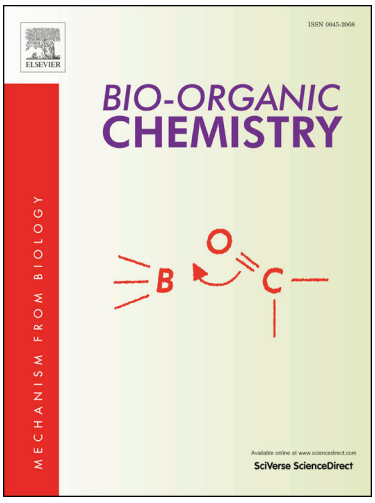
Journal Pre-proofs



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PII: S0045-2068(20)30504-6
DOI: https://doi.org/10.1016/j.bioorg.2020.103830
Reference: YBIOO 103830

To appear in: *Bioorganic Chemistry*

Received Date: 3 March 2020
Revised Date: 31 March 2020
Accepted Date: 5 April 2020

Please cite this article as: S.O. Kuranov, O.A. Luzina, O. Onopchenko, I. Pishel, S. Zozulya, M. Gureev, N.F. Salakhutdinov, M. Krasavin, Exploring bulky natural and natural-like periphery in the design of *p*-(benzyloxy)phenylpropionic acid agonists of free fatty acid receptor 1 (GPR40), *Bioorganic Chemistry* (2020), doi: https://doi.org/10.1016/j.bioorg.2020.103830

Exploring bulky natural and natural-like periphery in the design of *p*-(benzyloxy)phenylpropionic acid agonists of free fatty acid receptor 1 (GPR40)

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Abstract: Six derivatives of 3-phenylpropionic acid bearing various natural and natural-like, spatially defined peripheral motifs have been synthesized and evaluated in vitro for free fatty acid receptor 1 (FFA1) activation. Two frontrunner compounds (bearing a bornyl and cytosine groups) were evaluated in an oral glucose tolerance test in mice where both demonstrated the ability to sustain blood glucose levels following a glucose challenge. The bornyl compound displayed a somewhat superior, dose-dependent efficacy and, therefore, can be regarded as a lead compounds for further development as a therapeutic agent for type 2 diabetes mellitus. Its high affinity to FFA1 was rationalized by docking experiments.

Keywords: free fatty acid receptor 1; type 2 diabetes mellitus; natural products; natural-likeness; three-dimensional structure; glucose tolerance.

1. Introduction

Diabetes mellitus is a chronic metabolic disease affecting more than 400 million people worldwide, more than 90 per cent of this patient population suffering from type 2 diabetes mellitus (T2DM) [1]. T2DM is a complex pancreatic disorder characterized by impaired carbohydrate metabolism, high blood sugar levels, as well as insulin resistance and/or insulin deficiency [2]. The impact of T2DM is exacerbated by such complications as nephropathy [3], retinopathy [4], atherosclerosis [5] and cardiovascular disease [6]. Currently, numerous drugs are

in clinical use against T2DM [7,8], however, their suboptimal efficacy and potential side effects [9] warrant the development of new therapies.

One of the serious side-effects associated with antidiabetic glucose-lowering drugs in the risk of hypoglycemia which can be as debilitating condition as hyperglycemia treated by the drug themselves [10]. Free fatty acids have been discovered as potential targets for treatment of T2DM and other metabolic diseases [11-12]. In particular, free fatty acid receptor 1 (FFA1, also known as GPR40) expressed in pancreatic β-cells and enteroendocrine cells was shown to mediate glucose-stimulated insulin secretion (GSIS) [13]. The basal, healthy expression levels of FFA1 are relatively low; however, they dramatically increase in hyperglycemic state. Activation of FFA1 at this point by endogenous free fatty acids or therapeutic agonists leads triggers a signaling cascade which, in turn, increases insulin secretion and ultimately lowers the glucose levels. Once the latter return to normal, so does the expression of FFA1. Therefore, continued exposure to FFA1 agonists will have no effect and carry no risk of developing hypoglycemia [13-14]. Numerous FFA1 agonists have been developed over the last 15 years; these have been comprehensively summarized in the recent excellent reviews [15-17]. While no drug targeting FFA1 for diabetes is currently approved for clinical use, several drug candidates are noteworthy (Figure 1) as they all contain a pharmacophoric 3-phenylpropanoic acid motif responsible for mimicking the endogenous fatty acid ligands. The molecular periphery around this basic scaffold is typically optimized so as to increase compounds' affinity and specificity to the target [17].

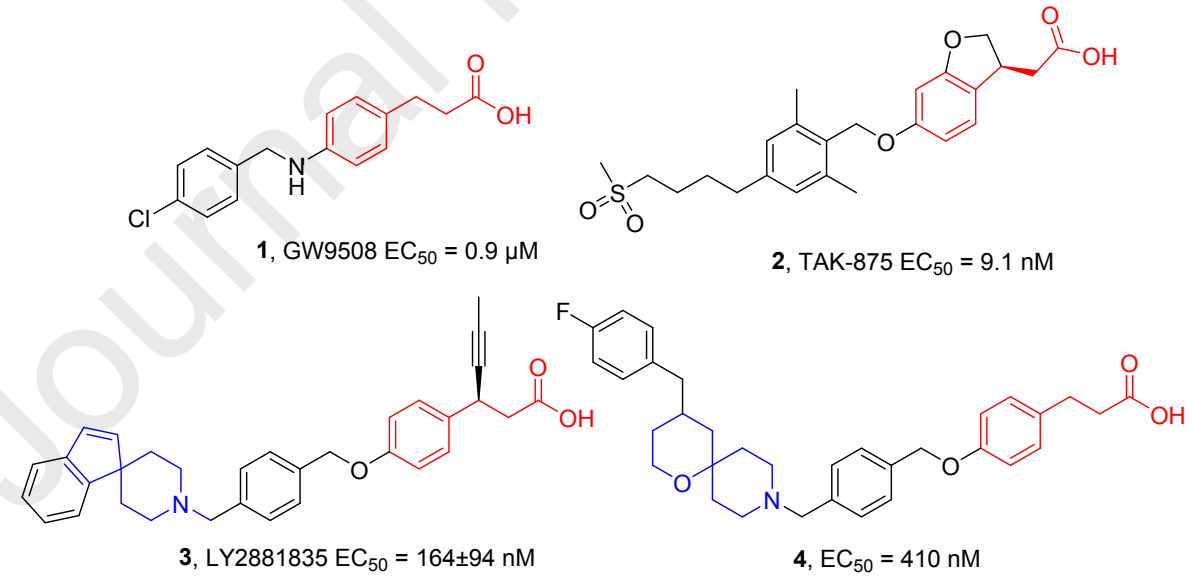


Figure 1. Drug candidates targeting FFA1 (at various stages of development).

An eloquent example of such elaborate periphery optimization is provided by the 100-fold improvement of potency on going from the nearly micromolar agonist **1** or GW9508 (also suffering from poor oral bioavailability) [18] to the advanced clinical candidate **2** or TAK-875

[19] which demonstrated promising efficacy in phase II clinical trials but failed in phase III due to idiosyncratic liver toxicity [20].

Two drug candidates – Eli Lilly's candidate LY2881835 (**3**) which was discontinued after phase I clinical trials [21] and early preclinical candidate **4** earlier developed by us [22] – illustrate a rather promising approach to the design of the 3-phenylpropanoic acid periphery (which is particularly relevant to the present study), namely, the use of spirocyclic motifs (highlighted in blue). The potential of spirocyclic motifs to provide a distinctly three-dimensional binding motifs with high degree of complementarity to the protein target signified them as an emerging type of privileged structures for drug design [23]. It is perhaps unsurprising that spirocycles frequently occur in natural products [24] which makes design of new spirocycles highly important from the standpoint of "natural-likeness" [25] of new chemical libraries for drug discovery.

