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Discovery of a Potent and Selective Free Fatty Acid Receptor 1 Agonist with Low Lipophilicity and High Oral Bioavailability

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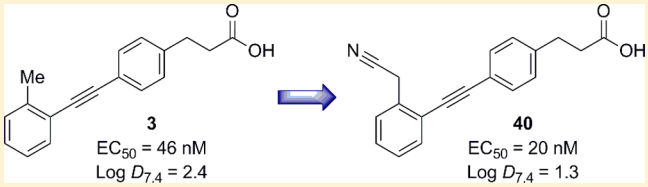
Supporting Information

ABSTRACT: The free fatty acid receptor 1 (FFA1, also known as GPR40) mediates enhancement of glucose-stimulated insulin secretion and is emerging as a new target for the treatment of type 2 diabetes. Several FFA1 agonists are known, but the majority of these suffer from high lipophilicity. We have previously reported the FFA1 agonist **3** (TUG-424). We here describe the continued structure−activity exploration and optimization of this compound series, leading to the discovery of the more potent agonist **40**, a compound with low lipophilicity, excellent in vitro metabolic stability and permeability, complete oral bioavailability, and appreciable efficacy on glucose tolerance in mice.



INTRODUCTION

Type 2 diabetes (T2D) is characterized by insulin resistance and insufficient insulin secretion by pancreatic β-cells, leading to dysfunctional control of plasma glucose and numerous long-term health consequences, such as increased risk of heart disease and stroke, kidney failure, blindness, neuropathy, and amputations. The global number of diabetics has now reached 350 million, of which 90% are type 2 diabetics.[1,2] Besides a healthy lifestyle, the most common treatments include insulin, metformin, and sulfonylureas, all of which are associated with problems such as weight gain, risk of hypoglycemia, and lack of sustained efficacy, and there is an urgent need for improved therapeutics.

The long-chain free fatty acid receptor 1 (FFA1, previously known as GPR40) is highly expressed in pancreatic β-cells and enhances glucose-stimulated insulin secretion (GSIS) but does not affect insulin secretion at low glucose levels.[3−6] This mechanism provides the potential for boosting insulin levels of type 2 diabetics without the risk of hypoglycemia associated with sulfonylureas and insulin administration. FFA1 is also expressed in enteroendocrine cells and has been implicated in the secretion of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).[7] Thus, the receptor may potentially enhance insulin secretion at high glucose levels through two independent mechanisms.

A number of synthetic FFA1 agonists have been reported in the literature (Chart 1),[5,8−26] of which the compounds TAK-875 and AMG-837 have reached clinical trials. A general problem with the currently known FFA1 ligands is their relatively high lipophilicity, which is associated with numerous problems, such as poor pharmacokinetic properties, metabolic instability, toxicity, and off-target effects, and correlates with attrition in clinical trials.[27−32] Studies have recommended that ClogP values should not exceed 4−5,[30,33] and ranking functions such as ligand lipophilicity efficiency (LLE) have been suggested to facilitate implementation of lipophilicity concerns in the optimization process.[27]

We previously reported the discovery of the alkyne agonist series with **3** (TUG-424, Chart 1) as the most potent compound.[13] The compound is, however, relatively lipophilic and exhibited only moderate in vitro metabolic stability and is thus not suitable as a drug candidate. We therefore aimed at improving the lipophilicity and metabolic stability of the compound by replacement of the terminal benzene ring by nitrogen-containing heterocycles, which led to the identification

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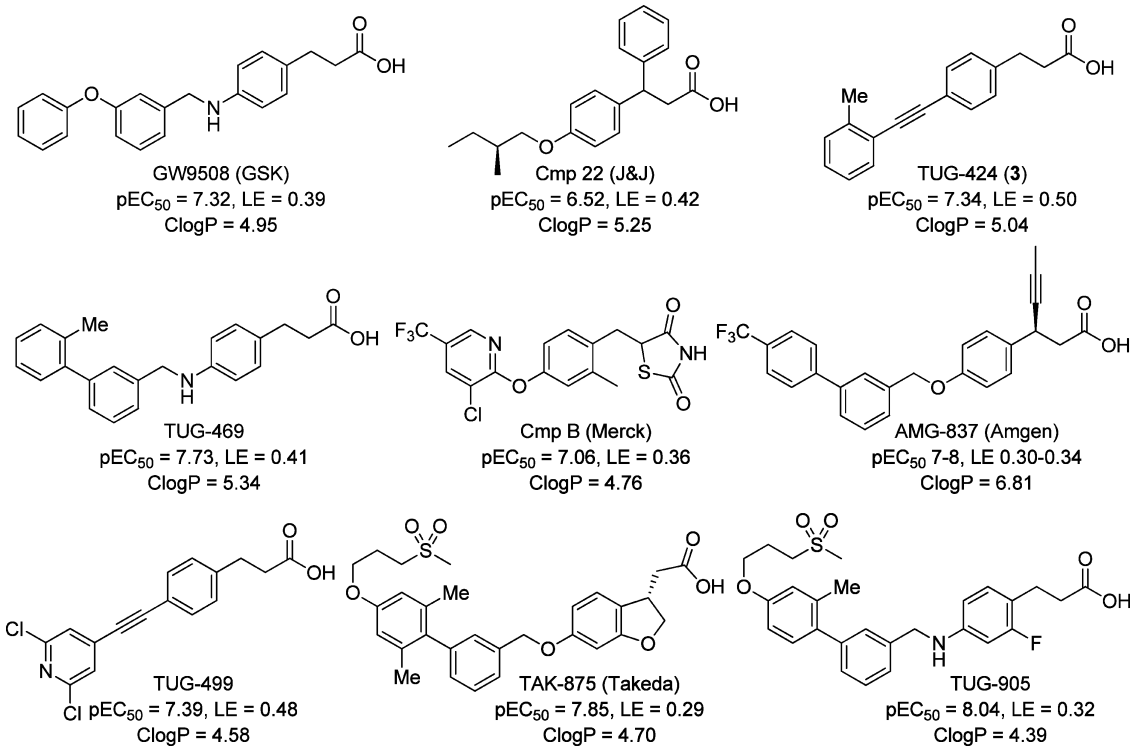
Chart 1. Representative FFA1 Agonists



