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Brief Article

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Discovery of a Potent Free Fatty Acid 1 Receptor Agonist with Low Lipophilicity, Low Polar Surface Area and Robust in Vivo Efficacy

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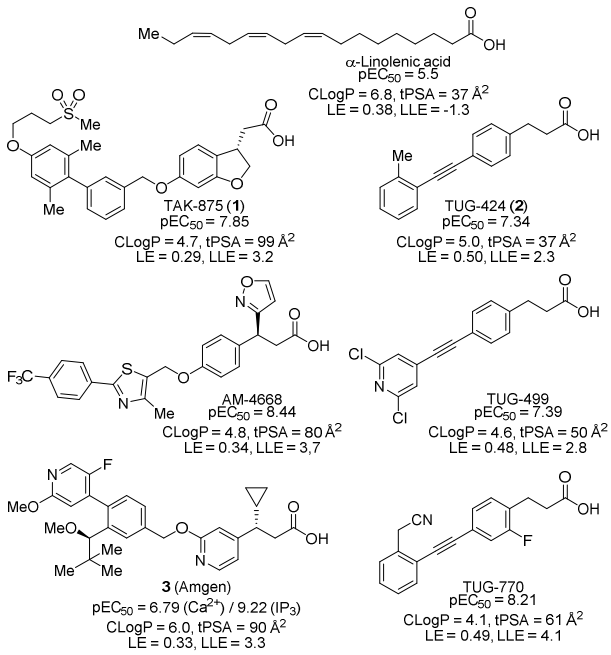
KEYWORDS. Type 2 diabetes, free fatty acid receptor, insulin secretagogue, FFA1, GPR40

ABSTRACT: The free fatty acid receptor 1 (FFA1 or GPR40) is established as an interesting potential target for treatment of type 2 diabetes. However, to obtain optimal ligands, it may be necessary to limit both lipophilicity and polar surface area, translating to a need for small compounds. We here describe the identification of **24**, a potent FFA1 agonist with low lipophilicity and very high ligand efficiency that exhibit robust glucose lowering effect.

The long-chain free fatty acid receptor FFA1 (formerly GPR40) is a G-protein coupled 7-transmembrane receptor that is expressed in pancreatic β-cells and that upon activation leads to insulin release in a glucose dependent manner.[1-5] Targeting the receptor with synthetic agonists can provide a viable treatment option for type 2 diabetes, averting the risk of hypoglycemia associated with other insulin secretagogues such as the sulfonylureas.[6] Phase II and III clinical trials with the selective FFA1 agonist **1** (TAK-875, fasiglifam) provided proof for this concept.[7] Unfortunately, **1** was withdrawn from phase III studies due to liver safety concerns even though disclosed results indicate only slightly higher incidence of 3-fold elevated alanine transaminase (ALT) between **1** and placebo-treated groups, and no difference in 5-fold elevated ALT.[8] The basis for the concerns of hepatotoxicity is currently not known. However, as FFA1 is not found to be expressed in human liver, it is widely presumed that the observed effects are compound related.[2,9-11] Most current FFA1 agonists have a somewhat high lipophilicity (e.g. Chart 1),[12] likely a consequence of the hydrophobic nature of the free fatty acid receptor binding site(s) and possibly due to the need for ligands to enter the binding site sideways via the lipid bilayer rather than from the top,[13] a factor that could preclude entrance of ligands with high polar surface area (PSA). A high lipophilicity is generally considered a deleterious property for potential drugs as it is correlated with poor absorption, toxicity, off-target effects and metabolic instability and thereby higher risk of failure in clinical trials.[14-16] In the case that both low lipophilicity and a low PSA are required features of safe and efficient FFA1 agonists, and since the surface of any ligand necessarily will be either lipophilic or polar, minimizing both would imply developing smaller ligands.