Our recent success employing natural products (camphor and cytisine) in the design of dipeptidyl peptidase-IV inhibitors with pronounced *in vitro* and *in vivo* hypoglycemic activities [26] prompted us to consider investigating the applicability of various natural and natural-like periphery motifs as a hitherto unexplored approach to the design of FFA1 agonists. The general design idea would be to introduce bulky, spatially defined groups derived from the natural (**5**-**8**) as well as natural-like adamantine (**9**-**10**) [27-28] polycyclic amines (Figure 2), very much in line with the successful employment of the spirocyclic motifs in compounds **3** and **4**. Herein, we report reducing this idea to practice.

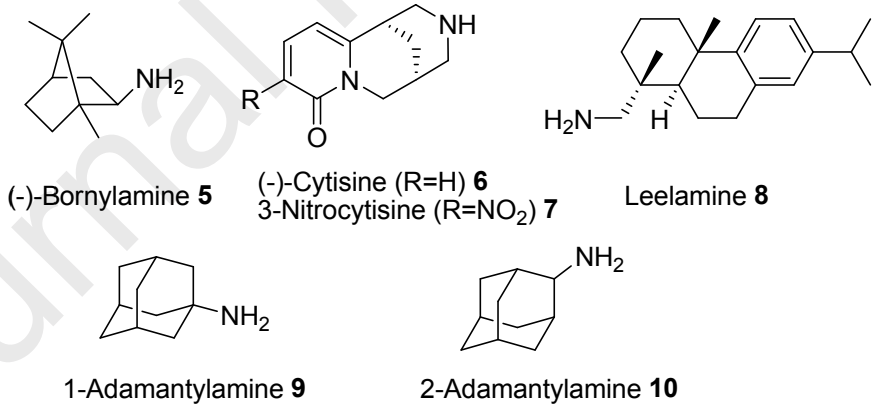


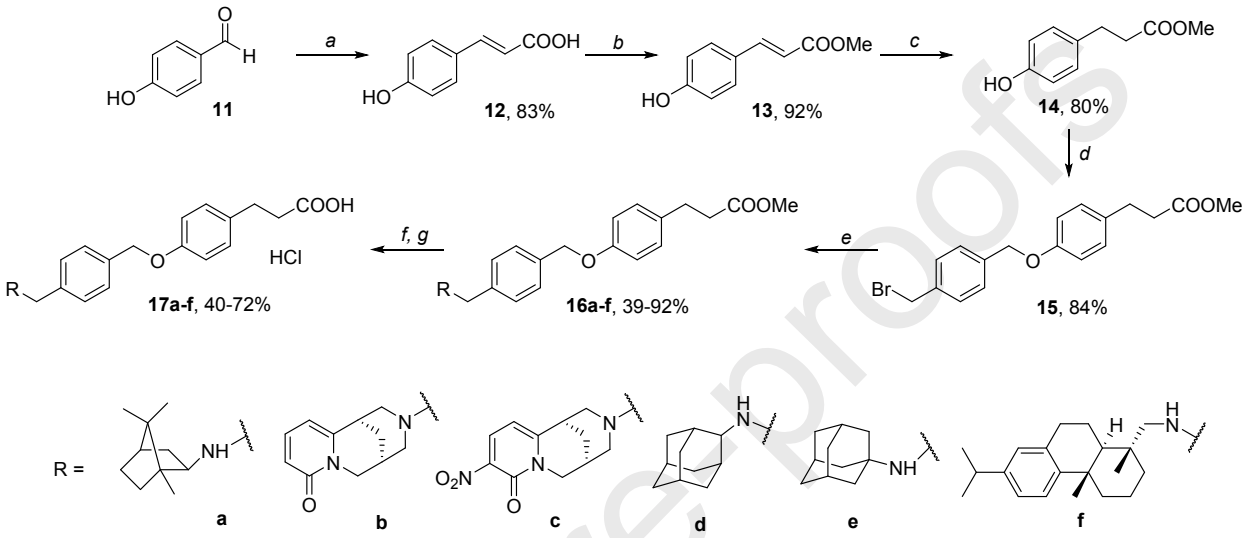
Figure 2. Natural (**5**-**8**) and non-natural (**9**-**10**) bulky amines for the design of FFA1 agonists.

2. Results and discussion

2.1 Synthesis

The key 3-(*p*-benzyloxy)phenylpropiponic acid building block **15** was prepared from *p*-hydroxybenzaldehyde (**11**) *via* condensation with malonic acid followed by decarboxylation which gave *p*-hydroxycinnamic acid (**12**) [29]. The latter was esterified ($MeOH/H_2SO_4$) to **13** and reduced at the double bond to give methyl 3-(*p*-hydroxyphenyl)propionate (**14**). The

phenolic group was alkylated by 1,4-bis(bromomethyl)benzene (taken in excess to reduce bis-substitution reaction) to give an excellent yield of the core building block **15**. The latter reacted with six amines shown in Figure 2 in acetonitrile in the presence of DIPEA as an HBr scavenger to give esters **16a-f** in modest to excellent yield. Alkaline hydrolysis of the latter followed by acidification with hydrochloric acid afforded target compounds **17a-f** as hydrochloride salts (Scheme 1).



Reagents and conditions: a. $CH_2(COOH)_2$, piperidine, pyridine, 65 °C; *b*. H_2SO_4, MeOH, reflux; c $NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH, 0 °C; *d. p*-$BrCH_2C_6H_4CH_2Br$, K_2CO_3, MeCN, reflux; *e*. RH (or RH·HCl), DIPEA, CH_3CN, reflux; *f*. $LiOH \cdot H_2O$, MeOH/THF/H_2O; *g*. HCl, H_2O.

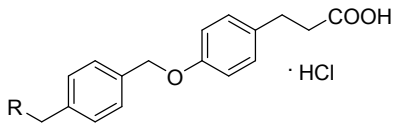
Scheme 1. Synthesis of target FFA1 agonists **17a-f**.

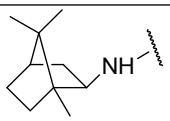
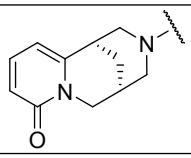
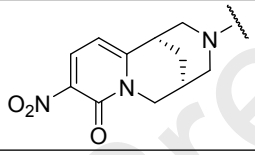
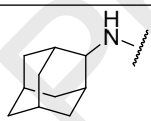
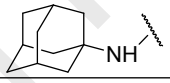
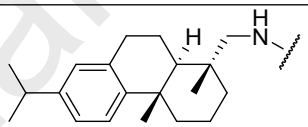
2.2. In vitro biological evaluation

Compound **17a-f** were tested for FFA1 activation in Chinese hamster ovary (CHO) cells stably overexpressing the receptor, using calcium flux essay (Table 1). To our delight, four out of six compounds (**17a-d**) displayed a full and dose-dependent agonism towards FFA1 as assessed relative to maximum efficacy relative to GW9508 (taken as a reference in 10 μM concentration). Interestingly, compound **17f** bearing the leelamine tail turned out to be virtually inactive. The difference in activity between 1-adamantyl (**17e**) and 2-adamntyl (**17d**) compounds is quite remarkable as well. The former compound, in contrast to the latter, displayed a 2.5-fold higher potency and full agonism while compound **17e** only gave a maximum of 53% FFA1 activation. Effect of the cytisine core nitration on the potency is noticeably negative (though it did not affect the maximum efficacy). Gratifyingly, the compounds decorated with bornyl (**17a**) and cytisinyl (**17b**) groups displayed full agonism in the submicromolar concentration ranger, very much in

line with our previous findings in the area of DPP-IV inhibitors [26]. These two frontrunner compounds were selected for in vivo efficacy experiments described below.

Table 1. In vitro agonistic activity of compounds **17a-f** towards FFA1.



Compound	R	% efficacy[a]	FFA1 EC_{50} (μM)
17a		108	0.75
17b		94	0.86
17c		116	2.74
17d		96	1.72
17e		53	5.26
17f		12	50

[a] % relative to maximum agonism displayed by GW9508 at 10 μM.

