2	83	68.1	3.85
11d	Cl	H	H	H	38.9	98	68.1	4.42
11e	CF_3	H	H	H	114	101	68.1	4.62
11f	H	F	H	H	11.1	98	68.1	3.85
11g	H	Cl	H	H	9.3	102	68.1	4.42
11h	H	OMe	H	H	24.3	91	77.4	3.70
11i	H	H	F	H	25.9	89	68.1	3.85
11j	H	H	H	F	25.2	95	68.1	3.85
11k	H	H	H	Cl	15.9	93	68.1	4.17
11l	H	H	H	OMe	22.9	99	77.4	3.14

[a] Calcium flux assay in GPR40-transfected CHO cells. Means of three experiments.

[b] % Compared to reference TAK-875.

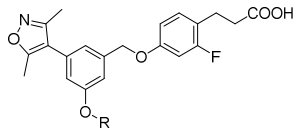
[c] Topological polar surface area values were calculated by ChemBioDraw Ultra 14.0.

[d] cLog P values were calculated by the BioByte's algorithm as implemented in ChemBioDraw Ultra 14.0.

[e] The EC_{50} value of compound **1** was presented in reference[17].

Table 2

Structures and GPR40 agonistic activities of compounds **22a–h**



Compound	R	hGPR40 EC_{50} (nM)[a]	E_{max} (%)[b]	tPSA[c]	cLog P[d]
22a	Me	53.1	99	77.4	3.70
22b	Et	69.1	101	77.4	4.23
22c	n-Pr	96.7	94	77.4	4.76
22d	i-Pr	79.2	99	77.4	4.54
22e	n-Bu	89.3	98	77.4	5.29
22f	i-Bu	143	102	77.4	5.16
22g	cyclopropyl methyl	90.9	92	77.4	4.68
22h	cyclopentyl	102	99	77.4	5.18

[a] Calcium flux assay in GPR40-transfected CHO cells. Means of three experiments.

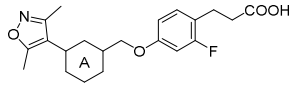
[b] % Compared to reference TAK-875.

[c] Topological polar surface area values were calculated by ChemBioDraw Ultra 14.0.

[d] cLog P values were calculated by the BioByte's algorithm as implemented in ChemBioDraw Ultra 14.0.

Table 3

Structures and GPR40 agonistic activities of compounds **28a–d**



Compound	R	hGPR40 EC_{50} (nM)[a]	E_{max} (%)[b]	tPSA[c]	cLog P[d]
28a		506	98	80.5	2.57
28b		4980	81	80.5	2.36
28c		862	108	80.5	2.57
28d		1930	85	80.5	2.36

[a] Calcium flux assay in GPR40-transfected CHO cells. Means of three experiments.

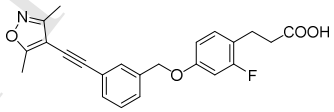
[b] % Compared to reference TAK-875.

[c] Topological polar surface area values were calculated by ChemBioDraw Ultra 14.0.

[d] cLog P values were calculated by the BioByte's algorithm as implemented in ChemBioDraw Ultra 14.0.

Table 4

Structure and GPR40 agonistic activity of compound **36**



Compound	hGPR40 EC_{50} (nM)[a]	E_{max} (%)[b]	tPSA[c]	cLog P[d]
36	214	105	68.1	5.03

[a] Calcium flux assay in GPR40-transfected CHO cells. Means of three experiments.

[b] % Compared to reference TAK-875.

[c] Topological polar surface area values were calculated by ChemBioDraw Ultra 14.0.

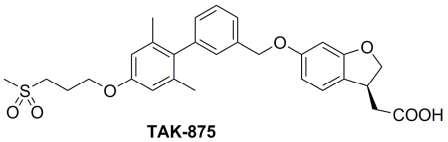
[d] cLog P values were calculated by the BioByte's algorithm as implemented in ChemBioDraw Ultra 14.0.