Chart 1. Structures of selected FFA1 agonists[a]



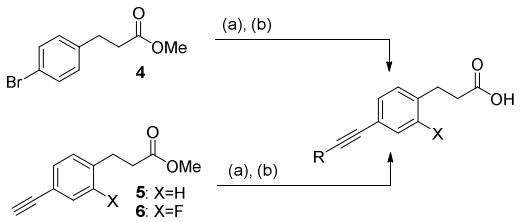
[a]ClogP and tPSA values calculated using ChemBioDraw 14.0. LE = RTlnK_D, presuming $EC_{50} \approx K_D$. Values are given in kcal mol^{-1} per non-hydrogen atom. LLE is calculated by pEC_{50} – ClogP.

Considerable efforts have been directed towards lowering lipophilicity in various compound series. For example, the series to which the previous clinical candidate **1** belongs was optimized by attachment of polar appendages,[17,18] the potent agonist TUG-469 was optimized further by adopting the same appendage,[19,20] and replacement of benzene rings with heteroaromatic rings has been used by Amgen for the same purpose in the development of AM-4668 and **3** (Chart 1).[21,22] In efforts to decrease lipophilicity and increase potency of the alkyne FFA1 agonist **2** (TUG-424),[23] we have employed both replacement of benzene rings with heterocycles and attachment of polar appendages,[24,25] leading to the discovery of TUG-770.[26] Herein, we report efforts at replacing the terminal aromatic ring of these moderately sized molecules with aliphatic moieties leading to further truncation and reduction of lipophilicity while keeping PSA at a minimum and preserving high potency.

RESULTS AND DISCUSSION

Using a modified Sonogashira procedure,[27] compounds were synthesized in two steps from the aryl bromide **4** or from the alkynes **5**[28] or **6**[29] (Scheme 1). The compounds were tested on human FFA1 and counter screened on human FFA4, another GPCR responding to long-chain free fatty acids that generally recognize ligands similar to FFA1.[4,5,30]

Scheme 1.[a]



[a]Reagents and conditions: (a) Alkyne or vinyl halide, Na_2PdCl_4, PIntB, CuI, TMEDA, water, 70 → 80 °C, 0.5–2 h, 45–84%; (b) LiOH (aq), THF, room temp, 2-12 h, 47–97%.

The use and validity of metrics such as ligand efficiency (LE)[31] and ligand lipophilicity efficiency (LLE)[14] to guide drug design has recently been debated.[32-34] Especially LE has been criticized for being more sensitive to changes in smaller molecules than in larger molecules and for treating all heavy atoms the same. Although values from such metrics should be treated as guidelines only and are not guaranteed to reflect the optimal compromise between potency, lipophilicity and size, we find both LE and LLE to be useful tools in the lead optimization process in general and especially when focus is on size and lipophilicity, as in the present case. Lipophilicity was calculated using BioByte's ClogP algorithm (ChemBioDraw), as this previously was found to correlate best with experimental log $D_{7.4}$ values for this series.[25]

The terminal phenyl ring of parent structure **7** is critical for activity on both FFA1 and FFA4, as evident from **8** (Table 1). Elongation by n-heptyl to produce fatty acid-like **6** unsurprisingly also resembles the natural long-chain free fatty acids in potency on the two receptors. Extending the alkyne of **7** by methylene and ethylene to homologues **10** and **11** results in erosion of activity on FFA1 and preserved or slightly gained activity on FFA4 with no improvement in overall properties. Of the smaller alicyclic terminal parts (**12**-**15**), the smallest cyclopropyl group (**12**) gave a loss in potency but preserved LLE due to its lower lipophilicity, whereas the larger cyclopentyl (**13**) and cyclohexyl (**14**) regained potency but also lipophilicity. The presence of a hydroxyl group in **15** abolished activity on both FFA1 and FFA4. Thus, none of the aliphatic or alicyclic terminal groups represented an advantage over the aromatic **7**. However, conjugating the alkyne to a double bond to reintroduce some of the features of the phenyl ring in the extension of the alkyne (**16**) boosted the potency on FFA1 back to the level of **7** whereas activity remained absent on FFA4. Due to its lower ClogP, **16** represented the first clear improvement over **7** in terms of LE and LLE.