2.3 In vivo efficacy evaluation

Based on the high potency and the full agonistic profile displayed by compounds **17a** and **17b** in the vitro screening experiments, these compounds have been selected for evaluation in vivo for hypoglycemic effects in oral glucose tolerance test (*o*GTT) in mice. To this end, doses escalating from 1 mg/kg to 25 mg/kg of these compounds were administered orally to C57BL/6NCrl mice prior to an oral glucose challenge. The blood levels were monitored over 2 h relative to control. As it is evident from the data, compound **17a** slightly reduced the plasma glucose levels at 1 mg/kg. Further increase led to a dose-dependent effect and 25 mg/kg dose of this compound essentially led to no noticeable blood glucose level increase following the glucose challenge (Figure 3A). On the contrary, compound **17b** proved less efficacious as even 25 mg/kg led to

noticeable (though certainly less significant than that of the control) increase in blood glucose levels, up to roughly 40% of the control (Figure 3B).

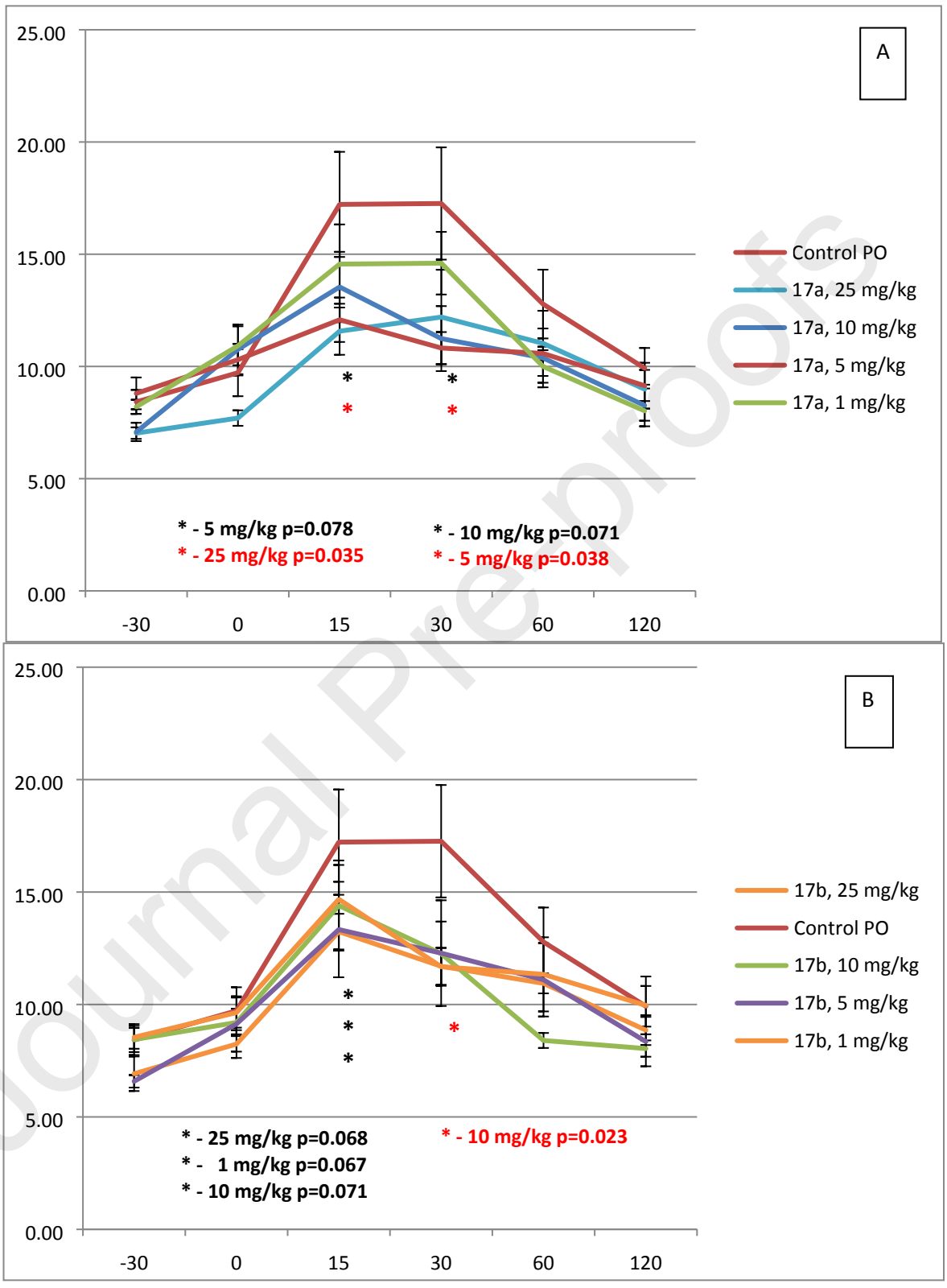
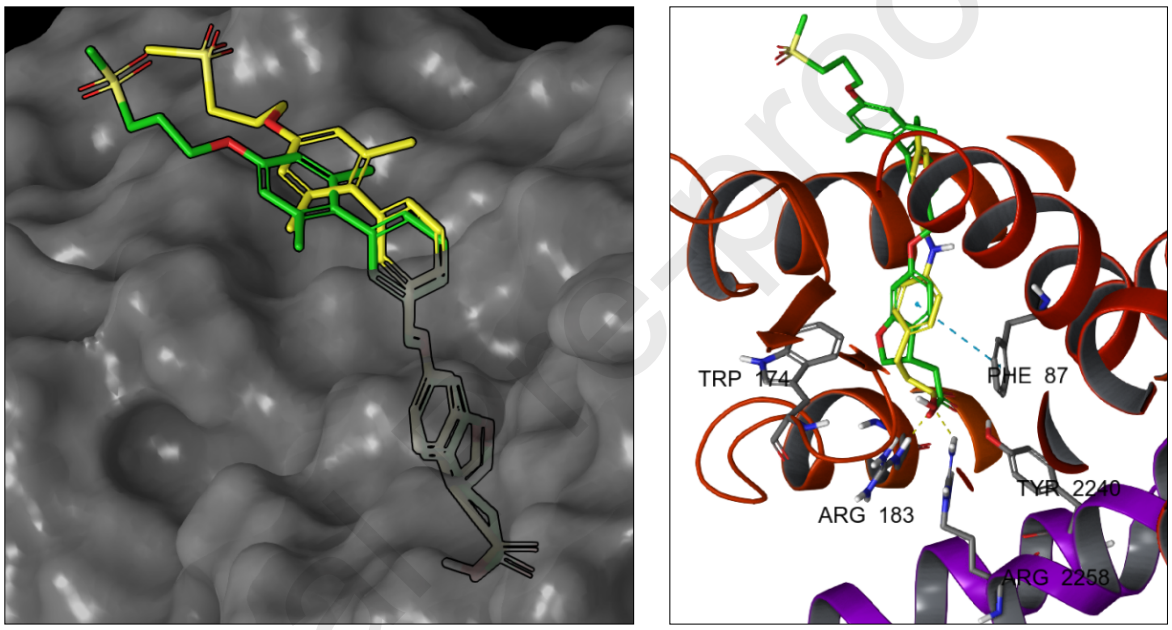


***Figure 3.** Plasma glucose concentration during the *o*GTT in C57BL/6NCrl mice (2 g/kg glucose challenge after 6 h fasting) performed after administration of 1-25 mg/kg of compounds **17a** (A) and **17b** (B). Results are mean ± SE (n = 6). The level of statistical significance was determined using Student's two-tailed t-test where the difference between the mean valus for two populations was considered.

2.4 In silico modeling

In order to rationalize the observed SAR trends for compounds 17a-f, we validated the docking protocol by performing de novo docking on TAK-875 into the crystal structure of FFA1 with bound TAK-875 (pdb code 4PHU). To our delight, the docking fully reproduced the binding mode observed in the crystal structure with RMSD < 2.5 Å (Figure 4A). Since GW9805 was used as a reference FFA1 agonist in this study, we also docked it into the binding site of FFA1 and we happy to see it fully reproduce the key interactions with the target, in particular, the variable π-stacking interaction with W174/F87, as well as principal hydrogen bonding to R183/R258(2258)/Y240 residues (Figure 4B).