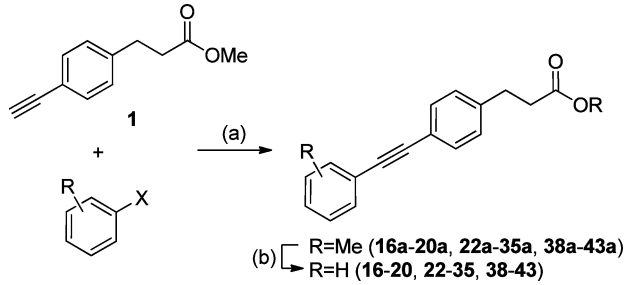
of TUG-499 (Chart 1).[19] Inspired by Takeda's clinical candidate TAK-875 (Chart 1), we recently explored the attachment of a mesylpropoxy appendage on TUG-469 (Chart 1), the previously reported compound from another series, and thereby identified TUG-905 (Chart 1), a compound with significant reduction in lipophilicity.[25] We have also continued the further optimization and exploration of structure–activity relationships (SAR) around **3** with focus on lowering lipophilicity and improving metabolic stability by the introduction of polar substituents. Herein, we report the results from these studies, which led to the identification of **40**, a compound with improved potency, lower lipophilicity than any previously reported FFA1 agonist, high selectivity, excellent in vitro ADME properties, complete bioavailability, and appreciable efficacy on glucose tolerance in mice.

RESULTS AND DISCUSSION

The majority of the alkyne ligands were synthesized directly by Sonogashira cross-coupling between the alkyne intermediate **1** and aryl halides (Scheme 1).[34] As the classical Sonogashira protocol (method A) in most cases works unsatisfactorily with **1**, we screened other methods and found that a method reported by Beller and co-workers (method B) gave improved results.[35] This method however also often gave low yields, especially with aryl iodides. We therefore optimized the method further and found that addition of 10% water (method C) resulted in suppression of Glaser–Hay dimerization of the alkyne and very rapid cross-coupling in high yields with both aryl bromides and iodides.[34] Method C is more convenient and has so far given significantly better results than other methods in all cases where iodo- and bromobenzenes are used as substrates.

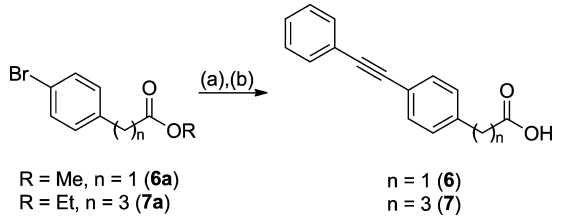
The phenylacetic acid (**6**) and 4-phenylbutanoic acid (**7**) analogues were synthesized from the corresponding aryl bromides by Sonogashira coupling with phenylacetylene (Scheme 2). Acrylic acid analogues **8** and **9** were synthesized from 4-bromocinnamic acid via esterification and Sonogashira cross-coupling reactions as for the propionic acids (Scheme 3).

Scheme 1[a]



[a]Reagents and conditions: (a) method A, $Pd(PPh_3)_2Cl_2$, CuI, aryl halide, Et_3N, DMF, 50 °C; method B, Na_2PdCl_4, PIntB, CuI, aryl halide, TMEDA, water, 80 °C; method C, Na_2PdCl_4, PIntB, CuI, aryl halide, TMEDA, water, 80 °C. (b) LiOH, THF, water, room temperature.

Scheme 2[a]



[a]Reagents and conditions: (a) Na_2PdCl_4, PIntB, CuI, phenylacetylene, TMEDA, water, 80 °C (78%). (b) LiOH, THF, water, room temperature (95–99%).

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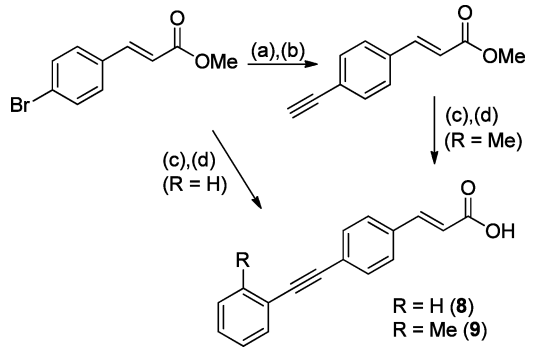
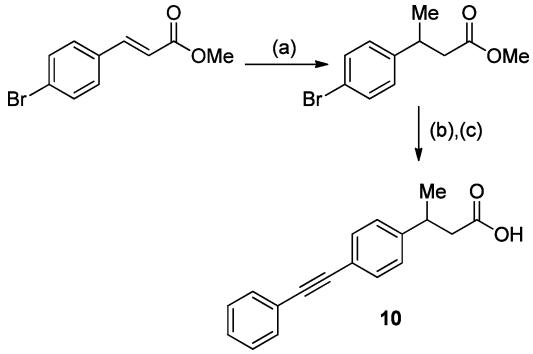
Scheme 3[a]



[a]Reagents and conditions: (a) trimethylsilylacetylene, Na_2PdCl_4, PIntB, CuI, TMEDA, water, 80 °C (77%). (b) K_2CO_3, MeOH, room temperature (95%). (c) Na_2PdCl_4, PIntB, CuI, 2-bromotoluene or phenylacetylene, TMEDA, water, 80 °C (68−84%). (d) LiOH, THF, water, room temperature (85−91%).