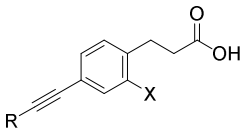
Further exploring the enyne system, a truncation to 2-propenyl (**17**) gave a moderate reduction in potency but the smaller size and lower lipophilicity increased both LE and LLE. It is also interesting to note an order of magnitude increase in potency between the isomers **12** and **17**, again demonstrating the beneficial effect of extending the alkyne π-system. Introduction of a terminal methyl in *E*- or *Z*-configuration (**18** and **19**) significantly boosted activity, for the *Z*-form **19** by close to an order of magnitude. Interestingly, the terminal methyl of **19** corresponds to the positioning of the *ortho*-methyl of **2** and with essentially identical potency (Chart 1). The combination of both terminal methyl substituents in **20** displayed lowered activity relative to **19**, probably due to steric issues in the narrow binding pocket.

We have previously seen a robust boost of potency in the alkyne series after introduction of a 2-fluoro substituent at the central benzene ring.[26] The same effect was observed upon introduction of the 2-fluoro substituent at **17**, **18** and **19**, with ΔpEC_{50} values of 0.71 for **21**, 0.38 for **23** and for **24** with 0.44 to 7.69. Activity on FFA4 was low or absent for all compounds and there was no sign of any 2-fluoro effect. To map the contributions of the various methyl groups, *Z*- and *E*-analogues **22** and **25** lacking the proximal methyl groups were investigated and confirmed that the *Z*-methyl contributes significantly to activity, in contrast to the *E*-methyl. Finally, introduction of a hydroxy group at the terminal methyl of **24** was investigated (**26**) leading to 20-fold deteriorated potency even if LLE increased due to the lower lipophilicity. Notably, all compounds except **15** and **26** have the same PSA (37 $Å^2$) as free fatty acids.

Altogether, it can be concluded that the truncated part of the terminal benzene ring of **2** contributes minimally to the potency of the compound on FFA1, but that the same part is important for activity at FFA4, as the truncated compounds in general are an order of magnitude less potent than the corresponding phenyl congeners.

Studies at Amgen have indicated the presence of multiple agonist binding sites at FFA1.[35] The recently published crystal structure of FFA1 bound to **1** indeed shows additional binding pockets besides the one occupied by **1** (PDB: 4PHU).[11,13,36] Both **2** and **24** docked well in the binding pocket of **1**. The docked **2** and **24** overlap with the methyl groups of both compounds filling a small lipophilic pocket that is left empty with for example **23** (Figure 1). Moreover, the part of **2** not overlapping with **24** is pointing out of the binding pocket (Figure S1), and thus appears not to contribute to binding to the receptor, whereas **24** docks best of all compounds and seems to perfectly fill the narrow part of the pocket.

Table 1. SAR of aliphatic and alicyclic substituents.