A B

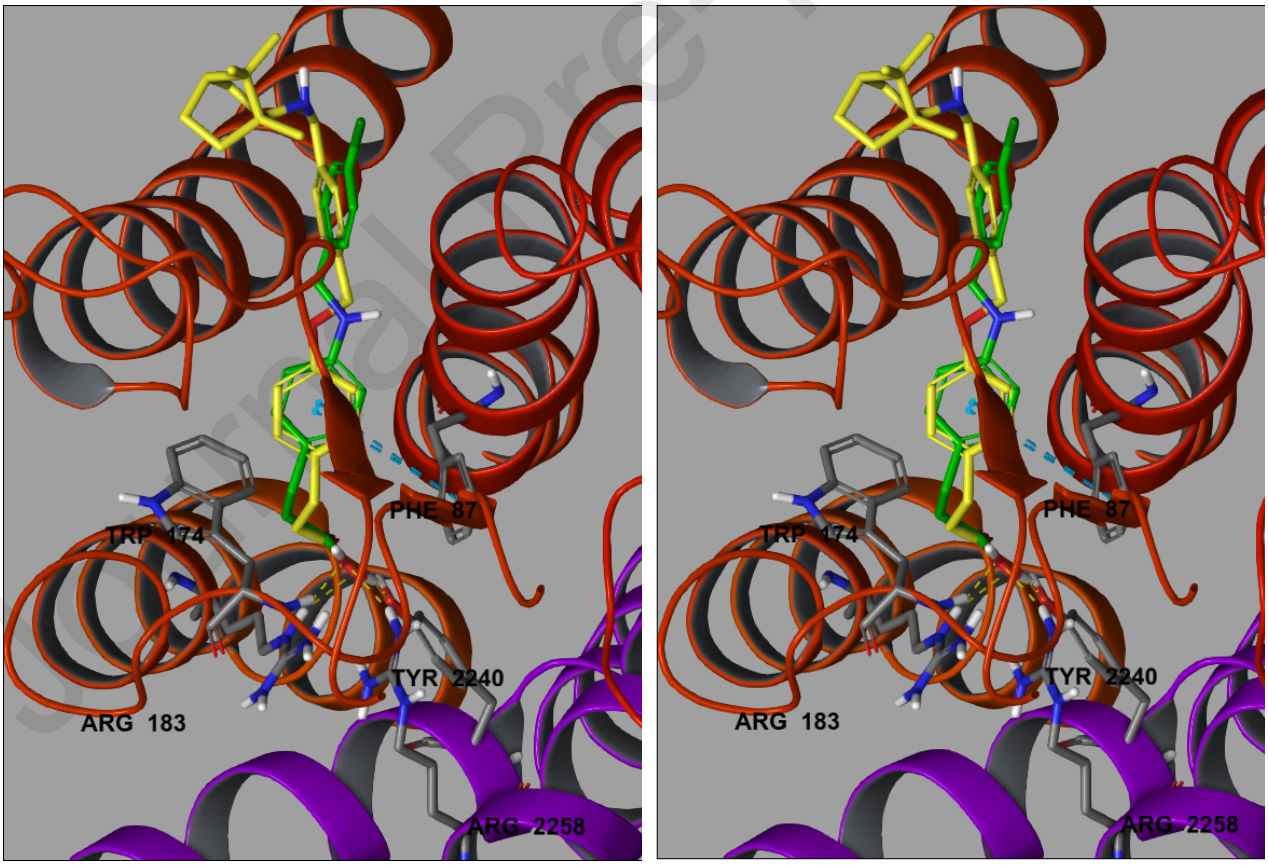
Figure 4. (A) Overlay of the docking pose of TAK-875 (yellow) with its crystal structure (green); (B) Docking of GW9805 into the binding site of FFA1.

Having validated the docking protocol, we proceeded to obtain Glide Score values for the compounds **17a-f** docked into the FFA1 binding site in comparison with reference ligands TAK-875 and GW9805. Unfortunately, Glide Score alone did not display any correlation with the observed EC_{50} values. Therefore, we calculated free energy values (ΔG) associated with the binding, using MMGBSA method which takes into account solvation energies of the protein-ligand complexes. To our delight, the ΔG values correlated well with the EC_{50} values with the most negative value observed for the most active compound (**17a**). At the same time the inactive compound (**17f**) did not even allow calculating relevant values associated with its binding to the target as its docking resulted in a number of high-energy poses which were deemed irrelevant (Table 2).

Table 2. EC_{50} values, Glide Score and DG values for compounds **17a-f** as well as reference ligands TAK-875 and GW9805 calculated based on docking experiments.

Compound	% efficacy	FFA1 EC_{50}, μM	GlideScore	ΔG (kcal/mol)
GW9508	100	0.90	-7.45	-80.69
TAK875	100	0.009	-10.616	-87.36
17a	108	0.75	-8.86	-82.19
17b	94	0.86	-9.70	-74.27
17c	116	2.74	-9.90	-73.28
17d	96	1.72	-9.22	-69.51
17e	53	5.26	-8.92	-70.51
17f	12	50	n/a	n/a

Thus, compounds **17a** displayed the free energy of binding closest, among the six compounds studied in this work, to those of GW9508 and TAK-875. This finding was also commensurate with the observation that **17a** very closely reproduced the docking pose of both GW9807 and TAK-875 in the FFA1 binding site (Figure 5).



A B

Figure 5. Overlay of the docking poses of compound **17a** (yellow) and (A) GW9805 (green) and (B) TAK-875 in the FFA1 binding site.

3. Conclusions

We have explored several natural and natural-like peripheral motif in the design of 3-pnehylpropionic acid-based agonists of free fatty acid receptor 1. The agonistic potency and the maximum efficacy displayed by the compounds synthesized appeared to be rather sensitive to the nature of the peripheral moiety. In particular, employing leelamine as a tail fragment, rendered the compound virtually inactive. The best, submicromolar in vitro activity and full agonism was displayed by two frontrunner compounds bearing a bornyl and cytisine moieties, respectively. Evaluation of these compounds at escalating doses in mice for the ability to sustain blood glucose levels following glucose challenge (i. e., the oral glucose tolerance test) demonstrated somewhat superior efficacy of the bornyl compound which represents a promising candidate for further evaluation as a therapeutic agent to treat type 2 diabetes mellitus.

4. Experimental section

4.1. General experimental

GC: 7820A gas chromatograph (Agilent Tech., USA); flame-ionisation detector; HP-5 capillary column, helium as carrier gas (flow rate 2 mL/min, flow division 99:1). Optical rotation: polar 3005 spectrometer; $CHCl_3$ soln. ^1H and ^{13}C NMR spectra: Bruker spectrometers AV-400 at 400.13 MHz (^1H) and 100.61 MHz (^{13}C), AV-600 at 600.30 MHz (^1H) and 150.95 MHz (^{13}C) in $CDCl_3$ or DMSO-d6; chemical shifts δ in ppm relative to residual $CHCl_3$ [δ($CHCl_3$) 7.26, δ($CHCl_3$) 77.00 ppm] or DMSO-d_6 [δ (DMSO-d_6) 2.50, δ (DMSO-d_6) 39.51 ppm], *J* in Hz. The structure of the products was determined by analyzing ^1H and ^{13}C NMR spectra. HR-MS: DFS Thermo Scientific spectrometer in a full scan mode (15–500 m/z, 70eV electron impact ionisation, direct sample administration). Column chromatography was performed on silica gel (60–200 l, Macherey-Nagel. All the target compounds reported in this paper have the purity of at least 95%. Spectral and analytical studies were carried out at the Collective Chemical Service Center of the Siberian Branch of the Russian Academy of Sciences. All chemicals were analytically pure and were used as received without purification prior to use. Solvents were purified according to literature procedures [30].