The *β*-methyl substituted analogue **10** was prepared by a conjugated addition of methyl Gilman reagent to methyl 4-bromocinnamate in the presence of chlorotrimethylsilane (Scheme 4).[36−38] All cyclopropyl analogues are racemic and

Scheme 4[a]



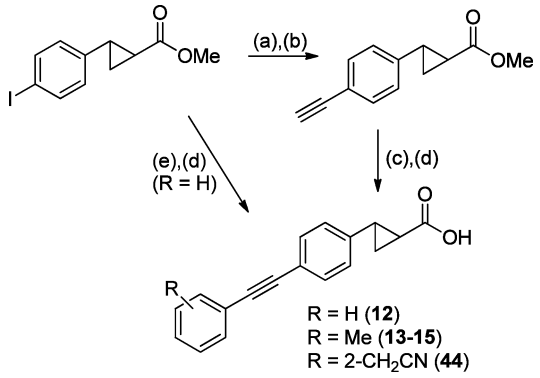
[a]Reagents and conditions: (a) MeMgCl, copper(I) thiophenolate, TMSCl, THF, −78 °C → 0 °C (47%). (b) Na_2PdCl_4, PIntB, CuI, phenylacetylene, TMEDA, water, 80 °C (57%). (c) LiOH, THF, water, room temperature (64%).

were prepared from methyl *trans*-2-(4-iodophenyl)-cyclopropanoate (Scheme 5).[19] The bicyclic alkyne **11** was synthesized from 5-bromodihydroindanone by a Horner−Wadsworth−Emmons reaction with triethyl phosphonoacetate, reduction with triethylsilane and TFA, and Sonogashira coupling with phenylacetylene followed by ester hydrolysis (Scheme 6). Aryl halides used to prepare **27**, **28**, **42**, and **43** were synthesized by alkylation of iodophenols. The methoxymethyl-substituted bromobenzene building blocks for **34** and **35** were synthesized following a $FeSO_4$-promoted ether synthesis of the corresponding bromobenzyl bromides.[39] The biphenylalkyne **21** was synthesized from the methyl ester **23a** by Suzuki cross-coupling (Scheme 7).

The mesylalkoxy-substituted alkynes were prepared from **26a** by a Williamson ether synthesis to form the bromoalkylated intermediate, which was subsequently mesylated by gentle heating with sodium methanesulfinate in PEG-400 (Scheme 8).

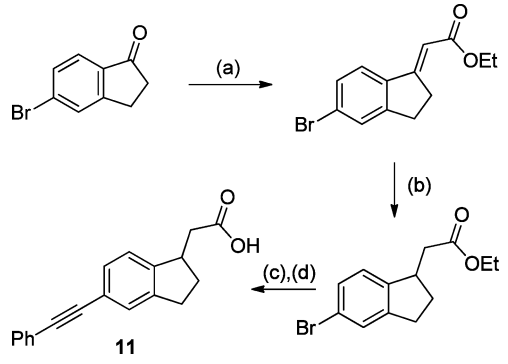
Compounds were screened on the human FFA1 in a calcium mobilization assay. Besides overall activity, ligand efficiency

Scheme 5[a]



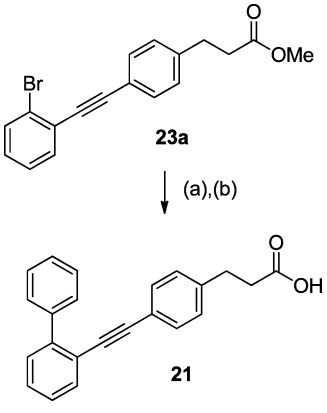
[a]Reagents and conditions: (a) trimethylsilylacetylene, Na_2PdCl_4, PIntB, CuI, TMEDA, water, 80 °C. (b) K_2CO_3, MeOH, room temperature (97% over two steps). (c) Na_2PdCl_4, PIntB, CuI, aryl halide, TMEDA (or TMEDA with 10% water for R = 2-CH_2CN), 80 °C (36−49%). (d) LiOH, THF, water, room temperature (97−99%). (e) $Pd(PPh_3)_2Cl_2$, CuI, phenylacetylene, Et_3N, DMF, 50 °C (53%).

Scheme 6[a]



[a]Reagents and conditions: (a) triethyl phosphonoacetate, 60% NaH, toluene, 0 °C → reflux (45%). (b) Et_3SiH, TFA, room temperature → 50 °C (58%). (c) Na_2PdCl_4, PIntB, CuI, phenylacetylene, TMEDA, water, 80 °C (57%). (d) LiOH, THF, water, room temperature (91%).