Table 5

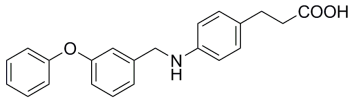
Pharmacokinetic profiles of **11k** in SD rats[a]

Route	Dose (mg/kg)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/mL)	$AUC_{0\text{-}8\ h}$ (μg·h/mL)
po	1.0	2.87 ± 0.33	1.0	0.84 ± 0.15	4.18 ± 0.71

[a] Data are expressed as mean ± SEM (n = 4).



TAK-875



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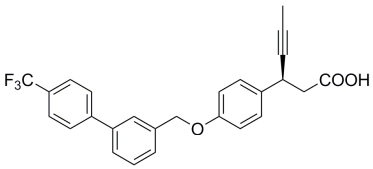

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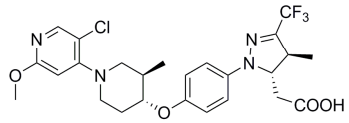

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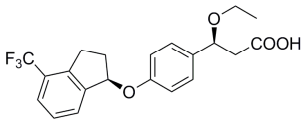

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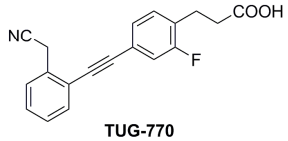

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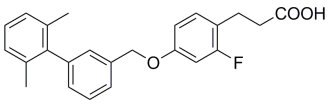

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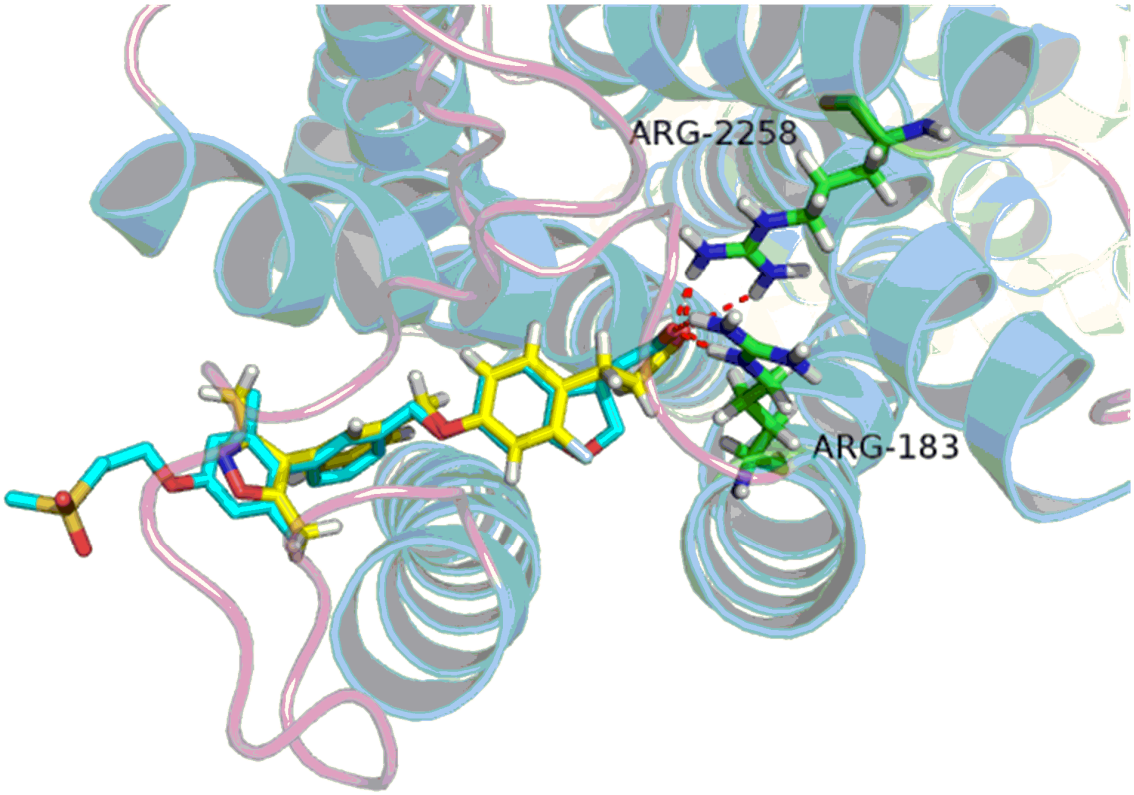