			pEC_{50} (efficacy, %)				
	R	X	hFFA1[a]	hFFA4[b]	ClogP[c]	LE_{FFA1}[d]	LLE_{FFA1}[e]
7	phenyl	H	6.70 ± 0.02 (108)	5.07 ± 0.08 (91)	4.54	0.48	2.16
8	H	H	<4	<4	2.17	-	-
9	*n*-heptyl	H	5.84 ± 0.04 (84)	5.39 ± 0.07 (102)	5.88	0.41	-0.04
10	benzyl	H	6.41 ± 0.03 (103)	4.98 ± 0.15 (113)	4.12	0.45	2.29
11	homobenzyl	H	5.74 ± 0.07 (128)	5.32 ± 0.10 (102)	4.65	0.37	1.09
12	*c*-propyl	H	5.30± 0.03 (128)	<4	3.15	0.47	2.15
13	*c*-pentyl	H	6.31 ± 0.04 (94)	4.47 ± 0.17 (105)	4.26	0.50	2.05
14	*c*-hexyl	H	6.29 ± 0.06 (98)	5.02 ± 0.03 (118)	4.82	0.47	1.47
15		H	>4	<4	2.74	-	-
16		H	6.70 ± 0.05 (100)	<4	4.37	0.50	2.33
17		H	6.38 ± 0.02 (108)	<4	3.18	0.57	3.20
18[f]		H	7.05 ± 0.02 (92)	4.47 ± 0.15 (103)	3.71	0.59	3.34
19[g]		H	7.25 ± 0.04 (95)	4.74 ± 0.10 (88)	3.71	0.60	3.54
20		H	7.08 ± 0.03 (105)	5.12 ± 0.05 (102)	4.10	0.56	2.98
21		F	7.09 ± 0.04 (106)	<4	3.32	0.59	3.77
22		F	6.68 ± 0.04 (97)	4.46 ± 0.03 (38)	3.45	0.54	3.27
23		F	7.43 ± 0.02 (97)	4.58 ± 0.02 (89)	3.85	0.59	3.58
24		F	7.69 ± 0.03 (103)	4.70 ± 0.03 (101)	3.85	0.61	3.84
25		F	7.32 ± 0.04 (91)	4.75 ± 0.22 (49)	3.45	0.61	3.87
26		F	6.37 ± 0.03 (105)	<4	2.06	0.48	4.31

[a]Values from a Ca^{2+} assay, efficacy is relative to TUG-20 (3-(4-(benzyloxy)phenyl)propanoic acid).[19] [b]Values from a β-arrestin mobilization assay, efficacy is relative to **2** (10 μM). [c]ClogP values calculated using the "ClogP" option of ChemBioDraw 12.0.3.[25] [d]LE is defined as = $RT\ln K_D$, presuming $EC_{50} \approx K_D$. Values are given in kcal mol^{-1} per non-hydrogen atom. [e]LLE is calculated by pEC_{50} – ClogP. [f]Contained 9% **19** as impurity. [g]Contained 5% **18** as impurity.

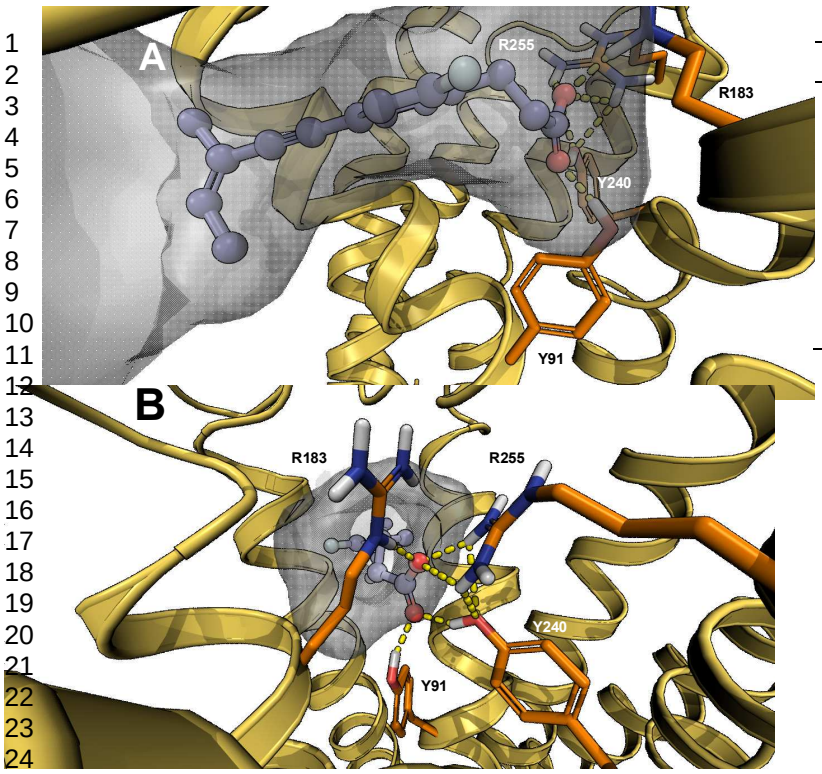


Figure 1. Docking of **24** in the FFA1 binding pocket of **1** viewed from the side (A) and from front (B).

