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Novel FFA1 (GPR40) agonists containing spirocyclic periphery: polar azine periphery as a driver of potency

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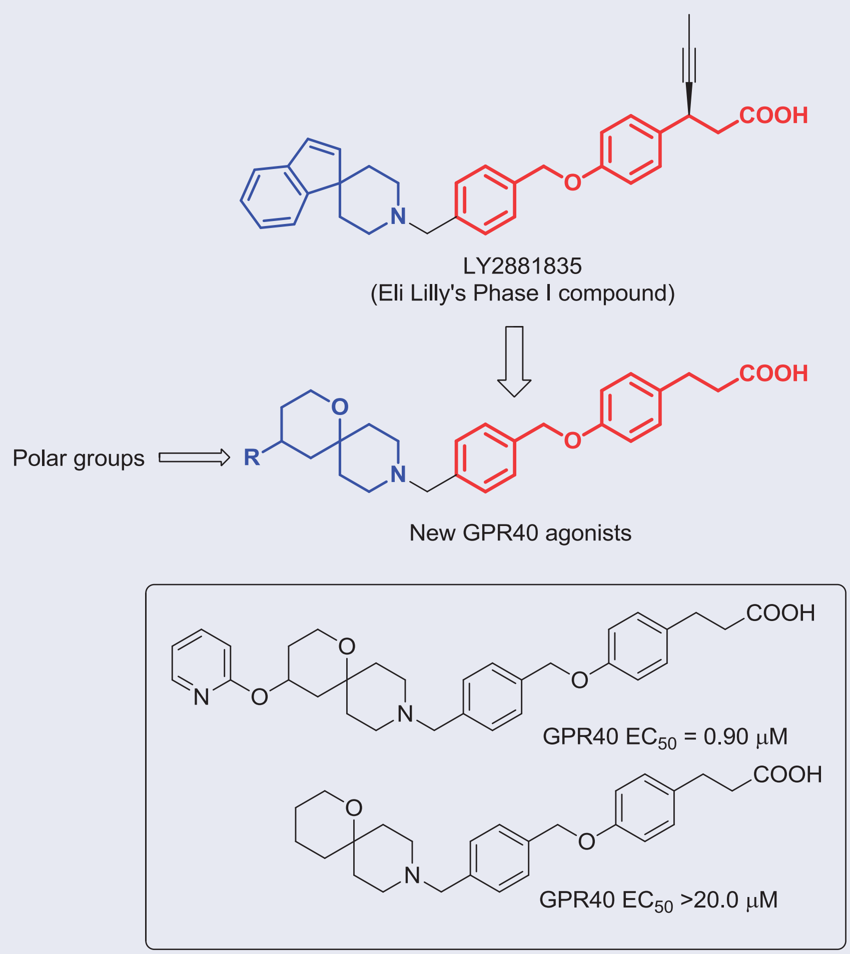
ABSTRACT

A series of nine compounds based on 3-[4-(benzyloxy)phenyl]propanoic acid core containing a 1-oxa-9-azaspiro[5.5]undecane periphery was designed, synthesized and evaluated as free fatty acid 1 (FFA1 or GPR40) agonists. The spirocyclic appendages included in these compounds were inspired by LY2881835, Eli Lilly's advanced drug candidate for type II diabetes mellitus that was in phase I clinical trials. These polar spirocyclic, fully saturated appendages (that are themselves uncharacteristic of the known FFA1 ligand space) were further decorated with diverse polar groups (such as basic heterocycles or secondary amides). To our surprise, while seven of nine compounds were found to be inactive (likely due to the decrease in lipophilicity, which is known to be detrimental to FFA1 ligand affinity), two compounds containing 2-pyridyloxy and 2-pyrimidinyloxy groups were found to have EC_{50} of 1.621 and 0.904 μM, respectively. This result is significant in the context of the worldwide quest for more polar FFA1 agonists, which would be devoid of liver toxicity effects earlier observed for a FFA1 agonist fasiglifam (TAk-875) in clinical studies.



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Supplemental data for this article can be accessed here.

Introduction

FFA1 or GPR40 is a cell surface G-protein coupled receptor expressed in pancreatic β-cells and is involved, through its activation, in regulation of insulin release and lowering of glucose levels[1]. The mechanism of glucose level regulation via FFA1 is quite unique as the therapeutic influence can only be exerted in hyperglycemic states when FFA1 expression levels are, in turn, upregulated. Once the normal glycemia is restored due to FFA1 activation, the FFA1 expression goes to basal levels and the insulin levels are no longer affected by the agonists still present in circulation. Therefore, therapeutic agents acting via this mechanism cannot cause hypoglycemia, a condition not less threatening than heightened glucose levels. Therefore, FFA1 agonists, if developed into clinically used drugs, would offer a much safer alternative to the currently available medicines for the treatment of type 2 diabetes mellitus (T2DM)[2]. Unfortunately, none of the compounds of this promising class have yet to claim their place in a clinical setting. The most advanced compound to-date, Takeda's fasiglifam (TAK-875) that had shown very promising efficacy results in phase II and III clinical trials, was stopped in development due to observable adverse liver toxicity in some patients. This has severely affected the field of FFA1 agonists, particularly from industry investment perspective[3]. At the time of writing this manuscript, only one clinical investigation of an FFA1 agonist was underway (Piramal's compound P11187 of unpublished structure; https://clinicaltrials.gov). One of the ways of restoring the dwindled promise of the new class of andidabetic compounds would be to keep in mind that the poof-of-concept was achieved for FFA1 inhibitors in the course of TAK-875 clinical research[4] and to tackle the toxicity profile of next-in-class compounds[5].