Leelamine **8** and 1-, 2-Adamantylamines **9, 10** were obtained from commercial sources.

4.2 Synthetic organic chemistry

4.2.1. (-)-Bornyl amine (**5**)

A mixture of (+)-camphor (13 mmol, 1.98 g), hydroxylamine hydrochloride (21.5 mmol, 1.77 g), and water (7 ml) was heated to 80°C, and methanol (7 mL) was added to dissolve the camphor. A solution of sodium acetate trihydrate (3.3 mmol, 4.5 g) in water (4 mL) was added, and the reaction mixture was heated under reflux at 100°C for 12 h. Upon removal of the methanol under reduced pressure, the white solid that precipitated was collected by filtration, washed with water (3x10 ml), and dried in vacuo to afford 1.95 g (90% yields) camphor oxime. The sodium borohydride (70.0 mmol , 2.66 g) was added portionwise to a solution of (1*S*)-camphor oxime (12 mmol, 1.95 g) and nickel dichloride hexahydrate (23 mmol, 5.54 g) in anhydrous methanol (40 ml) at –25°C over a period of 3 h. After completion of the addition, the resulting black slurry was stirred at this temperature overnight. The reaction mixture was then warmed to room temperature, and 25% ammonia solution (15 mL) in water (20 mL) was added with vigorous stirring. The resulting slurry was extracted with diethyl ether (3x70 mL), and the combined organic layers were washed with brine (20 mL), dried with anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (dichloromethane-methanol 100:0–90:10).

Foamy white solid, 33% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (s, 3H), 0.86 (s, 3H), 0.96 (s, 3H), 0.97 - 1.08 (m, 2H), 1.21 - 1.28 (m, 2H), 1.48 - 1.57 (m, 2H), 1.65 - 1.77 (m, 3H), 2.69 (dd, J = 8.9 Hz and J = 5.1 Hz, 1H).

4.2.2. 3-Nitrocytisine (**7**)

(–)-Cytisine **6** (0.5 g, 2.6 mmol) was dissolved in conc. H_2SO_4 (5 mL), treated with $NaNO_3$ (0.45 g, 5.26 mmol), stirred on a magnetic stirrer at room temperature until the starting material disappeared completely (controlled by TLC), neutralized with anhydrous Na_2CO_3, and extracted with ethyl acetate (3x5 mL). The extracts were combined, dried over Na_2SO_4, and evaporated. The solid was chromatographed over silicagel (C_6H_6–MeOH eluent, 9:1).

Yellow crystals, 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (br.s, 1H), 1.90 - 2.02 (m, 2H), 2.42 (br.s, 1H), 2.96 - 3.16 (m, 5H), 3.93 - 4.03 (m, 1H), 4.17 - 4.26 (m, 1H), 6.11 (d, J = 8.1 Hz, 1H), 8.32 (d, J = 8.1 Hz, 1H).

4.2.3. (*E*)-3-(4-Hydroxyphenyl)acrylic acid (**12**)

p-Hydroxybenzaldehyde **11** (1.512 g, 12.3 mmol) and malonic acid (2.83 g, 27.2 mmol) were placed in a 10 mL flask and dissolved in 6.8 ml of pyridine. Piperidine (0.115 mL, 1.2 mmol) was added, and the reaction was placed in pre-heated to 72°C bath for 5 days (controlled by TLC CH_2Cl_2-ethyl acetate 4:1). Then, the mixture was transferred to a beaker, and 100 mL of water

was added. Concentrated HCl was added drop-wise until pH 2-3. A white precipitate was collected using a Hirsch funnel and dried under vacuum. White powder, 83% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 6.27 (d, *J* = 15.9 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 2H), 7.40 - 7.56 (m, 3H), 9.84 - 10.27 (br.s, 1H)

4.2.4. (*E*)-Methyl 3-(4-hydroxyphenyl)acrylate (**13**)

Acid **12** (1.06 g 6.46 mmol) was dissolved in dry methanol. 2-3 drops of conc. sulfuric acid were added and solution obtained was refluxed for a two days until disappearing of starting material (controlled by TLC, CH_2Cl_2-acetic acid 100:1). After completion of the reaction methanol was evaporated under vacuum. A residue was re-dissolved in ethyl acetate (30 mL) and washed with water (10 mL), brine (10 mL) and dried over magnesium sulfate. Solvent was evaporated and the residue was used in following step without further purification.

White-off powder, 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 3H), 6.30 (d, *J* = 16.0 Hz, 1H), 6.53 (br.s, 1H), 6.82 - 6.91 (m, 2H), 7.41 (d, *J* = 8.6 Hz, 2H), 7.64 (d, *J* = 16.0 Hz, 1H).

4.2.5. Methyl 3-(4-hydroxyphenyl)propanoate (**14**)

To an ice-bath cooled solution of **13** (0.845 g, 5.1 mmol) and nickel dichloride hexahydrate (0.12 g, 0.5 mmol) in dry methanol (10 mL) a sodium borohydride (0.5 g, 13.2 mmol) was added in small portion. After completion of reaction (controlled by GC-MS), saturated ammonium chloride solution (2.5 mL) was added and methanol was evaporated under reduced pressure. Water (5 mL) and aqueous ammonia (10mL) were added to the residue, and the product was extracted with ethyl acetate (3x15 ml). The extracts were combined, dried over Na_2SO_4, and evaporated. The crude product was purified by column chromatography (hexane-ethyl acetate 7:3).

White solid, 0.67 g 80%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.60 (t, *J* = 7.70 Hz, 2H), 2.88 (t, *J* = 7.70 Hz, 2H), 3.67 (s, 3H), 5.55 (br.s, 1H), 6.70 - 6.80 (m, 1H), 7.00 - 7.09 (m, 1H).

4.2.6. Methyl 3-(4-(4-(bromomethyl)benzyloxy)phenyl)propanoate (**15**)

A solution of **14** (1.15 g, 6.4 mmol) and potassium carbonate (2.55 g, 18 mmol) in acetonitrile (20 mL) was stirred for 1 hour at reflux. Then stirring was stopped, the reaction mixture was allowed to cool down and a precipitate was allowed to settle. The solution was decanted into an addition funnel with additional acetonitrile (40 mL). A solution of p-dibromoxylene (4.80 g, 18.2 mmol) in acetonitrile (20 mL) was charged to the same reaction flask. Then the solution of **14** was slowly added to the reaction flask under reflux for 4 hours. The mixture obtained was

refluxed for additional 2 hours and then was cooled down. A precipitate was filtered off and washed with chloroform (3x30mL). Combined organic filtrate was evaporated and part of the unreacted p-dibromoxylene was recrystallized from acetone. The residue was purified by column chromatography with dry loading (hexane-ethyl acetate 20:1 to 10:1 after separation of *p*-dibromoxylene).

White solid, 81% yield.^{1}H NMR (400 MHz, $CDCl_3$) δ 2.60 (t, *J* = 7.8 Hz, 2H), 2.89 (t, *J* = 7.8 Hz, 2H), 3.67 (s, 3H), 4.50 (s, 2H), 5.03 (s, 2H), 6.89 (d, J=8.7 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 2H), 7.41 (s, 4H).

4.2.5. General procedure for reaction with amines **16a-f**

A solution of bromide **15** (1.18 mmol), amine (1.46 mmol) and *N*,*N*-diisopropylethylamine (2.01 mmol) in acetonitrile (7 mL) was refluxed for 2 hours. Ethyl acetate (20 mL) and 5% sodium hydroxide (10 mL) solution were added. Organic layer was separated and water layer was extracted with ethyl acetate (2x10 mL). Combined organic layers were washed with water (10 mL), brine (10 mL) and dried over magnesium sulfate. Solvent was evaporated under vacuum and residue was purified over silica gel column chromatography using chloroform-methanol 100:1.