With its small size (246.3 Da) and high potency, **24** likely to be the FFA1 agonist with the highest LE known and appears to represent an optimal combination of high potency, low lipophilicity and low PSA. Further investigations revealed good aqueous solubility (191 μM in PBS using turbidimetric solubility[37] at a maximum concentration of 200 μM) and lipophilicity in the optimal range (log $D_{7.4}$ = 1.79).[15]

We were somewhat concerned about the chemical stability of the enyne system even though this is found in certain drugs such as the antifungal terbinafine. Gratifyingly, recovery of **24** after 24 h in PBS or 2 h in simulated gastric and intestinal fluid was high to quantitative (100% after 24 h in 0.1 M PBS at 37 °C; 84-87% in FaSSGF, 95-99% in FeSSIF, 100% in FaSSIF, all after 2 h at 37 °C).

The two vinylic methyl groups of **24** are potentially exposed for CYP450 oxidation, thus the stability of **24** was examined in a human liver microsome assay,[38] resulting in 38% recovery of **24** after 1 h. Although lower than ideal, this indicates a useful and higher than expected metabolic stability of **24.** In comparison, **2** has shown a metabolic stability of 26% in the same assay, possibly due to its higher lipophilicity,[25] whereas the reference compounds verapamil and terfenadine gave 23% and 6% recovery, respectively.

The in vivo pharmacokinetic properties of **24** were investigated in mice and showed fast oral absorption with a high maximal plasma concentration after 15 min >1000-fold higher than EC_{50}, and a decent pharmacokinetic profile, a satisfactory plasma half-life of 1½ hour, moderate clearance and good overall exposure (Table 2). The effect of **24** on glucose tolerance was investigated in mice, revealing a significant glucose lowering effect and increased insulin response following oral dosing at 10 mg/kg (Figure 2).

Table 2. Pharmacokinetic properties of **24**

Pharmacokinetic properties[a]	**24**
C_{max}	44.7 μM
	(11009 ng/mL)
t_{max}	15 min
$t_{½}$	99 min
$AUC_{0-\infty}$	8295 μM·min
	(2043 μg/mL·min)
Clearance	0.49 mL·kg/min