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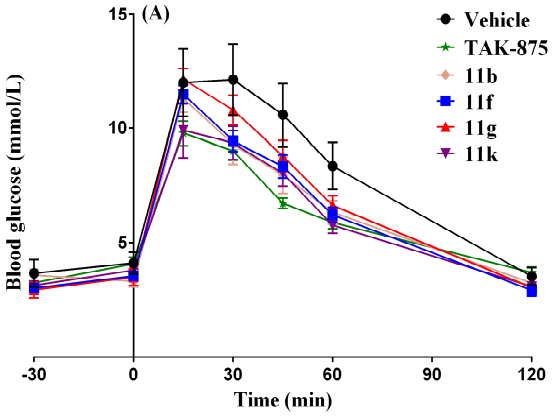

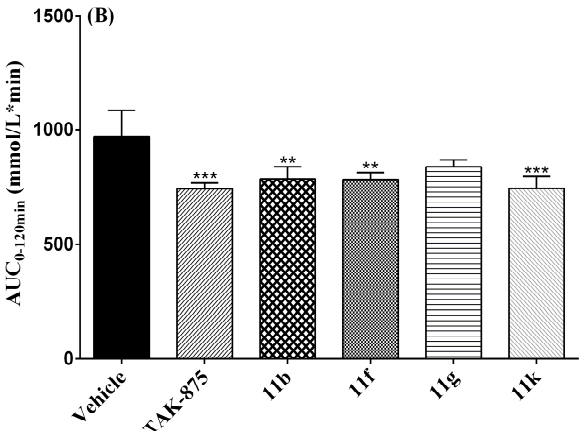

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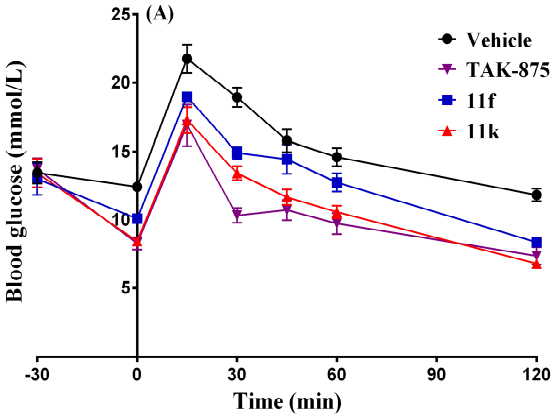