One of the most promising strategies toward reducing liver toxicity profile of FFA1 is to reduce the overly lipophilic character of the advanced FFA1 agonists, including TAK-875[5]. On the one hand, lowering lipophilicity could negatively affect the potency profile of more polar compounds as the receptor has medium-to-long chain fatty acids as endogenous ligands and the majority of the known agonist are considered mimics of the latter. On the other hand, we have already demonstrated that decoration of the 3-phenylpropanoic acid core (which is additionally substituted with a *p*-benzyloxy substituent in many advanced FFA1 agonists, including TAK-875, Figure 1) with polar heterocyclic appendages[6] or replacing the phenyl ring in this core with heterocyclic motifs[7,8] can result in compounds having potency comparable to that of the most advanced compounds reported. In the more recent study, we continued exploring the former (polar periphery) approach and drew inspiration from Eli Lilly's compounds LY2881835 that contains spirocyclic tertiary amine periphery. We reasoned that if we simplify the pharmacophoric core to the unsubstituted 3-[4-(benzyloxy)phenyl]propanoic acid and decorate aldehyde ester building block **1** (which we had made synthetic available on multigram scale[9]) with various 1-oxa-9-azaspiro[5.5]undecane building blocks **2** (which are available, in turn, via secondary alcohol manipulation of the earlier reported[10] *N*-Boc-protected 1-oxa-9-azaspiro[5.5]undecan-4-ol (**3**), this may provide a series of novel spirocyclic analogs of LY2881835 (**4**) some of which may be endowed with agonist activity toward FFA1 (Figure 2). We have already found[11] that if the 1-oxa-9-azaspiro[5.5]undecane periphery is decorated with lipophilic periphery (R = benzyl), the agonist potency falls in the nanomolar range (i.e. becomes comparable to that of TAK-875 that has $EC_{50} = 0.014\,\mu M$[12]). Such a result was predictable in a sense that lipophilicity is a known driver of potency of free fatty acid receptors[13]. It is also notable that the unsubstituted 1-oxa-9-azaspiro[5.5]undecane moiety (4, R = H) prepared and tested by us earlier was found inactive[11]. In this study, we undertook decoration of the 1-oxa-9-azaspiro[5.5]undecane periphery with diverse polar R groups, including basic heterocyclic and secondary amide moieties. Herein, we disclose our positive findings in this area.

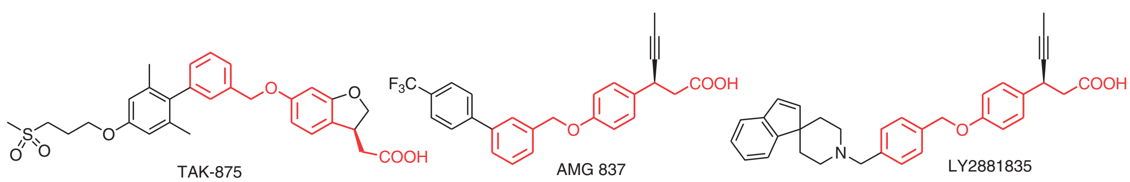


Figure 1. Advanced GPR40 agonists containing the 3-[4-(benzyloxy)phenyl]propanoic acid core.

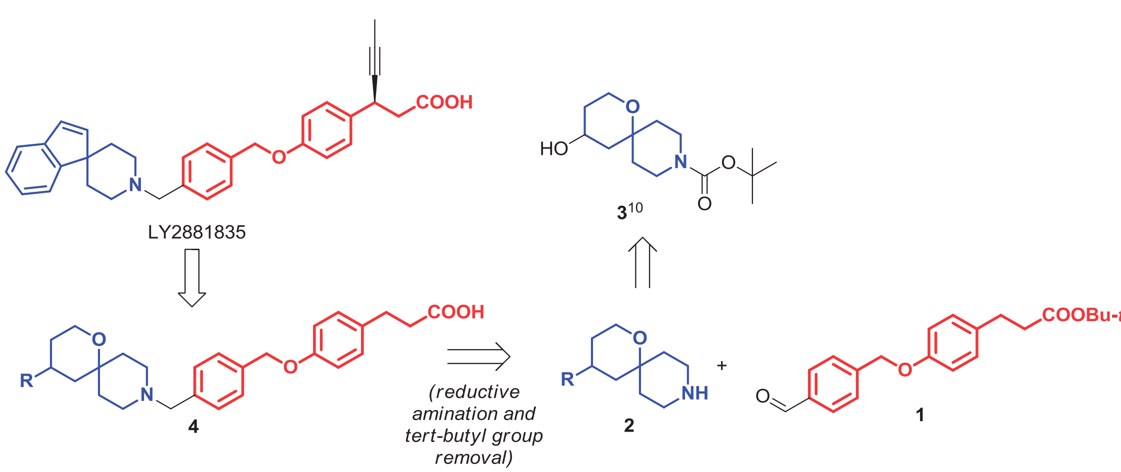


Figure 2. Design and retrosynthetic analysis of new series of FFA1 agonists 4.

Materials and methods

Chemical syntheses – general

All reactions were conducted in oven-dried glassware in atmosphere of nitrogen. Melting points were measured with a Büchi (Flawil, Switzerland) B-520 melting point apparatus and were not corrected. Analytical thin-layer chromatography was carried out on Silufol UV-254 silica gel plates using appropriate mixtures of ethyl acetate and hexane. Compounds were visualized with short-wavelength UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker MSL-300 spectrometers in DMSO-d_6 using TMS as an internal standard. Mass spectra were recorded using Shimadzu LCMS-2020 system (Kyoto, Japan) with electron impact (EI) ionization. All reagents and solvents were obtained from commercial sources and used without purification.

General procedure for the preparation of compounds 2a–d

A solution of *tert*-butyl 1-oxa-9-azaspiro[5.5]undecan-4-ol (**3**, 4 g, 14.8 mmol) in DMF (20 mL) was added dropwise to a 0 °C suspension of NaH (1.3 g, 32.6 mmol, 60% dispersion in mineral oil) in dry DMF (100 mL) under argon. The resulting mixture was stirred at 0 C for 30 min whereupon a solution of the respective heteroaryl halide (19.2 mmol) – in DMF (10 mL) was added dropwise. The resulting mixture was allowed to warm up to r.t. and stirred at that temperature for 18 h. The reaction mixture was poured into water (200 mL) and the aqueous phase was extracted with ethyl acetate (3 × 200 mL). The combined organic extracts were washed with 3% aqueous citric acid, 5% aqueous $NaHCO_3$, brine and water, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was fractionated on silica gel using 0 → 5% ethyl acetate in hexanes as an eluent and the fractions containing the desired product (according to LC–MS analysis) were combined and concentrated in dryness. Without further purification, the residue was dissolved in CH_2Cl_2 (3 mL/mmol calculated assuming 100% purity of the material obtained in the previous step), the solution was cooled to 0 °C and TFA (1 mL/mmol) was added. The mixture thus obtained was stirred at 0 °C for 6 h and then concentrated to dryness to provide, after crystallization from isopropyl alcohol, the target spirocyclic piperidine as a trifluoroacetate salt. Compound **2d** was converted to hydrochloride salts by treatment of their r.t. suspensions in 1,4-dioxane with 4 M HCl in 1,4-dioxane followed by stirring for 3 h, evaporation of the volatiles *in vacuo* and crystallization from isopropyl alcohol.