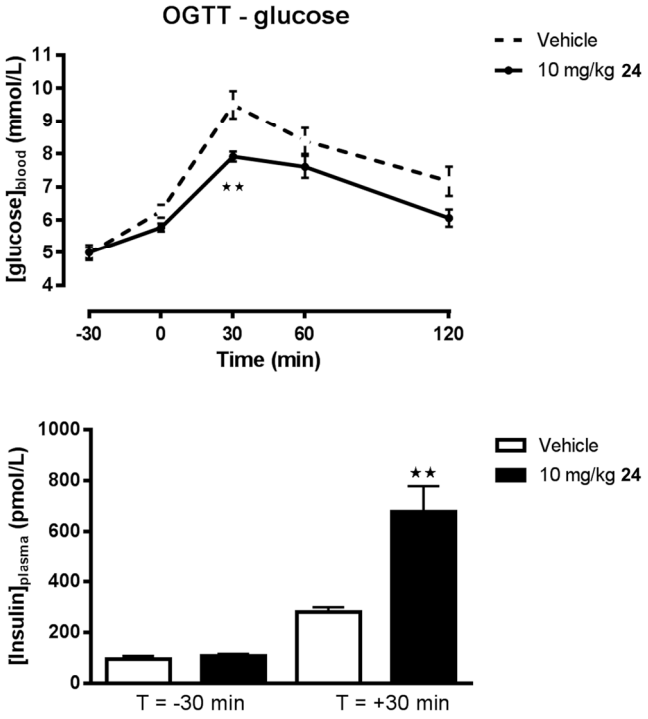


Figure 2. Effect of **24** on glucose tolerance in C57Bl/6 wildtype mice (10 mg/kg p.o., n=8). (A) Effect on plasma glucose levels. (B) Effect on plasma insulin levels. **, p<0.01.

CONCLUSION

Further exploration of the alkyne FFA1 agonist series represented by **2** with focus on minimization of both lipophilicity and PSA resulted in identification of a series of small but potent enyne FFA1 agonists where **24** displayed the optimal combination of high potency, low PSA and low lipophilicity, as well as good overall physicochemical properties including a satisfactory chemical and metabolic stability, good pharmacokinetic properties and robust effect on plasma glucose and insulin levels in an OGTT in mice after oral dosing. It is notable that **24** is carrying the carboxylic acid as the only polar functional group and thus has a PSA identical to that of the endogenous free fatty acid agonists but approximately 100-fold more potent and 1000-fold less lipophilic than for example, α-linolenic acid (Chart 1), and should therefore represent the best of both worlds in terms of receptor binding site access and potency.

EXPERIMENTAL SECTION

General remarks. Commercial starting materials and solvents were used without further purification. THF was freshly distilled from sodium/benzophenone. MeOH was freshly distilled from Mg. TLC was performed on TLC Silica gel 60 F254 plates and visualized at 254 nm and/or by staining with phosphomolybdic acid, vanillin, or $KMnO_4$ stains. Petroleum ether (PE) refers to alkanes with bp 60-80 ^oC. Purification by flash chromatography was carried out using silica gel 60 (0.040-0.063 mm, Merck). ^1H, ^{13}C and ^{19}F NMR spectra were recorded at 400, 101, and 376 MHz respectively on Bruker Avance III 400 at 300 K. Purity was determined by HPLC and confirmed by inspection of NMR spectra (^1H, ^{13}C, ^{19}F NMR). HPLC analysis was performed using a Dionex 120 C18 column (5 µm, 4.6x150 mm); flow: 1 mL/min; 10% MeCN in water (0-1 min), 10-100% MeCN in water (1-10 min), 100% MeCN (11-15 min), with both solvents containing 0.05% TFA or 0.1% HCOOH as modifier; UV detection at 254 nm. High-resolution mass spectra (HRMS) were obtained on a Bruker micrOTOF-Q II (ESI). All test compounds were of ≥95% purity unless otherwise stated.

(*Z*)-3-(2-Fluoro-4-(3-methylpent-3-en-1-yn-1-yl)phenyl)-propanoic acid (24).

A Schlenk flask charged with Na_2PdCl_4 (1 mol%), PIntB (2 mol%), CuI (2 mol%), **6** (105 mg, 0.51 mmol), (*Z*)-2-bromobut-2-ene (60 µL, 0.59 mmol), H_2O (0.2 mL/mmol) and TMEDA (1.8 mL/mmol) was evacuated and backfilled with argon three times. The reaction mixture was heated to 80 °C. After 30 min, the reaction mixture was added water and extracted with EtOAc (x3). The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated, and the residue was purified by flash chromatography (EtOAc:petroleum ether, 1:6) to give methyl (*Z*)-3-(2-fluoro-4-(3-methylpent-3-en-1-yn-1-yl)phenyl)-propanoate (60 mg, 45%) as a pale yellow oil: R_f = 0.62 (EtOAc:petroleum ether, 1:2); ^1H NMR ($CDCl_3$) δ 7.18–7.06 (m, 3H), 5.86–5.76 (m, 1H), 3.67 (s, 3H), 2.97 (t, *J* = 7.6 Hz, 2H), 2.63 (t, *J* = 7.7 Hz, 2H), 1.96–1.90 (m, 3H), 1.90–1.84 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 173.0, 160.6 (d, *J* = 246.9 Hz), 131.3, 130.5 (d, *J* = 5.7 Hz), 127.5 (d, *J* = 16.1 Hz), 127.3 (d, *J* = 3.2 Hz), 123.7 (d, *J* = 9.9 Hz), 118.5, 118.1 (d, *J* = 23.6 Hz), 91.9 (d, *J* = 3.1 Hz), 89.3, 51.7, 34.0 (d, *J* = 1.3 Hz), 24.6 (d, *J* = 2.3 Hz), 22.8, 16.3; ESI-MS *m/z* 261.1 ($M+H^+$).