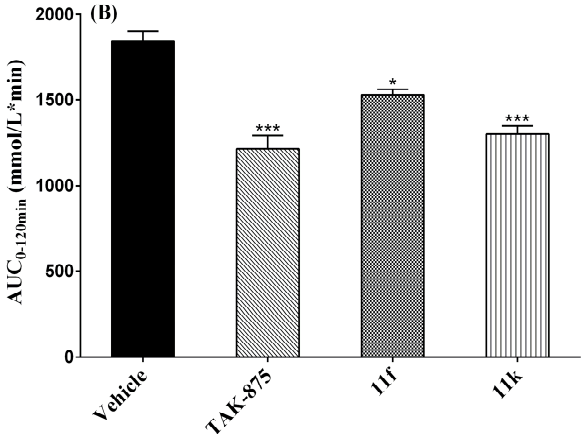

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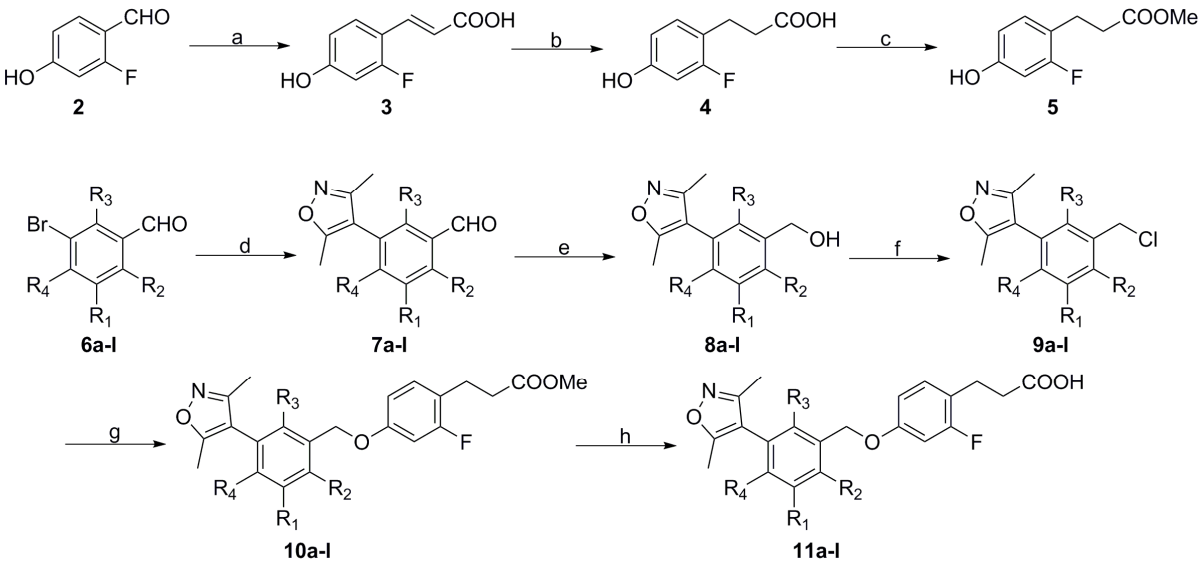

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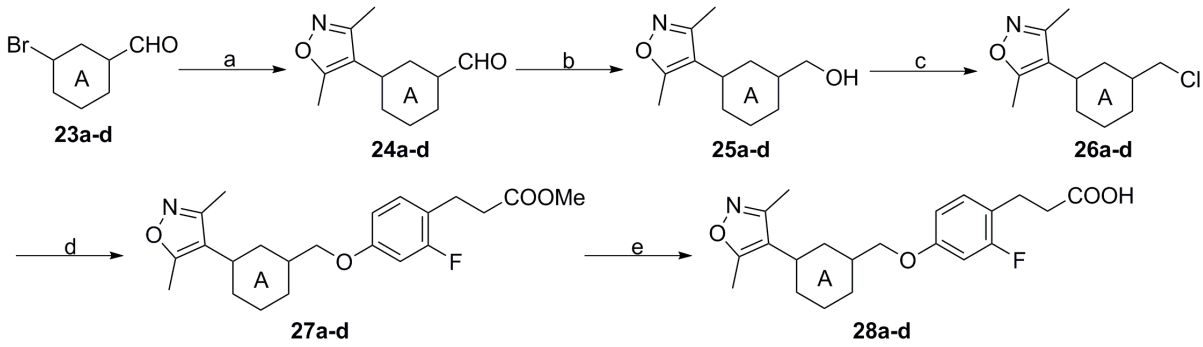