4-(Pyrazin-2-yloxy)-1-oxa-9-azaspiro[5.5]undecane ditrifluoroacetate (2a)

Yield 2.73 g (5.72 mmol, 39%). Sticky hygroscopic solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.59 (d, J = 4.78 Hz, 1H), 8.57–8.26 (m, 1H), 7.12 (t, J = 4.78 Hz, 1H), 5.34–5.23 (m, 1H), 3.87–3.79 (m, 1H), 3.71–3.61 (m, 1H), 3.15–2.90 (m, 2H), 2.19 (d, J = 13.23 Hz, 1H), 2.08–1.87 (m, 2H), 1.74–1.49 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.9, 162.4, 159.8, 115.6, 69.3, 69.0, 58.0, 39.1, 33.5, 31.1, 28.3. MS *m/z* 250.0 (M + H^{+}).

4-(Pyrimidin-2-yloxy)-1-oxa-9-azaspiro[5.5]undecane ditrifluoroacetate (2b)

Yield 2.70 g (5.66 mmol, 38%). Sticky hygroscopic solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.57 (s, 1H), 8.39–8.35 (m, 1H), 8.26 (t, J = 2.93 Hz, 1H), 8.19 (s, 2H), 5.36–5.27 (m, 1H), 3.85–3.77 (m, 1H), 3.71–3.60 (m, 1H), 3.13–2.87 (m, 4H), 2.24–2.19 (m, 1H), 2.06–1.88 (m, 3H), 1.73–1.50 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.9, 140.8, 136.9, 135.8, 69.3, 68.3, 66.4, 58.3, 39.1, 33.5, 31.1, 28.2. MS *m/z* 250.1 (M + H^{+}).

4-(Pyridin-2-yloxy)-1-oxa-9-azaspiro[5.5]undecane trifluoroacetate (2c)

Yield 3.12 g (8.61 mmol, 58%). Sticky hygroscopic solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (d, J = 62.13 Hz, 2H), 8.19–8.12 (m, 1H), 7.76–7.66 (m, 1H), 7.00–6.92 (m, 1H), 6.79 (d, J = 8.35 Hz, 1H), 5.40–5.26 (m, 1H), 3.81 (dt, J = 11.66, 4.17 Hz, 1H), 3.74–3.60 (m, 1H), 3.17–2.84 (m, 4H), 2.20 (d, J = 14.56 Hz, 1H), 2.07–1.86 (m, 3H), 1.76–1.44 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.3, 146.6, 139.8, 117.2, 111.3, 69.3, 67.4, 58.4, 39.2, 33.5, 31.4, 28.5. MS *m/z* 248.9 (M + H^{+}).

4-(Pyridin-4-yloxy)-1-oxa-9-azaspiro[5.5]undecane dihydrochloride (2d)

Yield 2.13 g (6.63 mmol, 45%). White crystalline solid, m.p. = 109–114 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.38–8.33 (m, 2H), 6.99–6.96 (m, 2H), 4.91–4.80 (m, 1H), 3.77–3.62 (m, 2H), 2.80–2.57 (m, 4H), 2.03–1.87 (m, 3H), 1.56–1.30 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.0, 151.0, 111.1, 71.7, 69.6, 57.7, 41.5, 41.3, 40.6, 32.1, 31.6. MS *m/z* 249.1 (M + H^{+}).

Tert-butyl 4-oxo-1-oxa-9-azaspiro[5.5]undecane-9-carboxylate (5)

To a solution of **3** (47.0 g, 173 mmol) in dichloromethane (500 mL) pyridinium dichromate (PDC) (130 g, 346 mmol) was added in small portions. The reaction mixture was stirred at r.t. for 18 h. The precipitate formed was filtered off and washed with dichloromethane (150 mL). The combined filtrate and washings were washed with 5% aqueous HCl, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using chloroform as eluent to provide the title compound.

Yield 35.4 g (131 mmol, 76%). White crystalline solid, m.p. = 61–64 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.00 (t, J = 6.1 Hz, 2H), 3.75 (dd, J = 10.1, 3.3 Hz, 2H), 3.22–3.09 (m, 2H), 2.46 (t, J = 6.0 Hz, 2H), 2.36 (s, 2H), 1.80 (d, J = 12.9 Hz, 2H), 1.50 (dd, J = 14.6, 2.8 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 206.8, 154.8, 79.6, 74.9, 60.4, 52.7, 41.7, 39.1, 34.4, 28.4. MS *m/z* 270.2 (M + H^{+}).

General procedure for the preparation of compounds (2e–f)

To a thoroughly stirred solution of **5** (2.0 g, 7.43 mmol) and respective secondary amine (0.58 g, 8.15 mmol) in CH_2Cl_2 (50 mL) was added, in small portions, sodium triacetoxyborohydride (11.0 g, 52.0 mmol). The reaction mixture was stirred for 18 h, poured into sat. aq. K_2CO_3 (100 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was fractionated on silica gel using 0 → 10% methanol in CH_2Cl_2 as an eluent. The fractions containing the target compound (according to LC–MS analysis) were pooled and concentrated *in vacuo*. The residue was dissolved in 1,4-dioxane (15 mL) and treated with 4 M HCl in 1,4-dioxane (1 mL). After stirring at r.t. for 6 h, the reaction mixture was concentrated to dryness *in vacuo* and the residue was crystallized from isopropyl alcohol to provide analytically pure title compound.

4-Pyrrolidin-1-yl-1-oxa-9-azaspiro[5.5]undecane dihydrochloride (2e)

Yield 1.66 g (5.61 mmol, 76%). White crystalline solid, m.p. = 171–173 °C. ^{1}H NMR (300 MHz, D_2O) δ 3.99–3.93 (m, 1H), 3.74–3.59 (m, 4H), 3.30–3.25 (m, 3H), 3.17–3.09 (m, 3H), 2.49–2.43 (m, 1H), 2.21–1.88 (m, 8H), 1.77–1.57 (m, 3H). ^{13}C NMR (75 MHz, D_2O) δ 70.0, 59.2, 57.5, 51.7, 51.6, 39.4, 39.3, 37.7, 34.6, 28.7, 25.6, 22.5. MS *m/z* 225.4 (M + H$^+$).

4-Morpholin-4-yl-1-oxa-9-azaspiro[5.5]undecane dihydrochloride (2f)

Yield 1.32 g (4.25 mmol, 57%). White crystalline solid, m.p. > 250 °C. ^{1}H NMR (300 MHz, D_2O) δ 4.25–4.19 (m, 2H), 4.10–4.00 (m, 1H), 3.88–3.72 (m, 5H), 3.69–3.60 (m, 2H), 3.36–3.20 (m, 5H), 2.57–2.51 (m, 1H), 2.29–2.20 (m, 2H), 2.06–1.67 (m, 5H). ^{13}C NMR (75 MHz, D_2O) δ 70.2, 60.0, 64.0, 59.4, 48.9, 39.4, 39.3, 35.5, 34.7, 26.5, 25.6. MS *m/z* 241.2 (M + H$^+$).