Scheme 7[a]



[a]Reagents and conditions: (a) $PhB(OH)_2$, $Pd(OAc)_2$, SPhos, K_3PO_4, toluene, 100 °C (74%). (b) LiOH, THF, water, room temperature (94%).

(LE)[40] and calculated lipophilicity (ClogP), as well as LLE[27] calculated as the difference between pEC_{50} and ClogP, were taken advantage of in the evaluation of analogues. Since the

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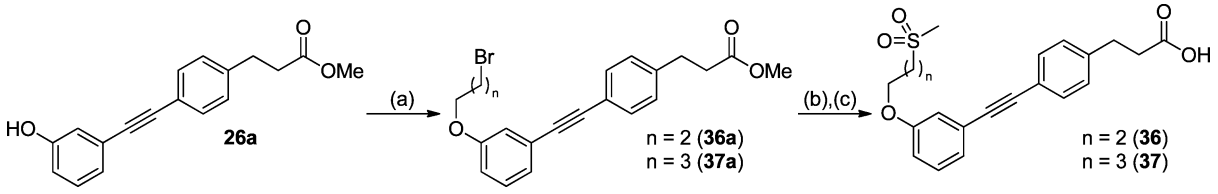
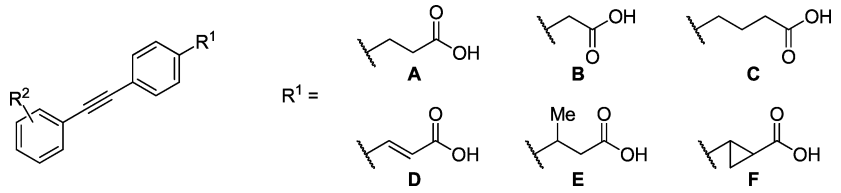
Scheme 8[a]



[a]Reagents and conditions: (a) $Br(CH_2)_{n+1}Br$, K_2CO_3, acetone, reflux (79−93%). (b) $MeSO_2Na$, PEG-400, 45 °C (38−51%). (c) LiOH, THF, water, room temperature (58−80%).

Table 1. SAR Exploration of the Propionic Acid Chain



compd	R^1	R^2	FFA1, calcium pEC_{50} (% efficacy)[a]	LE[b]	ClogP[c]	log $D_{7.4}$[d]	LLE[e]
2[f]	A	H	6.70 ± 0.03 (106)	0.48	4.54		2.16
3[f]	A	2-Me	7.34 ± 0.07 (103)	0.50	5.04	2.44 ± 0.01	2.86
4[f]	A	3-Me	7.13 ± 0.05 (97)	0.49	5.04		2.75
5[f]	A	4-Me	6.55 ± 0.04 (109)	0.45	5.04	2.13 ± 0.02	2.17
6	B	H	6.01 ± 0.03 (93)	0.46	4.05		1.96
7	C	H	6.00 ± 0.02 (99)	0.41	4.92		1.08
8[g]	D	H	5.03 ± 0.08 (83)	0.36	4.88		0.15
9	D	2-Me	5.64 ± 0.04 (104)	0.39	5.38		0.26
10	E	H	6.70 ± 0.03 (103)	0.46	4.94		1.76
11	(Scheme 6)		6.46 ± 0.04 (99)	0.42	5.05		1.42
12	F	H	6.84 ± 0.04 (107)	0.47	4.59		2.25
13	F	2-Me	6.86 ± 0.02 (99)	0.45	5.09		1.78
14	F	3-Me	7.03 ± 0.02 (102)	0.46	5.09		1.95
15	F	4-Me	6.51 ± 0.04 (107)	0.42	5.09		1.43

[a]Efficacy is given as percent response relative to 10 μM TUG-20.[18] [b]Ligand efficiencies (LE) were calculated by $LE = RT \ln K_D$, presuming that $EC_{50} \approx K_D$. Values are given in kcal mol^{-1} per non-hydrogen atom.[40] [c]Calculated by BioByte's algorithm as implemented in ChemBioDraw Ultra 12.0 (the "ClogP" option). [d]Determined by the shake flask method.[25] [e]Ligand lipophilicity efficiencies (LLE) were calculated by the formula $LLE = pEC_{50} - ClogP$.[27] [f]Previously reported.[13] [g]Tested with 0.05% bovine serum albumin (BSA).

ClogP calculation method was used extensively in the optimization, a validation and comparison of alternative methods using related compounds with experimental log $D_{7.4}$ values was performed, and log $D_{7.4}$ values from representative new alkynes were subsequently added to strengthen the validation. Of 11 methods, the ClogP method showed the best correlation with experimental values (R^2 = 0.96; see the Supporting Information).

We have previously reported the discovery of the potent and selective FFA1 agonist **3** (Chart 1) by screening of a focused library of constrained fatty acid analogues and optimization of a 4-(phenylethynyl)phenoxyacetic acid hit.[13] The optimization study revealed the phenylpropanoic acid analogue **2** (Table 1) to be clearly favored over the phenoxyacetic acid of the initial hit and that introduction of methyl substituents at the terminal phenyl showed the 2-position (**3**) was preferred over the 3-position (**4**), while a 4-methyl (**5**) did not result in increased potency relative to the unsubstituted **2**.[25] Shortening or elongating the propionic acid chain by one methylene group (**6** and **7**) resulted in significant decrease in potency. The planar acrylic acid **8** showed a further order of magnitude reduced potency, which to some degree was regained by introduction of the 2-methyl on the terminal phenyl ring (**9**). The introduction of a β-methyl group (**10**) did not affect potency, but is disfavored relative to **2** as it results in lower LE and higher lipophilicity and introduces a chiral center that complicates synthesis. Constraining the propionic acid by connecting the methyl group of **10** back to the benzene ring via a methylene group to form a dihydroindane system (**11**, Scheme 6), in analogy to the structures reported by Takeda,[21] yielded reduced potency. In contrast, connecting the β-methyl to the α-carbon to form a cyclopropyl constraint of the propionic acid chain (**12**) results in a moderate gain of activity. Unfortunately, the gain was not maintained with the introduction of methyl substituents at the terminal benzene ring (**13**−**15**).