4.2.5.1. Methyl 3-(4-(4-(((1*R*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylamino)methyl)-benzyloxy)phenyl)propanoate (**16a**)

White solid 76% yield. M.p. 89.7-90.0°C. $[\alpha]_D^{24}$ -46 (c 0.140, $CHCl_3$). IR (KBr,) ν/cm^{-1}: 831, 1012, 1178, 1232, 1516, 1729. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.90 (s, 3H), 1.01 - 1.12 (m, 5H), 1.44 - 1.76 (m, 6H), 2.56 - 2.66 (m, 3H), 2.90 (t, *J* = 7.8 Hz, 2H), 3.62 (d, *J* = 13.4 Hz, 1H), 3.67 (s, 3H), 3.79 (d, *J* = 13.4, 1H), 5.02 (s, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.5 Hz, 2H), 7.31 - 7.41 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 12.2, 20.5, 20.6, 27.3, 30.1, 36.0, 36.8, 38.7, 45.3, 46.7, 48.4, 51.6, 52.2, 66.1, 69.9, 114.8 (2C), 127.5 (2C), 128.3 (2C), 129.2 (2C), 132.8, 135.4, 141.1, 157.3, 173.4. HRMS for $C_{28}H_{37}O_3N_1^+$ calcd 435.2768, found 435.2760 $[M]^+$.

4.2.5.2. Methyl 3-{4-[(4-{[(1*S*,9*R*)-6-oxo-7,11-diazatricyclo[7.3.1.0^{2,7}]trideca-2,4-dien-11-yl]methyl}phenyl)methoxy]phenyl}propanoate (**16b**)

Colorless oil, 85% yield. $[\alpha]_D^{24}$ -168 (c 0.140, $CHCl_3$). IR (neat), ν/cm^{-1}: 798 1142, 1153, 1240, 1512, 1547, 1568, 1543, 1734. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.73 - 1.97 (m, 2H), 2.28 - 2.48 (m, 3H), 2.54 - 2.64 (m, 2H), 2.80 - 3.00 (m, 5H), 3.34 - 3.53 (m, 2H), 3.65 (s, 3H), 3.81 - 3.95 (m, 1H), 4.04 - 4.16 (m, 1H), 4.95 (s, 2H), 5.91 (dd, *J* = 6.9 and *J* = 1.2 Hz, 1H), 6.48 (dd, *J* =

9.0 and J = 1.4 Hz, 1H), 6.83 - 6.91 (m, 2H), 7.00 (d, J = 8.0, 2H), 7.07 - 7.14 (m, 2H), 7.22 - 7.31 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 28.0, 30.0, 35.4, 35.9, 49.9, 51.5, 59.9 (2C), 61.6, 69.8, 104.7, 114.8 (2C), 116.5, 127.4 (2C), 128.4 (2C), 129.2 (2C), 132.8, 135.7, 137.7, 138.6, 151.2, 157.2, 163.6, 173.3. HRMS for $C_{29}H_{32}O_4N_2^+$ calcd 472.2357, found 472.2362 $[M]^+$.

4.2.5.3. Methyl 3-{4-[(4-{[(1*S*,9*R*)-5-nitro-6-oxo-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-dien-11-yl]methyl}phenyl)methoxy]phenyl}propanoate (**16c**)

Orange oil, 82% yield. $[\alpha]_D^{24}$ -186 (c 0.140, $CHCl_3$). IR (neat), ν/cm^{-1}: 1155, 1238, 1300, 1317, 1511, 1550, 1677, 1733. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82 - 1.96 (2H, м, H-24), 2.39 (2H, д, J = 8.5), 2.51 (br.s, 1H), 2.59 (t, J = 7.8 Hz, 2H), 2.85 - 3.00 (m, 4H), 3.08 (br s, 1H), 3.43 (d, J = 12.0 Hz, 2H), 3.66 (s, 3H), 3.98 (dd, J = 15.8 Hz and J = 6.4 Hz, 1H), 4.19 (d, J = 15.7 Hz, 1H), 4.96 (s, 2H), 6.04 (d, J = 8.1 Hz, 1H), 6.88 (d, J = 8.60 Hz, 2H), 6.99 (d, J = 5.4 Hz. 2H), 7.11 (d, J = 8.6, 2H), 7.24 - 7.32 (m, 2H), 8.33 (d, J = 8.1 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 25.1, 27.6, 30.0, 35.8, 36.3, 51.1, 51.4, 59.0, 59.5, 61.5, 69.6, 102.6, 114.8 (2C), 127.4 (2C), 128.3 (2C), 129.1 (2C), 132.8, 134.4, 136.1, 137.1, 137.6, 155.1, 157.2, 160.5, 173.2. HRMS for $C_{29}H_{31}O_6N_3^+$ calcd 517.2207, found 517.2222 $[M]^+$

4.2.5.4. Methyl 3-{4-[(4-{[(adamantan-1-yl)amino]methyl}phenyl)methoxy]phenyl}-propanoate (**16d**)

White solid, 56% yield. M.p. 108.5°C. IR (KBr,) ν/cm^{-1}: 1169, 1178, 1250, 1271, 1516, 1732. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 - 1.78 (m, 13H), 2.10 (m, 3H), 2.59 (t, J = 7.7 Hz, 2H), 2.89 (t, J = 7.7 Hz, 2H), 3.66 (s, 3H), 3.76 (s, 2H), 5.01 (s, 2H), 6.89 (d, J = 8.5 Hz, 2H), 7.10 (d, J = 8.5 Hz, 2H), 7.36 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 29.6 (3C), 30.0, 35.9, 36.7 (3C), 42.7 (3C), 44.8, 50.9, 51.5, 69.8, 114.8 (2C), 127.5 (2C), 128.5 (2C), 129.2 (2C), 132.7, 135.5, 141.2, 157.2, 173.4. HRMS for $C_{28}H_{35}O_3N_1^+$ calcd 433.2612, found 433.2610 $[M]^+$.

4.2.5.5. Methyl 3-{4-[(4-{[(adamantan-2-yl)amino]methyl}phenyl)methoxy]phenyl}-propanoate (**16e**)

White solid, 69% yield. M.p. 86.3-86.9°C. IR (KBr,) ν/cm^{-1}: 1178, 1244, 1514, 1726. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (d, J = 12.1 Hz, 4H), 1.64 - 1.76 (m, 4H), 1.77 - 1.96 (m, 7H), 2.04 (d, J = 12.9 Hz, 2 H), 2.60 (t, J = 7.8 Hz, 2H), 2.79 (br.s, 1H), 2.90 (t, J = 7.8 Hz, 2H), 3.67 (s, 3H), 3.79 (s, 2H), 5.02 (s, 2H), 6.86 - 6.95 (m, 2H), 7.11 (d, J = 8.6 Hz, 2H), 7.32 - 7.45 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 27.6, 27.8, 30.1, 31.3 (2C), 32.0 (2C), 35.9, 37.5 (2C), 37.9, 50.7,

51.6, 61.1, 69.9, 114.8 (2C), 127.5 (2C), 128.3 (2C), 129.2 (2C), 132.8, 135.4, 141.1, 157.3, 173.4. HRMS for $C_{28}H_{35}O_3N_1^+$ calcd 433.2612, found 433.2604 $[M]^+$.