2-(9-(Tert-butoxycarbonyl)-1-oxa-9-azaspiro[5.5]undecan-4-yl)acetic acid (6)

To a 0 °C, vigorously stirred suspension of NaH (6.54 g, 163 mmol, 60% dispersion in mineral oil) in THF (300 mL) thriethylphosphonoacetate (45 g, 200 mmol) was added dropwise under argon. The stirring continued at that temperature for 1 h, whereupon a solution of **10** (40 g, 149 mmol) in THF (100 mL) was added. The reaction mixture was allowed to reach r.t. and was stirred at that temperature for 18 h. The reaction mixture was poured into water (500 mL) and the aqueous phase was extracted with ethyl acetate (3 × 200 mL). The combined organic extracts were washed with 3% aqueous citric acid, 5% aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was fractionated on silica gel using 0 → 5% ethyl acetate in hexanes as an eluent. The fractions containing the olefination product (according to LC–MS analysis) were pooled and concentrated to dryness (yielding 32.7 g of the material). The residue (10.9 g) was dissolved in EtOH (200 mL), $HCOONH_4$ (2.8 g, 0.44 mmol) and 10% Pd on carbon (300 mg) were added and the resulting mixture was heated at reflux for 12 h. The mixture was cooled to r.t. and filtered through a plug of Celite (subsequently washing the latter with EtOH). The combined filtrate and washings were concentrated to dryness. The residue was partitioned between water (150 mL) and ethyl acetate (150 mL). The organic layer was separated and the aqueous layer was additionally extracted with ethyl acetate (2 × 150 mL). The combined organic extracts were washed with 3% aqueous citric acid, 5% aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in MeOH (100 mL) and a solution of KOH (5.42 g, 96.7 mmol) in water (20 mL) was added. The mixture was stirred at r.t. for 18 h and concentrated to dryness *in vacuo*. The residue was dissolved in water (100 mL), the aqueous solution was extracted with ether (2 × 50 mL) and then acidified to pH 5.0 with 3% aqueous HCl. The solution thus obtained was extracted with ethyl acetate (3 × 100 mL) and the combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to provide analytically pure **6**.

Yield 7.35 g (49%). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 12.03 (s, 1H), 3.67–3.45 (m, 4H), 3.16–2.83 (m, 2H), 2.16–1.95 (m, 4H), 1.63–1.51 (m, 2H), 1.49–1.30 (m, 2H), 1.38 (s, 9H), 1.28–0.89 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 173.2, 153.9, 78.4, 69.7, 59.7, 41.6, 41.1, 38.6, 32.0, 28.7, 28.1, 26.8. MS *m/z* 314.5 (M + H$^+$).

General procedure for the preparation of compounds 2g–i

To a solution of [9-(*tert*-butoxycarbonyl)-1-oxa-9-azaspiro[5.5]undec-4-yl]acetic acid (6, 0.50 g, 1.59 mmol) in CH_2Cl_2 (50 mL) carbonyldiimidazole (0.28 g, 1.71 mmol) was added in small portions. The mixture was stirred for 1 h, whereupon a solution of the respective amine (1.75 mmol) in CH_2Cl_2 (10 mL) was added dropwise, and the stirring continued for 18 h. The mixture was poured into water (200 mL) and the slurry extracted with CH_2Cl_2 (3 × 200 mL). The combined organic extracts were washed with 1% aq. citric acid, 5% aq. $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was fractionated on silica gel using 0 → 10% methanol in CH_2Cl_2 as an eluent. The fractions containing the target compound (according to LC–MS analysis) were pooled and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (3 mL/mmol calculated assuming 100% purity of the material obtained in the previous step), the solution was cooled to 0 °C and TFA (1 mL/mmol) was added. The mixture thus obtained was stirred at 0 °C for 6 h and then concentrated to dryness to provide, after crystallization from isopropyl alcohol, the target spirocyclic piperidines **2g**–**i** as trifluoroacetate salts. Compound **2g** was converted to hydrochloride salts by treatment of their r.t. suspensions in 1,4-dioxane with 4 M HCl in 1,4-dioxane followed by stirring for 3 h, evaporation of the volatiles *in vacuo* and crystallization from isopropyl alcohol.

4-[2-(Methylamino)-2-oxoethyl]-1-oxa-9-azaspiro[5.5]undecane hydrochloride (4g)

Yield 0.21 g (0.81 mmol, 51%). White crystalline solid, m.p. = 103–105 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 9.19 (s, 1H), 7.88 (s, 1H), 6.40 (s, 1H), 3.63 (dd, J = 11.81, 4.46 Hz, 1H), 3.55 (s, 3H), 3.47 (dd, J = 11.95, 10.60 Hz, 1H), 3.06–2.69 (m, 4H), 2.32 (d, J = 13.29 Hz, 1H), 2.12–1.69 (m, 4H), 1.58–1.42 (m, 4H), 1.14–0.89 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 171.1, 68.2, 66.4, 60.0, 42.6, 41.7, 38.8, 35.1, 31.9, 27.1, 25.4, 25.3. MS *m/z* 227.4 (M + H$^+$).

4-{2-Oxo-2-[(pyridin-4-ylmethyl)amino]ethyl}-1-oxa-9-azaspiro[5.5]undecane ditrifluoroacetate (4h)

Yield 0.76 g (1.42 mmol, 75%). Sticky hygroscopic solid. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 8.87 (d, J = 6.68 Hz, 2H), 8.75 (t, J = 5.91 Hz, 1H), 7.87 (d, J = 6.63 Hz, 1H), 4.61–4.46 (m, 2H), 3.69–3.63 (m, 1H), 3.54–3.45 (m, 1H), 3.12–2.79 (m, 4H), 2.39–2.32 (m, 1H), 2.15–2.07 (m, 3H), 1.70–1.35 (m, 3H), 1.23–0.99 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 172.0, 160.6, 141.9, 124.9, 68.3, 60.2, 42.5, 42.0, 41.8, 39.3, 35.5, 32.0, 27.2, 25.7. MS *m/z* 304.4 (M + H$^+$).