Although methyl substituents in both 2- and 3-position resulted in increased potency, the initial studies showed that the 2,3-dimethyl as well as the 3,5-dimethyl pattern gave a compound with lower potency than the corresponding monomethyl analogues.[13] To complete this study, we synthesized the 2,5-dimethyl (**16**), 2,6-dimethyl (**17**), and 4-chloro-2-methyl (**18**) analogues, all of which exhibited reduced activity relative to **3** (Table 2).

We found that **3** had only moderate stability toward human liver microsomes (HLM, see below) and suspected that the 2-

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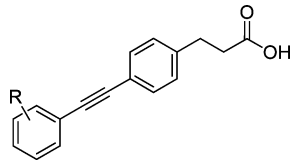
Table 2. Exploration of Substitution on the Terminal Phenyl Ring



compd	R	FFA1, calcium pEC_{50} (efficacy, %)[a]	LE[b]	ClogP[c]	log $D_{7.4}$[d]	LLE[e]
16	2-Me, 5-Me	6.91 ± 0.06 (102)	0.45	5.54		1.37
17	2-Me, 6-Me	6.38 ± 0.04 (103)	0.42	5.54		0.84
18	2-Me, 4-Cl	6.86 ± 0.02 (103)	0.45	5.75		1.11
19	4-F	6.28 ± 0.05 (96)	0.43	4.68		1.60
20	2-Et	7.05 ± 0.03 (101)	0.46	5.57		1.48
21	2-Ph	6.00 ± 0.02 (91)	0.33	6.43		−0.43
22	2-Cl	6.82 ± 0.04 (107)	0.47	5.25		1.57
23	2-Br	7.08 ± 0.06 (99)	0.49	5.40		1.68
24	2-CF_3	6.47 ± 0.02 (98)	0.39	5.42		1.05
25	2-OH	4.79 ± 0.07 (83)	0.33	3.87		0.92
26	3-OH	6.35 ± 0.04 (103)	0.43	3.87		2.48
27	2-OMe	6.66 ± 0.03 (99)	0.44	4.46		2.20
28	3-OMe	7.15 ± 0.03 (101)	0.47	4.46		2.69
29	2-OCF_3	6.65 ± 0.03 (106)	0.38	5.57	2.66 ± 0.01	1.08
30[f]	3-OCF_3	7.02 ± 0.04 (103)	0.40	5.57	2.83 ± 0.01	1.45
31	2-COMe	6.73 ± 0.04 (101)	0.42	3.98		2.75
32	2-CH_2OH	6.24 ± 0.02 (111)	0.41	3.50		2.74
33	2-C_2H_4OH	5.69 ± 0.02 (106)	0.35	3.73		1.96
34	2-CH_2OMe	7.39 ± 0.03 (108)	0.46	4.34	1.29 ± 0.00	3.05
35	3-CH_2OMe	7.42 ± 0.04 (99)	0.46	4.34	1.43 ± 0.01	3.08
36	3-$O(CH_2)_3Ms$	6.09 ± 0.03 (100)	0.31	3.55		2.55
37	3-$O(CH_2)_4Ms$	6.37 ± 0.03 (106)	0.31	3.33	0.88 ± 0.01	3.04
38	2-CN, 5-Me	6.33 ± 0.02 (103)	0.39	4.47		1.86
39	2-Me, 5-CN	7.40 ± 0.03 (105)	0.45	4.47	1.75 ± 0.01	2.93
40	2-CH_2CN	7.70 ± 0.04 (103)	0.48	3.96	1.28 ± 0.01	3.76
41	3-CH_2CN	6.72 ± 0.04 (98)	0.42	3.96	0.98 ± 0.01	2.76
42	2-OCH_2CN	7.33 ± 0.03 (105)	0.44	3.45		3.89
43	3-OCH_2CN	6.58 ± 0.03 (105)	0.39	3.45		3.14
44	(Scheme 5)	7.45 ± 0.03 (99)	0.45	4.01		3.44

[a]Efficacy is given as percent response relative to 10 μM TUG-20.[18] [b]Ligand efficiencies (LE) were calculated by LE = $RT \ln K_D$, presuming that $EC_{50} \approx K_D$. Values are given in kcal mol^{-1} per non-hydrogen atom.[40] [c]Calculated by ChemBioDraw Ultra 12.0 with the "ClogP" option. [d]Determined by the shake flask method.[25] [e]Calculated by the formula LLE = pEC_{50} − ClogP.[27] [f]Tested with 0.05% BSA.

methyl group might be implicated. A 4-fluoro substituent on the terminal phenyl ring (**19**) to stabilize the terminal phenyl ring toward oxidation came with a penalty in terms of lower potency. Increasing the steric bulk of the 2-methyl group of **3** by replacement with ethyl (**20**) or phenyl (**21**) resulted in reduced potency. These analogues also had increased lipophilicity and were unlikely to improve the HLM stability. Replacement of the 2-methyl by metabolically stable chloro (**22**), bromo (**23**), or trifluoromethyl (**24**) substituents did not preserve potency, while lipophilicity was increased in all cases.