23a-d **24a-d** **25a-d** **26a-d**

27a-d **28a-d**

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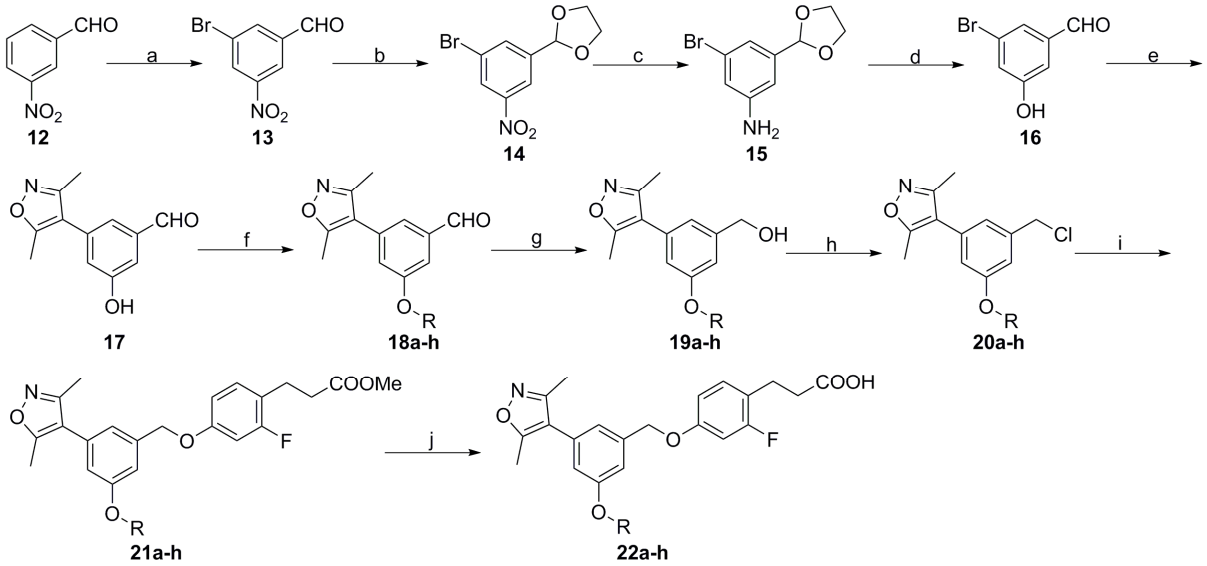

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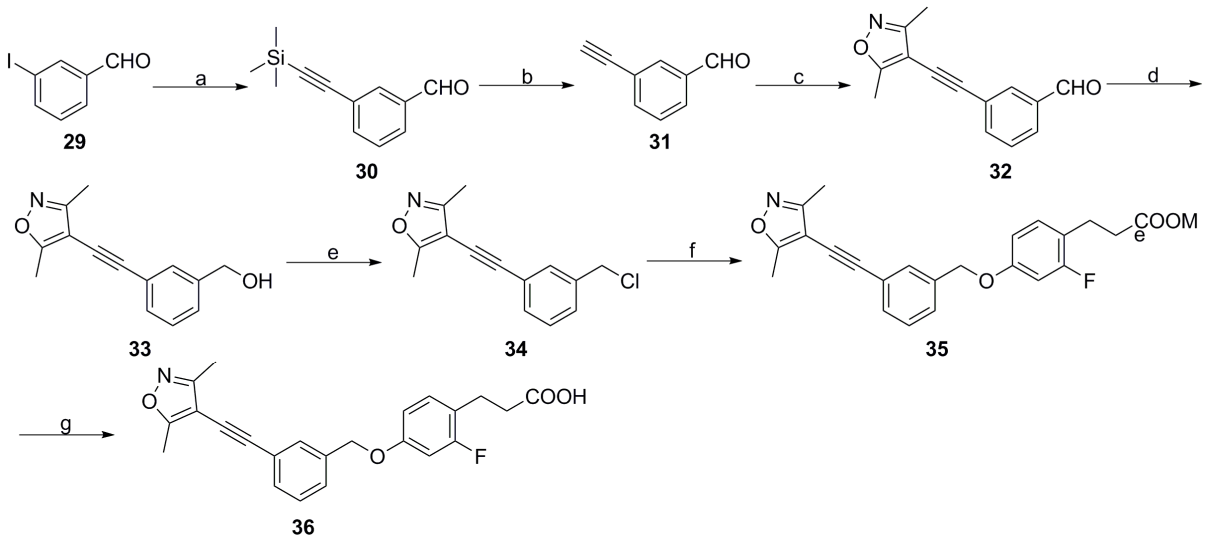

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Highlights

Synthesis of a series of novel 3,5-dimethylisoxazole derivatives with ideal tPSA values is described.

GPR40 agonist activity screening in vitro for all the targeted compounds was conducted.

Compound **11k** (EC_{50} = 15.9 nM) exhibited robust potency of glycemic control.

Compound **11k** showed similar potency to TAK-875 in vitro and in vivo.

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