4-{2-Oxo-2-[(pyridin-3-ylmethyl)amino]ethyl}-1-oxa-9-azaspiro[5.5]undecane ditrifluoroacetate (2i)

Yield 1.76 g (1.42 mmol, 89%). Sticky hygroscopic solid. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 8.84–8.79 (m, 2H), 8.66 (t, J = 5.76 Hz, 1H), 8.42–8.37 (m, 1H), 8.04–7.99 (m, 1H), 4.51–4.37 (m, 2H), 3.68–3.61 (m, 1H), 3.54–3.43 (m, 1H), 3.11–2.78 (m, 4H), 2.39–2.32 (m, 1H), 2.13–2.03 (m, 3H), 1.67–1.33 (m, 5H), 1.23–0.96 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 172.0, 144.6, 141.0, 139.8, 127.1, 68.4, 60.2, 42.6, 41.8, 39.6, 39.4, 35.5, 32.0, 31.4, 27.3, 25.7. MS *m/z* 304.1 (M + H$^+$).

General procedure for preparation of compounds 4a–i

A solution of the respective spirocyclic piperidine salt **2a**–**i** (0.46 mmol) in CH_2Cl_2 (5 mL) was treated with trimethylamine

($n \times 0.46$ mmol, where $n =$ number of salt parts per molecule) followed by a solution of **1**[11] (0.44 mmol) in CH_2Cl_2 (5 mL). After a brief stirring (15 min), sodium triacetoxyboohydride (STAB, 1.32 mmol) was added and the stirring continued for 12 h at r.t. The reaction was poured into 10% aqueous $NaHCO_3$ (20 mL). Organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was fractionated on silica gel using 0 → 1% MeOH in CH_2Cl_2. The fractions containing the reductive amination product (according to LC–MS analysis) were pooled and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (3 mL) and treated with TFA (1 mL). The mixture was stirred at r.t. for 18 h and concentrated *in vacuo*. Two molar HCl in ether (3 mL) was added to the residue and the later was triturated (with occasional sonication) until a crystalline hydrochloride salt formed. The latter was separated by filtration, washed with ether and dried *in vacuo* to provide analytically pure compounds **4a**–**i**.

3-{4-[(4-{[4-(Pyrazin-2-yloxy)-1-oxa-9-azaspiro[5.5]undec-9-yl]methyl}benzyl)oxy]phenyl}propanoic acid dihydrochloride (4a)

Yield 89 mg (0.15 mmol, 33%). White crystalline solid, m.p. = 70–72 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.01 (s, 1H), 8.60 (t, $J = 5.4$ Hz, 2H), 7.66 (d, $J = 8.1$ Hz, 2H), 7.50 (d, $J = 8.0$ Hz, 2H), 7.17–7.09 (m, 3H), 6.92 (d, $J = 8.6$ Hz, 2H), 5.31–5.20 (m, 1H), 5.09 (s, 2H), 4.40–4.26 (m, 2H), 3.87–3.78 (m, 1H), 3.71–3.60 (m, 1H), 3.18–2.87 (m, 4H), 2.75 (t, $J = 7.7$ Hz, 2H), 2.47 (t, $J = 7.4$ Hz, 2H), 2.31 (d, $J = 14.8$ Hz, 1H), 2.07–1.81 (m, 5H), 1.71–1.49 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.8, 163.8, 159.7, 156.6, 138.5, 133.1, 131.6, 131.1, 129.3, 129.2, 127.8, 115.5, 114.6, 68.9, 68.7, 58.4, 58.3, 46.9, 46.7, 35.5, 33.6, 31.1, 29.5, 28.1. HRMS (ESI) *m/z* calcd for $C_{30}H_{35}N_3O_5$ $[M + H]^+$ 518.2655, found 518.2621.

3-{4-[(4-{[4-(Pyrimidin-2-yloxy)-1-oxa-9-azaspiro[5.5]undec-9-yl]methyl}benzyl)oxy]phenyl} propanoic acid dihydrochloride (4b)

Yield 81 mg (0.14 mmol, 30%). White crystalline solid, m.p. = 119–121 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.19 (s, 1H), 8.29–8.26 (m, 1H), 8.23–8.18 (m, 2H), 7.58 (dd, $J_1 = 8.05$ Hz, $J_2 = 53.97$ Hz, 4H), 7.04 (dd, $J_1 = 8.55$ Hz, $J_2 = 65.91$ Hz, 4H), 5.34–5.27 (m, 1H), 5.08 (s, 2H), 4.38–4.27 (m, 2H), 3.84–3.78 (m, 1H), 3.69–3.60 (m, 1H), 3.14–2.91 (m, 4H), 2.75 (t, $J = 7.55$ Hz, 2H), 2.48 (t, $J = 7.55$ Hz, 2H), 2.33–2.27 (m, 1H), 2.04–1.88 (m, 5H), 1.67–1.48 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.9, 158.9, 156.6, 140.8, 138.5, 136.9, 135.8, 133.2, 131.6, 129.4, 129.3, 127.8, 114.6, 70.2, 69.0, 68.7, 68.3, 58.4, 58.2, 46.9, 46.7, 35.6, 33.4, 31.0, 29.5, 28.3. HRMS (ESI) *m/z* calcd for $C_{30}H_{40}N_2O_5$ $[M + H]^+$ 518.2655, found 518.2664.

3-{4-[(4-{[4-(Pyridin-2-yloxy)-1-oxa-9-azaspiro[5.5]undec-9-yl]methyl}benzyl)oxy]phenyl}propanoic acid dihydrochloride (4c)

Yield 82 mg (0.14 mmol, 30%). Amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.02 (s, 1H), 8.18–8.14 (m, 1H), 7.76–7.69 (m, 1H), 7.66 (d, $J = 8.0$ Hz, 2H), 7.49 (d, $J = 7.9$ Hz, 2H), 7.14 (d, $J = 8.5$ Hz, 2H), 7.01–6.95 (m, 1H), 6.92 (d, $J = 8.4$ Hz, 2H), 6.81 (d, $J = 8.4$ Hz, 1H), 5.36–5.25 (m, 1H), 5.09 (s, 2H), 4.42–4.23 (m, 2H), 3.85–3.76 (m, 1H), 3.64 (t, $J = 9.8$ Hz, 1H), 2.99 (dt, $J = 21.8$, 10.3 Hz, 4H), 2.74 (t, $J = 7.5$ Hz, 2H), 2.47 (t, $J = 7.6$ Hz, 2H), 2.30 (d, $J = 14.7$ Hz, 1H), 2.05–1.81 (m, 5H), 1.66–1.45 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.7, 162.1, 156.6, 146.5, 139.7, 138.5, 133.1, 131.5, 129.3, 129.2, 127.7, 117.1, 114.6, 111.3, 68.8, 68.7, 67.3, 62.6, 58.4, 58.2, 46.9, 46.7, 35.5, 33.4, 31.2, 29.5, 28.4. HRMS (ESI) *m/z* calcd for $C_{31}H_{36}N_2O_5$ $[M + H]^+$ 517.2702, found 517.2697.