It is well-known that lipophilicity generally correlates with poor metabolic stability, a consequence of the increased tendency of more lipophilic molecules to seek out of the aqueous phase and into the active sites of the metabolic enzymes. We have previously described our efforts to optimize **3** by replacing the terminal phenyl group with hydrophilic heterocycles,[19] and we recently described the effects of introducing a polar substituent on an FFA1 agonist, resulting in reduced lipophilicity and higher HLM stability together with preserved potency.[25] Although initial attempts to introduce polar substituents on the terminal phenyl ring of **3** had resulted in compounds with low or no activity,[13] we decided to continue the exploration of this strategy. Introduction of a hydroxyl group in the 2-position (**25**) resulted in a 2 orders of magnitude erosion of activity. A 3-hydroxy substituent (**26**) gave a moderate reduction in potency relative to **2**, but a higher LLE. It is thus clear that the positioning of a polar group is critical. A rationale for this was provided by the modeling study below. Methoxy groups in the 2- and 3-positions (**27**, **28**) improved the situation significantly compared to the hydroxyls. In the case of the 3-MeO analogue **28**, the reduction in lipophilicity compensated for the lower potency and gave the compound an LLE of 2.69 based on ClogP, compared to 2.86 for **3**. As methoxy substituents imply a risk of metabolic instability, the more stable trifluoromethoxy group was explored in the same positions (**29**, **30**). The activities of **29** and **30** paralleled those of methoxy analogues **27** and **28**, but the increased lipophilicity and decreased LE and LLE made the compounds less attractive. Continuing the screen for more polar substituents, the 2-acetyl (**31**) turned out equipotent with the unsubstituted **2**, but significantly less potent than **3**. Fishing for hydrogen-bond interactions further away from the scaffold,

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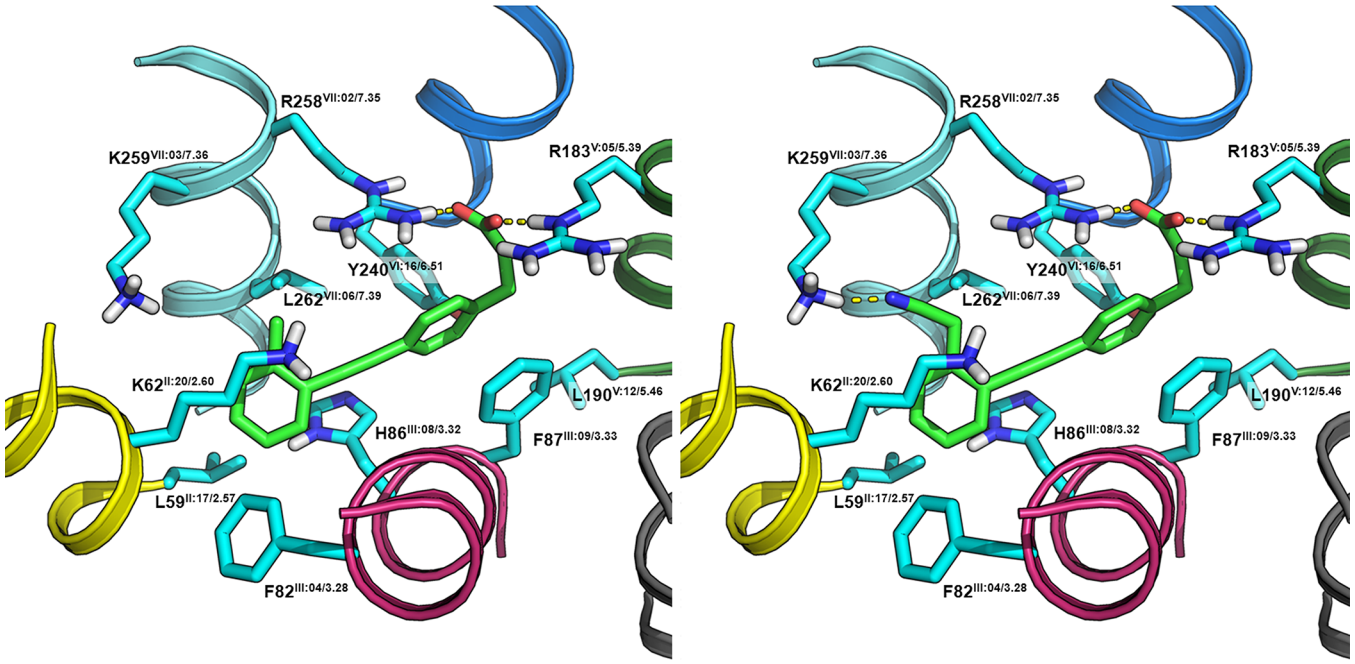

Figure 1. Complex of **3** (left) and **40** (right) with a homology model of the human FFA1. Residues situated close to the ligands are labeled with sequence number, and Schwartz–Baldwin[41] and Ballesteros–Weinstein[42] notations are given as superscripts.