The methyl ester (54 mg, 0.21 mmol) in THF (1 mL) was added a solution of $LiOH{\cdot}H_2O$ (25 mg, 0.6 mmol) in water (0.5 mL) and the reaction mixture was stirred until consumption of the ester (approx. 1 hour), adjusted to pH 1 with 1 M HCl (aq), and extracted with EtOAc (x3). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated to give 44 mg (86%) of **24** as a pale yellow solid: t_R = 12.56 min (HPLC purity: 99.5%); ^1H NMR ($CDCl_3$) δ 7.18–7.08 (m, 3H), 5.86–5.77 (m, 1H), 2.97 (t, *J* = 7.6 Hz, 2H), 2.68 (t, *J* = 7.7 Hz, 2H), 1.93–1.90 (m, 3H), 1.90–1.84 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 178.4, 160.6 (d, *J* = 247.0 Hz), 133.4, 130.5 (d, *J* = 5.8 Hz), 127.4 (d, *J* = 3.2 Hz), 127.2 (d, *J* = 16.1 Hz), 123.8 (d, *J* = 9.8 Hz), 118.5, 118.2 (d, *J* = 23.5 Hz), 91.8 (d, *J* = 3.3 Hz), 89.4, 33.9, 24.2 (d, *J* = 2.3 Hz), 22.8, 16.4; ESI-HRMS calcd for $C_{15}H_{15}FO_2Na$ ($M+Na^+$) 269.0948, found 269.0955.

ASSOCIATED CONTENT

Supporting Information. Synthetic procedures and compound characterization, procedures for in vitro biological assays and for in vivo pharmacokinetic and OGTT studies, molecular formula strings, coordinates for **2** and **24** docked in FFA1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

[1]These authors contributed equally.

Notes

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BDH, GM and TU are shareholders in Caldan Therapeutics.

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ABBREVIATIONS

ALT, alanine transaminase; FaSSGF, fasting state simulated gastric fluid; FaSSIF, fasting state simulated intestinal fluid; FeSSIF, fed state simulated intestinal fluid; FFA1, free fatty acid receptor 1 (GPR40); FFA4, free fatty acid receptor 4 (GPR120); LE, ligand efficiency; LLE, ligand lipophilicity efficiency; OGTT, oral glucose tolerance test; PBS, phosphate buffered saline; PIntB, 2-(di-*tert*-butylphosphino)-*N*-phenylindole; PSA, polar surface area; T2D, type 2 diabetes; TMEDA, *N*,*N*,*N'*,*N'*-tetramethylethylenediamine.

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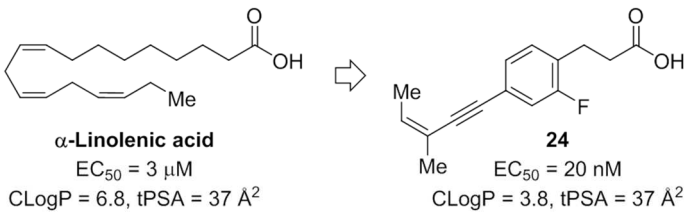

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