3-{4-[(4-{[4-(Pyridin-4-yloxy)-1-oxa-9-azaspiro[5.5]undec-9-yl]methyl}benzyl)oxy]phenyl}propanoic acid dihydrochloride (4d)

Yield 50 mg (0.085 mmol, 19%), amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.46 (s, 1H), 7.03–8.68 (m, 2H), 7.66–7.59 (m, 4H), 7.47– 7.41 (m, 2H), 7.08 (d, $J = 6.64$ Hz, 2H), 6.86 (d, $J = 6.86$ Hz, 2H), 5.27–5.23 (m, 1H), 5.03 (s, 2H), 4.40–4.20 (m, 2H), 3.78–3.63 (m, 2H), 3.11–2.87 (m, 4H), 2.68 (t, $J = 7.55$ Hz, 2H), 2.43 (t, $J = 7.55$ Hz, 2H), 2.38–2.31 (m, 1H), 2.04–1.83 (m, 5H), 1.55–1.16 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.9, 169.4, 156.7, 143.0, 138.6, 133.2, 131.8, 129.4, 128.0, 127.9, 114.7, 113.8, 72.9, 69.1, 68.9, 66.5, 58.4, 58.0, 46.8, 35.8, 33.3, 30.9, 29.6, 28.5. HRMS (ESI) *m/z* calcd for $C_{31}H_{36}N_2O_5$ $[M + H]^+$ 517.2702, found 517.2687.

3-[4-({4-[(4-Pyrrolidin-1-yl-1-oxa-9-azaspiro[5.5]undec-9-yl)methyl]benzyl}oxy)phenyl]propanoic acid dihydrochloride (4e)

Yield 181 mg (0.32 mmol, 73%). Amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.17 (s, 1H), 7.66 (d, $J = 7.91$ Hz, 2H), 7.49 (d, $J = 7.82$ Hz, 2H), 7.02 (dd, $J_1 = 8.46$ Hz, $J_2 = 65.92$ Hz, 2H), 5.08 (s, 2H), 4.43–4.24 (m, 2H), 3.85–3.72 (m, 1H), 3.56–3.38 (m, 4H), 3.16–2.84 (m, 6H), 2.74 (t, $J = 7.55$ Hz, 2H), 2.48 (t, $J = 7.55$ Hz, 1H), 3.37–2.30 (m, 1H), 2.05–1.57 (m, 11H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.9, 156.6, 138.6, 133.2, 131.7, 129.3, 128.0, 127.9, 114.6, 68.7, 58.6, 58.5, 56.7, 50.4, 50.1, 46.7, 37.4, 35.6, 35.2, 29.5, 28.3, 25.5, 22.6. HRMS (ESI) *m/z* calcd for $C_{30}H_{40}N_2O_4$ $[M + H]^+$ 493.3066, found 493.3072.

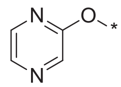
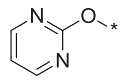
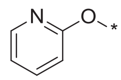
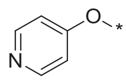
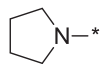
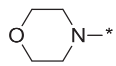
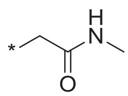
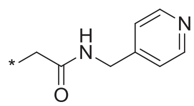
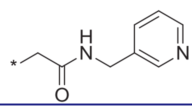
3-[4-({4-[(4-Morpholin-4-yl-1-oxa-9-azaspiro[5.5]undec-9-yl)methyl]benzyl}oxy)phenyl]propanoic acid dihydrochloride (4f)

Yield 79 mg (0.14 mmol, 31%). White crystalline solid, m.p. > 250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.34 (s, 1H), 10.96 (s, 1H), 7.64 (d, $J = 6.90$ Hz, 2H), 7.50 (d, $J = 7.18$ Hz, 2H), 7.14 (d, $J = 7.82$ Hz, 2H), 6.92 (d, $J = 7.68$ Hz, 2H), 5.09 (s, 2H), 4.41–4.28 (m, 2H), 3.98–3.75 (m, 5H), 3.60–3.39 (m, 5H), 3.17–2.86 (m, 4H), 2.75 (t, $J = 7.55$ Hz, 2H), 2.48 (t, $J = 7.55$ Hz, 2H), 2.43–2.34 (m, 1H), 2.08–1.92 (m, 4H), 1.80–1.54 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.8, 156.6, 138.6, 133.2, 131.6, 129.2, 127.8, 114.6, 68.9, 68.7, 66.3, 63.3, 58.8, 48.1, 46.7, 46.6, 35.5, 35.3, 29.5, 26.1, 25.6. HRMS (ESI) *m/z* calcd for $C_{30}H_{40}N_2O_5$ $[M + H]^+$ 509.3015, found 509.3013.

3-(4-{[4-({4-[2-(Methylamino)-2-oxoethyl]-1-oxa-9-azaspiro[5.5]undec-9-yl}methyl)benzyl]oxy}phenyl)propanoic acid hydrochloride (4g)

Yield 107 mg (0.19 mmol, 43%). Amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.00 (s, 1H), 7.78 (d, $J = 3.9$ Hz, 1H), 7.65 (d, $J = 7.9$ Hz, 2H), 7.48 (d, $J = 7.9$ Hz, 2H), 7.13 (d, $J = 8.4$ Hz, 2H), 6.91 (d, $J = 8.5$ Hz, 2H), 5.08 (s, 2H), 4.41–4.22 (m, 2H), 3.63 (dd, $J = 11.7$, 4.8 Hz, 1H), 3.52–3.41 (m, 1H), 3.15–2.81 (m, 4H), 2.74 (t, $J = 7.5$ Hz, 2H), 2.54 (d, $J = 4.2$ Hz, 3H), 2.46 (t, $J = 7.5$ Hz, 2H), 2.44 (d, $J = 8.2$ Hz, 1H), 2.10–1.83 (m, 4H), 1.71–1.33 (m, 4H), 1.14–0.93 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.1, 171.5, 156.7, 138.8, 133.3, 131.8, 129.5, 129.4, 128.1, 114.8, 68.8, 68.0, 60.2, 58.7, 47.3, 47.2, 42.8, 41.8, 35.7, 35.7, 32.0, 29.7, 27.4, 25.8, 25.6. HRMS (ESI) *m/z* calcd for $C_{29}H_{38}N_2O_5$ $[M + H]^+$ 495.2859, found 495.2844.

Table 1. Compounds **4a–i** synthesized and FFA1 activation data obtained in this work.