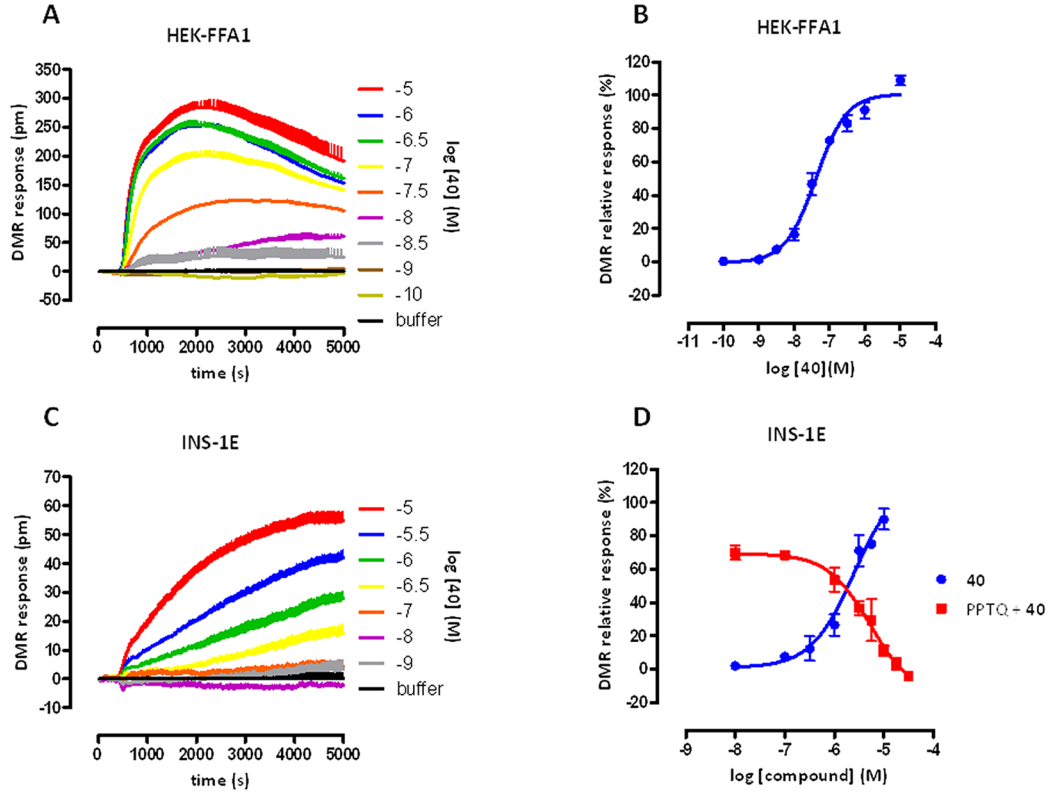

Figure 2. Activity of **40** on FFA1-transfected HEK-293 cells and in the rat β-cell line INS-1E that endogenously expresses FFA1. (A) Representative traces from the dynamic mass redistribution (DMR) assay of **40** on HEK-FFA1 cells. (B) Concentration–response curves of **40** in FFA1-HEK cells from the DMR assay (pEC_{50} = 7.4 ± 0.05, n = 3). (C) Traces from the DMR assay of **40** in INS-1E cells. (D) Concentration–response curve of **40** in INS-1E cells (pEC_{50} = 5.6 ± 0.16, n = 5) and inhibition of **40** (3 μM) by the FFA1 antagonist PPTQ (pIC_{50} = 5.3 ± 0.13, n = 3).

the 2-hydroxymethyl (**32**) and 2-hydroxyethyl (**33**) analogues were investigated, but these did not display sufficient potency. The methoxymethyl substituent in the 2- or 3-position (**34**, **35**), however, brought the activity up to the same level as **3** while lower lipophilicity was maintained, thus increasing LLE. Mesylalkoxy substituents, successfully applied on another compound series,[25] were investigated with various chain lengths (**36**, **37**). The 3-mesylbutoxy substituent (**37**) resulted in a

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small decrease in activity relative to **2** but more than an order of magnitude reduced lipophilicity, and thereby a significantly increased LLE relative to both **2** and **3**. The concept of introducing mesylalkoxy substituents to lower lipophilicity while potency was maintained thus seems to work well, although the potencies of these analogues were too low to place them among the preferred compounds.

The introduction of a polar cyano substituent on the terminal ring together with a methyl group (**38**, **39**) resulted in **39** being equipotent with **3** with somewhat reduced lipophilicity. Gratifyingly, combining the cyano and the methyl into a 2-cyanomethyl substituent (**40**) produced a significant increase in potency and a pronounced reduction in lipophilicity relative to **3**. The 3-cyanomethyl analogue (**41**) turned out an order of magnitude less potent. Thus, the substituent position is important, as for the hydroxyl analogues **25** and **26**, but the favored position is reversed. Extending the substituent to cyanomethoxy (**42**, **43**) led to reduced potency to the same degree in both the 2- and 3-postion together with further reduced lipophilicity. The preference for the 2-position is thus in agreement with the methyl (**3**, **4**) and cyanomethyl (**40**, **41**) analogues, but reversed in comparison to the other analogues where oxygen is attached directly to the phenyl ring (**25**−**29**). A combination of 2-cyanomethyl with the cyclopropyl constraint of the propionic acid chain (**44**) resulted in a drop in potency, as for the methyl substituents. Altogether, **40** remained the preferred compound, despite the slightly higher LLE of **42**.