4.2.5.6. Methyl 3-(4-{[4-({[(1*R*,4a*S*)-1,4a-dimethyl-7-(propan-2-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]amino}methyl)phenyl]methoxy}phenyl)propanoate (**16f**)

White solid, 57% yield. M.p. 67.7°C. $[\alpha]_D^{26}$ +21 (c 0.048, $CHCl_3$). IR (neat), ν/cm^{-1}: 822, 1014, 1178, 1242, 1514, 1728. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (s, 3H), 1.20 - 1.28 (m, 9H), 1.36 - 1.53 (m, 4H), 1.61 - 1.87 (m, 5H), 2.24 - 2.33 (m, 2H), 2.54 (d, *J* = 11.8 Hz, 1H), 2.62 (t, *J* = 7.5 Hz, 2H), 2.78 - 2.95 (m, 5H), 3.68 (s, 3H), 3.73 - 3.84 (m, 2H), 5.03 (s, 2H), 6.88 - 6.95 (m, 3H), 7.00 (dd, *J* = 8.1 Hz and *J* = 1.6 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 2H), 7.19 (m, *J* = 8.2 Hz, 1H), 7.31 - 7.42 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 18.8, 18.9, 19.3, 24.0 (2C), 25.4, 30.1, 30.3, 33.4, 35.9, 36.2, 37.1, 37.4, 38.5, 45.2, 51.5, 54.6, 61.0, 69.9, 114.9 (2C), 123.7, 124.3, 126.8, 127.5 (2C), 128.1 (2C), 129.2 (2C), 132.8, 134.8, 135.5, 140.9, 145.4, 147.6, 157.4, 173.4. HRMS for $C_{38}H_{49}O_3N^+$ calcd 567.3707, found 567.3710 $[M]^+$.

4.2.6. General procedure for hydrolysis

To an ice-cooled solution of methyl ester (0.195 mmol) in THF (2 mL) a solution of lithium hydroxide monohydrate (0.390 mmol) in water (2 mL) was added. After completion of the reaction, THF was evaporated under reduced pressure. 2M hydrochloric acid was added drop-wise until pH 2-3. Precipitate was filtered and washed with water.

4.2.6.1. 3-(4-{[4-({[(1*R*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl]amino}methyl)-phenyl]methoxy}phenyl)propanoic acid hydrochloride (**17a**)

White solid, 65% yield. M.p. 153.2°C. $[\alpha]_D^{26}$ -35 (c 0.052, EtOH). IR (KBr,) ν/cm^{-1}: 827, 1244, 1383, 1423, 1512, 1709. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.72 - 0.82 (m 3H), 0.85 - 1.04 (m, 8H), 1.41 - 1.54 (m, 2H), 1.55 - 1.65 (m, 1H), 1.71 (t, *J* = 4.1 Hz, 1H), 2.06 - 2.18 (m, 1H), 2.42 - 2.53 (m, 2H), 2.67 - 2.79 (m, 2H), 2.91 (dd, *J* = 8.5 Hz and *J* = 5.4, 1H), 4.05 - 4.21 (m, 2H), 5.09 (s, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 7.9 Hz, 2H), 7.67 (d, *J* = 7.9 Hz, 2H), 8.7 (br.s, 2H) 12.05 (br.s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 11.8, 19.9, 20.4, 26.3, 29.6, 34.7, 35.7, 36.3, 44.3, 47.0, 48.6, 50.1, 64.2, 68.8, 114.8 (2C), 127.8 (2C), 129.3 (2C), 131.0 (2C), 133.2, 138.2, 156.6, 173.9. HRMS for $C_{27}H_{35}O_3N_1^+$ calcd 421.2615, found 421.2610 $[M]^+$. Anal calcd for $C_{27}H_{36}ClNO_3$: Cl 7.74. Found: Cl 8.06.

4.2.6.2. 3-{4-[(4-{[(1S,9R)-6-oxo-7,11-diazatricyclo[7.3.1.0^{2,7}]trideca-2,4-dien-11-yl]methyl}-phenyl)methoxy]phenyl}propanoic acid hydrochloride (**17b**)

White solid, 42% yield. M.p. 184.5°C. $[\alpha]_D^{26}$ -123 (c 0.132, EtOH). IR (KBr,) ν/cm^{-1}: 1236, 1510, 1549, 1647, 1724. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.79 - 1.95 (m, 2H), 2.42 - 2.51 (m, 2H), 2.69 - 2.81 (m, 3H), 3.09 - 3.54 (m, 5H), 3.77 - 3.89 (m, 1H), 3.92 - 4.04 (m, 1H), 4.19 - 4.42 (m, 2H), 5.08 (s, 2H), 6.28 (d, *J* = 6.6 Hz, 1H), 6.42 (d, *J* = 8.6 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 2H), 7.13 (d, *J* = 8.5, 2H), 7.43 (dd, *J* = 8.8 Hz and *J* = 7.1 Hz, 1H), 7.49 (d, *J* = 7.9 Hz, 2H), 7.58 (d, *J* = 7.9 Hz, 2H), 10.06 (br.s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.5, 25.8, 29.5, 31.9, 35.6, 48.0, 55.8, 56.1, 59.7, 68.7, 106.5, 114.6 (2C), 116.9 , 127.8 (2C), 128.5, 129.3 (2C), 132.1 (2C), 133.2, 138.8, 139.6, 147.5, 156.6, 162.3, 173.8. HRMS for $C_{28}H_{30}O_4N_2^+$ calcd 458.2200, found 458.2195 $[M]^+$. Anal calcd for $C_{28}H_{31}ClN2O_4$: Cl 7.16. Found: Cl 7.37.

4.2.6.3. 3-{4-[(4-{[(1*S*,9*R*)-5-Nitro-6-oxo-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-dien-11-yl]methyl}phenyl)methoxy]phenyl}propanoic acid hydrochloride (**17c**)

Orange solid, 40% yield. M.p. 115.5°C. $[\alpha]_D^{26}$ -173 (c 0.060, EtOH). IR (KBr,) ν/cm^{-1}: 1304, 1321, 1510, 1552, 1680, 1730. ^{1}H NMR (300 MHz, DMSO-d_6): 1.90 (m, 2H), 2.43 - 2.52 (m, 2H), 2.70 - 2.86 (m, 3H), 3.16 - 3.55 (m, 5H), 3.84 - 4.09 (m, 2H), 4.30 (m, 2H), 5.07 (s, 2H), 6.40 (d, *J* = 7.8 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 2H), 7.14 (d, *J* = 8.6 Hz, 2H), 7.51 (m, 4H), 8.39 (d, *J* = 8.1 Hz, 1H), 9.99 - 10.19 (m, 1H), 11.46 - 12.63 (br.s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.8, 25.5, 29.5, 32.6, 35.6, 49.1, 54.9, 55.7, 59.7, 68.7, 104.1, 114.6 (2C), 127.8 (2C), 128.3, 129.3 (2C), 132.1 (2C), 133.2, 135.4, 138.0, 138.9, 154.6, 156.3, 156.6, 173.8. Anal calcd for $C_{28}H_{30}ClN_3O_6$: C 62.28; H 5.60; N 7.78; Cl 6.57. Found: C 62.20; H 5.64; N 7.77; Cl 6.61.

4.2.6.4. 3-{4-[(4-{[(Adamantan-1-yl)amino]methyl}phenyl)methoxy]phenyl}propanoic acid hydrochloride (**17d**)

White solid, 42% yield. M.p. 264.7-268.1°C. IR (KBr,) ν/cm^{-1}: 827, 1250, 1514, 1697. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.56 - 1.72 (m, 6H), 1.99 (m, 6H), 2.13 (m, 3H), 2.43 - 2.53 (m, 2H), 2.70 - 2.78 (m, 2H), 4.07 (s, 2H), 5.10 (s, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 2H), 7.47 (d, *J* = 7.9 Hz, 2H), 7.62 (d, *J* = 8.2 Hz, 2H), 9.18 (br.s, 2H), 12.12 (br.s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 28.5 (3C), 29.5, 35.3 (3C), 35.6, 37.4 (3C), 42.3, 57.0, 68.6, 114.7 (2C), 127.7 (2C), 129.3 (2C), 130.5 (2C), 132.2, 133.1, 138.0, 156.5, 173.8. HRMS for $C_{27}H_{35}O_3N_1^+$ calcd 419.2455, found 419.2447 $[M]^+$. Anal calcd for $C_{27}H_{34}ClNO_3$: Cl 7.77. Found: Cl 8.02.