Compound	R	Isolated yield, %	% FFA1 activation at 5 μM*	EC_{50} (μM)	% Efficacy*
4a		33	32.5	>20.0	n/a
4b		30	80.0	1.62	80
4c		30	61.4	0.90	61
4d		19	13.6	>20.0	n/a
4e		73	9.1	>20.0	n/a
4f		31	10.2	>20.0	n/a
4g		43	9.9	>20.0	n/a
4h		22	10.0	>20.0	n/a
4i		29	11.6	>20.0	n/a

*Relative to GPR40 activation by 20 μM of GW9509, $n = 2$.

3-[4-({4-[(4-{2-Oxo-2-[(pyridin-4-ylmethyl)amino]ethyl}-1-oxa-9-azaspiro[5.5]undec-9-yl)methyl]benzyl}oxy)phenyl]propanoic acid dihydrochloride (4h)

Yield 61 mg (0.095 mmol, 22%). Amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.94 (s, 1H), 8.60–8.55 (m, 4H), 7.64 (d, $J = 7.50$ Hz, 2H), 7.48 (d, $J = 8.09$ Hz, 2H), 7.15–7.07 (m, 4H), 6.91 (d, $J = 8.60$ Hz, 2H), 5.08 (s, 2H), 4.38–4.24 (m, 4H), 3.68–3.61 (m, 1H), 3.53–3.43 (m, 1H), 3.12–2.82 (m, 4H), 2.75 (t, $J = 7.55$ Hz, 2H), 2.48 (t, $J = 7.55$ Hz, 2H), 2.44–2.40 (m, 1H), 2.04–1.93 (m, 1H), 1.89–1.77 (m, 1H), 1.71–1.48 (m, 6H), 1.23–1.01 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.8, 164.6, 159.6, 156.6, 138.5, 133.2, 131.6, 129.4, 129.3, 127.8, 114.6, 68.7, 67.9, 64.3, 60.1, 58.4, 47.0, 47.0, 41.9, 35.6, 35.5, 35.4, 32.0, 29.5, 26.7, 25.6. HRMS (ESI) *m/z* calcd for $C_{34}H_{41}N_3O_5$ $[M + H]^+$ 572.3124, found 572.3100.

3-[4-({4-[(4-{2-Oxo-2-[(pyridin-3-ylmethyl)amino]ethyl}-1-oxa-9-azaspiro[5.5]undec-9-yl)methyl]benzyl}oxy)phenyl]propanoic acid dihydrochloride (4i)

Yield 83 mg (0.13 mmol, 29%). Amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.80–8.72 (m, 2H), 8.38–8.32 (m, 1H), 8.04–7.97 (m, 1H), 7.57 (dd, $J_1 = 8.01$ Hz, $J_2 = 48.02$ Hz, 4H), 7.03 (dd, $J_1 = 8.60$ Hz, $J_2 = 66.41$ Hz, 4H), 5.09 (s, 2H), 4.44–4.25 (m, 4H), 3.67–3.60 (m, 1H), 3.52–3.43 (m, 1H), 3.10–2.83 (m, 4H), 2.75 (t, $J = 7.55$ Hz, 2H), 2.48 (t, $J = 7.55$ Hz, 2H), 2.45–2.41 (m, 1H), 2.15–1.90 (m, 4H), 1.67–1.00 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.8, 171.4, 156.6, 143.6, 141.2, 139.3, 138.5, 133.2, 131.6, 129.4, 129.3, 127.8, 126.6, 114.6, 68.7, 67.9, 60.1, 60.0, 58.4, 47.0, 42.4, 41.7, 35.6, 35.4, 31.9, 29.5, 27.3, 25.5. HRMS (ESI) *m/z* calcd for $C_{34}H_{41}N_3O_5$ $[M + H]^+$ 572.3124, found 572.3110.

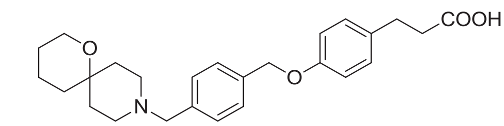
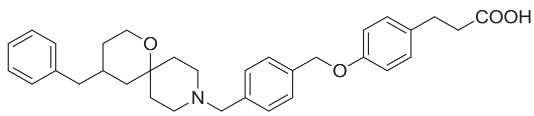
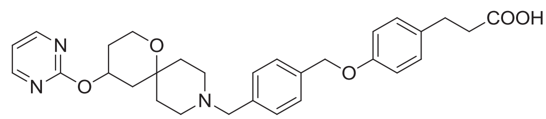
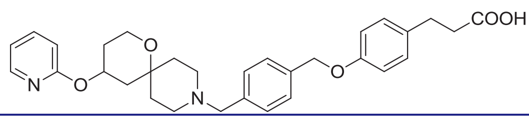
Determination of agonistic activity of compounds against FFA1 (GPR40) receptor

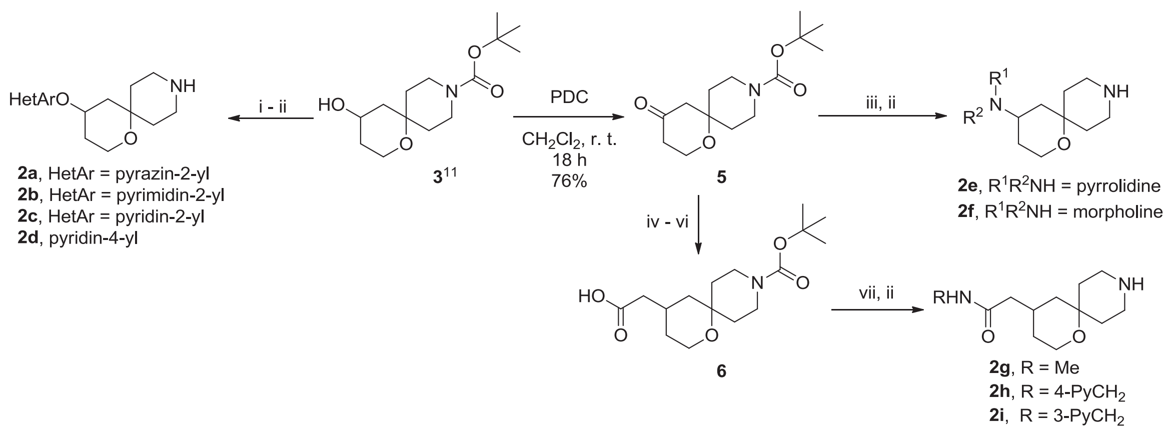
CHO cells stably expressing human GPR40 (stable CHO-GPR40 line created at Enamine Ltd., Kyiv, Ukraine) were seeded (12 500 cells/well) into 384-well black-wall, clear-bottom microtiter plates 24 h prior to assay. Cells were loaded for 1 h with fluorescent calcium dye (Fluo-8 Calcium Assay kit, Abcam, ab112129, Cambridge, UK) and tested using fluorometric imaging plate reader (FLIPR Tetra® High Throughput Cellular Screening System, Molecular Devices Corp., Sunnyvale, CA). Maximum change in fluorescence over base line was used to determine agonist response. A potent and selective agonist for FFA1 (GPR40) GW9508 (Selleckchem, S8014) was tested with the test compounds as a positive control. Concentration response curve data were fitted using Molecular Devices ScreenWorks® System Control Software (Molecular Devices). The half-maximal effective concentration was determined from these curves plotted in "% FFA1 activation – log[drug]" coordinates and % maximum efficacy was related to that of the reference compounds GW9508.

Results and discussion

The spirocyclic building blocks for subsequent use in decorating the pharmacophore core building block 1[10] were synthesized from common precursor **3**, which we prepared on multigram scale as described earlier[10]. Sodium alkoxide generated from **3** on treatment with NaH was an effective nucleophile in S_NAr-type reaction

Table 2. cLogP and EC_{50} values for selected spirocyclic derivatives reported earlier (**7** and **8**) and investigated in this work (**4b–c**).

Compound	Structure	cLogP[14]	FFA1 EC_{50} (μM)
7[12]		4.69	>20.0
8[12]		6.36	0.055
4b		4.03	1.82
4c		4.95	0.90



Scheme 1. Synthesis of spirocyclic building blocks **2a–i** from common precursor **3**.

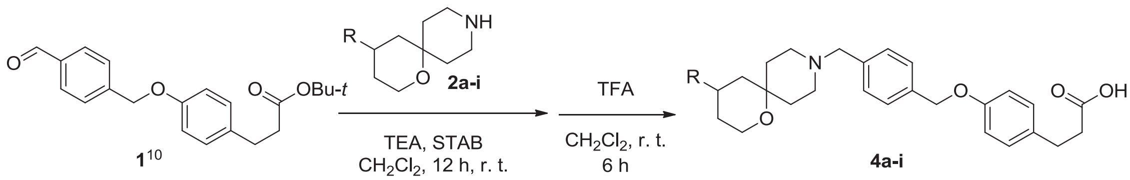
with heteroaryl halides furnishing, after Boc group removal, building blocks **2a–d**. The secondary alcohol functionality in **3** underwent a facile oxidation with PDC to furnish a good yield of respective ketone **5**. The carbonyl group in **5** was reductively aminated with pyrrolidine and morpholine in the presence of sodium triacetoxyborohydride (STAB) and gave, after Boc group removal, building blocks **2e–f**. The same keto group a competent partner in Horner–Wadsworth–Emmons olefination, which led, after hydrogenation of the resulting olefin and ethyl ester hydrolysis, to carboxylic acid **6**. The latter was a common precursor to amides **2g–i** obtained via a standard CDI-promoted amidation followed by Boc group removal (Scheme 1).

Reagents and conditions: (i) NaH, DMF, 0 °C, 30 min; HetArHal, DMF, 0 C → r.t., 18 h; (ii) TFA, CH_2Cl_2, 0 °C, 6 h; (iii) R^1R^2NH, STAB, CH_2Cl_2, r.t., 18 h; (iv) $EtOOCCH_2P(O)(OEt)_2$, NaH, THF, 0 °C → r.t., 18 h; (v) $HCOONH_4$, 10% Pd–C, EtOH, reflux, 12 h; (vi) KOH, aq. MeOH, r.t., 18 h; (vii) RNH_2, CDI, CH_2Cl_2, r.t. 18 h.

Spirocyclic piperidine building blocks **2a–i** were used in the reductive amination reaction of aldehyde **1** in presence of STAB and final compounds **4a–i** were obtained after a facile tert-butyl ester hydrolysis on treatment with TFA followed by salt form exchange, see Material and methods (Scheme 2).

Compounds **4a–i** were preliminarily tested at 5 μM concentration for activation of FFA1. Only two compounds (**4b** and **c**) demonstrated >50% activation of the receptor at that concentration. To see how the single-concentration data translate into EC_{50} values, compounds **4a–i** were also tested in dose–response (% FFA1 activation) mode. As can be seen from the data thus obtained (Table 1), the % activation data (5 μM) were quite predictive of the compounds' ability to activate FFA1 in a broad concentration range as the only meaningful EC_{50} values were determined for the same two compounds **4b** and **c**, while the rest of compounds were virtually inactive.

These results are not unexpected as the majority of the compounds containing polar appendages are perhaps too polar to mimic the endogenous ligands of FFA1, that is medium-to-long chain fatty acids. The low-micromolar and even submicromolar EC_{50} values obtained for compounds **4b** and **c** are, therefore, very surprising and encouraging. From examination of the superimposed cLogP and FFA1 potency data presented in Table 2 it becomes clear that the unexpectedly high agonist activity of compounds **4b** and **c** is not associated with a lipophilicity increase (a known FFA1 potency driver[13]) and is likely due to specific interactions of the ligands with the protein, due to the presence of the azin-2-yloxy moieties. We have shown earlier[11] that grafting a benzyl group only a spirocyclic scaffolds of the inactive compound **7** resulted in a highly potent compound **8**. While such a potency boost could be rationalized by the nearly 100-fold increase in lipophilicity (as gauged by cLogP values), we demonstrate d_6[2] that the benzyl group in **8** also



Scheme 2. Preparation of FFA1 agonists **4a–s** studied in this work.

forms a network of hydrophobic and π-stacking interactions with the target. Compounds **4b** and **c** could, in principle, be considered as more polar isosteres of compound **8** and the observed potency of the former very likely results from similar additional contacts with the receptor. Lipophilicity is clearly not responsible for the observed SAR as the cLogP values of **4b** and **c** are comparable and even lower than the same value for inactive compound **7** (Table 2).

Conclusion

Highly lipophilic character of the known advanced FFA1 agonists limited their progression through clinical development. In this study, we achieved a significant result in designing new FFA1 agonists, namely, the polar-appendage versions of spirocyclic 1-oxa-9-azaspiro[5.5]undecanes, which activated FFA1 in low-micromolar and submicromolar range. This finding significantly broadens the chemistry space and medicinal chemistry freedom-to-operate in today's worldwide quest for more polar and potentially less toxic FFA1 agonists as a fundamentally novel type of therapeutic agents to treat T2DM.

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Disclosure statement

The authors declare no conflict of interest. The authors are solely responsible for the content and results presented in this paper.

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