4.2.6.5. 3-{4-[(4-{[(Adamantan-2-yl)amino]methyl}phenyl)methoxy]phenyl}propanoic acid hydrochloride (**17e**)

White solid, 42% yield. M.p. 160.6.1°C. IR (KBr,) ν/cm^{-1}: 825, 1244, 1290, 1418, 1462, 1512, 1585, 1705. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.46 - 1.64 (m, 4H), 1.68 (br.s, 2H), 1.81 (m, 4H),

2.09 - 2.29 (m, 4H), 2.40 - 2.58 (m, 2H), 2.74 (t, *J* = 7.5 Hz, 2H), 3.13 (br.s, 1H), 4.17 (s, 2H), 5.09 (s, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 7.9 Hz, 2H), 7.66 (d, *J* = 7.9 Hz, 2H), 9.31 (br.s, 2H), 11.63 - 12.33 (br.s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.2, 26.4, 28.2 (2C), 29.5, 29.6 (2C), 35.6, 36.3 (2C), 36.7, 47.4, 60.7, 68.7, 114.7 (2C), 127.7 (2C), 129.2 (2C), 130.6 (2C), 131.3, 133.1, 138.0, 156.5, 173.8. HRMS for $C_{27}H_{33}O_3N_1$ $^+$ calcd 419.2455, found 419.2447 $[M]^+$. Anal calcd for $C_{27}H_{34}ClNO_3$: Cl 7.77. Found: Cl 8.07.

4.2.6.6. 3-(4-{[4-({[(1R,4aS)-1,4a-Dimethyl-7-(propan-2-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]amino}methyl)phenyl]methoxy}phenyl)propanoic acid hydrochloride (**17f**)

White solid, 58% yield. M.p. 187.3-189.2°C. $[\alpha]_D^{26}$ +33 (c 0.157, EtOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (s, 3H), 1.05 - 1.19 (m, 9H), 1.21 - 1.76 (m, 8H), 2.24 (d, *J* = 12.5 Hz, 1H), 2.42 - 2.50 (m, 2H), 2.53 - 2.92 (m, 7H), 4.04 - 4.16 (m, 2H), 5.10 (s, 2H), 6.81 - 6.86 (m, 1H), 6.87 - 6.98 (m, 3H), 7.08 - 7.16 (m, 3H), 7.48 (d, *J* = 7.9 Hz, 2H), 7.62 (d, *J* = 7.8 Hz, 2H), 8.74 - 9.30 (m, 2H), 11.88 (br.s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 17.9, 18.1, 18.3, 23.9 (2C), 24.9, 28.7, 29.5, 32.9, 35.2, 35.5, 35.9, 36.9, 37.3, 43.8, 50.6, 56.1, 68.7, 114.7 (2C), 123.5, 123.8, 126.3, 127.7 (2C), 129.2 (2C), 130.6 (2C), 132.7, 133.1, 134.3, 138.1, 145.1, 146.7, 156.5, 173.8. HRMS for $C_{27}H_{35}O_3N^+$ calcd 553.3551, found 553.3550 $[M]^+$. Anal calcd for $C_{38}H_{48}ClNO_3$: Cl 6.01. Found: Cl 6.16

4.3 In vitro FFA1 activation assay

CHOcells stably expressing human FFA1 (stable CHO-GPR40 line created at Enamine Ltd.) 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 dye-loading solution containing Fluo-8AM (Abcam, ab142773), Probenecid (Sigma, P8761), Pluronic F-127 (Sigma, P2443), Tartrazine (Sigma, T0388), Amaranth (Sigma, A1016) and tested using fluorometric imaging plate reader (FLIPR Tetra® High Throughput Cellular Screening System, Molecular De- vices Corp.). Maximum change in fluorescence over base line was used to determine agonist response. A potent and selective agonist for FFA1 GW9508 (Selleckchem, S8014) was tested with the test compounds as a positive control. To obtain concentration response curve data nonlinear regression with variable slope (four paramters) were fitted using GraphPad Prism version 7.03 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

4.4. In silico modeling

The structure of FFA1 receptor was downloaded from RCSB ProteinDataBank (Pdb id: 4PHU). Using the ProteinPrep Wizard module within Schrödinger Suite, errors contained in the pdb file (such as missing amino acids, incorrect bonds, irrelevant protonation) were corrected and the structures were optimized using the Ramachandran plot and minimized for energy.

4.5. In vivo studies

4.5.1. Animals

Male C57BL/6NCrl mice were maintained on a 12:12-h light-dark cycle under standard animal housing conditionswith temperature and humidity control. All animal procedures were performedin accordance with the approved standard procedures and animal care guidelines of Bioethics Commission of the Educational and Scientific Center "Institute of Biology" at Shevchenko National University, Kyiv, Ukraine. The“3Rs”principles were applied for conducting all animal studies.

4.5.2. Administration of test compounds

All compounds were formulated in Labrasol® (Gattefosse, France) solution in saline (50%-50%, v/v). Compounds were administered to mice by oral gavage using 20 gastainless steel animal feeding tube (InstechLaboratories, Inc., USA), at a volume dose of 5 ml/kg. Mice from the control groups received the same volume of the vehicle alone. All mice were dosed at 30 min before starting the OGTT test. GW9508 (Cayman Chemical) was used as reference compound at the dose of 100 mg/kg.

4.5.3. Determination of blood glucose levels

Determining the level of blood glucose was performed after 6-hour fasting. On Call Plus glucometer (Acon Laboratories, Inc., USA) and specific test strips (REF G133-111) were used for determining glucose level. Tail vein blood sampling was done by incision of the tail tip; 5-6 µl of blood was used for each glucose assay.

4.5.3. Glucose tolerance test

For glucose tolerance test (OGTT), mice were orally treated with 2 g/kg of glucose in a volume of 10 ml/kg after a 6-hour fasting. Glucose measurements were performed right before the compound treatment (point “-30 min”) as well as immediately before and at 15, 30, 60 and 120 min after glucose administration.

Acknowledgements

Authors would like to acknowledge the Multi-Access Chemical Service Center of the Siberian Branch of the Russian Academy of Science for spectral and analytical measurements.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/xxx.

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Exploring bulky natural and natural-like periphery in the design of *p*-(benzyloxy)phenylpropionic acid agonists of free fatty acid receptor 1 (GPR40)

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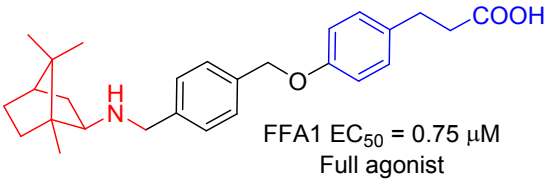
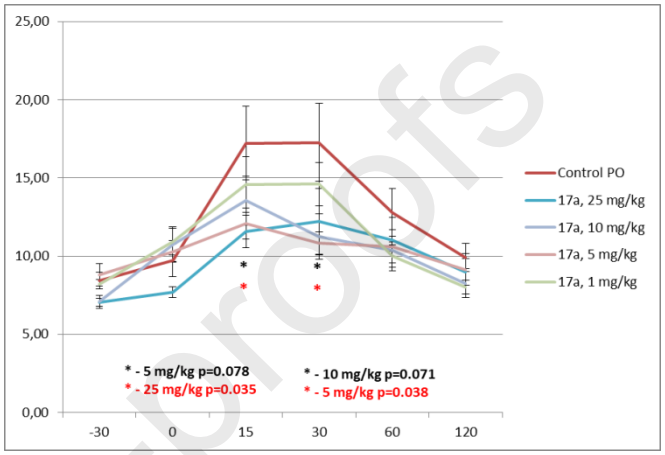


FFA1 EC_{50} = 0.75 μM
Full agonist



Dose-dependent efficacy in oral glucose tolerance test

- FFA1 is a clinically validated antidiabetic target with no approved drugs on the market.
- 3-Phenyl propionic acid is a known mimetic of endogenous ligands of FFA1.
- Natural and natural-like amines have been employed as periphery motifs.
- Bornyl- and cytisine-bearing compounds were most active in vitro as FFA1 agonists.
- In vivo efficacy testing (oGTT) showed the bornyl compound to be the lead compound.