A molecular modeling study was performed to explore the basis for the interesting effects observed with the different substituent positions. The complex of **3** and **40** with a homology model of hFFA1 was generated as described previously.[19] The carboxylic acid of the compounds interacts with the two arginine residues Arg183 and Arg258 and the diphenylacetylene part extends toward TM2 (Figure 1). The benzene rings are twisted with almost 90° between them, as also observed in the crystal structure of **3**,[13] which favors interaction between the central ring and Phe87 and places the methyl group in a small hydrophobic cavity created by Leu262. This hydrophobic cavity provides a rationale for the preference of the 2-methyl substituent (**3**) over the 3-methyl (**4**) and the unsubstituted compound (**2**), and for the highly disfavored interaction with the 2-hydroxy-substituted **25** compared to the 3-hydroxy analogue **26**. Likewise, the lower potency of the 2-cyano-5-methyl-substituted **38** compared to the 5-cyano-2-methyl-substituted **39** can be rationalized by the disfavored polar ortho-substituent. The 2-cyanomethyl moiety of **40** fit well into the hydrophobic cavity and, in addition, provides a hydrogen bond acceptor that is perfectly situated to form a hydrogen bond interaction with Lys259 (distance ∼ 2.0 Å) with Lys62 as an alternative interaction point, thus providing a rationale for the high potency of this compound.

The 2-cyanomethyl analogue **40** was the most potent agonist by good margin and also had lower lipophilicity and was thus chosen for further examination. The compound was evaluated using a dynamic mass redistribution (DMR) assay, which monitors real-time protein activity in the living cell without the need of labeling.[43] Potent activity in HEK293 cells transfected with hFFA1 was confirmed [Figures 2 and S1 (Supporting Information)]. Further testing of **40** with the insulin-secreting rat β-cell line INS-1E endogenously expressing FFA1 showed a concentration-dependent effect that was confirmed to be FFA1-mediated by treatment with the FFA1 antagonist *trans*-1-oxo-3-(4-phenoxyphenyl)-2-propyl-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (PPTQ).[44]

Counterscreens demonstrated >100-fold selectivity for FFA1 over FFA2 (GPR43, $pEC_{50} < 5$), FFA3 (GPR41, $pEC_{50} < 5$), GPR120 ($pEC_{50} = 5.32 \pm 0.03$), PPARγ ($pEC_{50} < 5$), and 55 other receptors, enzymes, and transporters (see the Supporting Information). Physicochemical and in vitro ADME properties of **40** were obtained for a full comparison with the lead compound **3** (Table 3). Both **3** and **40** have good solubility and demonstrated excellent chemical stability. The experimental lipophilicity of **40** as measured by log $D_{7.4}$ was reduced by an order of magnitude relative to **3**. Stability toward HLM was drastically improved, supporting that the 2-methyl group was involved in the insufficient stability observed with **3** and demonstrating that reduction of lipophilicity is an efficient means for mending unsatisfactory metabolic stability. No significant cell toxicity or inhibition of the most important CYP enzymes or P-glycoprotein (P-gp) was found. Both compounds exhibited high permeability in Caco-2 cells. The permeability of **40** was also examined on the mucus-secreting cell-line HT29-MTX,[45] an improved model system for the intestinal epithelium, and was found to be significantly higher [$P_{app} = (2.54 \pm 0.07) \times 10^{-5}$ cm/s, 78% recovered] than the readily absorbed drug ketoprofen [$P_{app} = (1.51 \pm 0.05) \times 10^{-5}$ cm/s, 80% recovered].

Table 3. Physicochemical and in Vitro ADME Properties of 3 and 40

assay	3	40
aqueous solubility (PBS, pH 7.4)[a]	174 μM	188 μM
chemical stab. (PBS, 37 °C, 12 days)	99.9%	99.8%
log D (*n*-octanol/PBS, pH 7.4)[b]	2.44 (2.34)	1.28 (1.32)
plasma protein binding (human)[c]	97.7%	>99.9%
metabolic stability (HLM)[a]	26%	81%
CYP inhibition (10 μM)[a]		
CYP1A2	−8%	−3%
CYP2C9	−25%	11%
CYP2C19	−1%	−2%
CYP2D6	0%	5%
CYP3A4	−4%	8%
P-gp inhibition (% @ 30/100 μM)[a]	6.6/23.3	−4.0/−1.8
Caco-2 (A to B, TC7, pH 6.5/7.4)[a]	87×10^{-6} cm/s	91×10^{-6} cm/s

[a]Determined at Cerep Inc. [b]Determined by the shake-flask method.[25] The values given in parentheses were determined at Cerep Inc. [c]Determined by equilibrium dialysis at Cerep Inc.

Pharmacokinetic investigations of **3** and **40** in mice revealed rapid and complete absorption of both compounds after oral dosing (Table 4). The half-life in mice is as expected rather short, but **40** showed a somewhat longer half-life than **3**. The exposure of **40** after oral dosing was 3.5-fold higher than for **3**. The relatively low volume of distribution for **40** can be rationalized by the high plasma protein binding and contributes to **40** having a half-life only moderately longer than **3** despite higher metabolic stability. A low clearance was confirmed with **40** compared to a quite high clearance for **3**, corresponding to approximately 15% and 60% of the hepatic blood flow in mice, respectively. Both compounds were well-tolerated by the mice in acute exposures up to 250 mg/kg po. A satisfactory overall pharmacokinetic profile was found for **40**.

The effects of **3** and **40** on glucose tolerance in normal mice after oral administration 30 min prior to glucose challenge were

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Table 4. Pharmacokinetic Profiles of 3 and 40 in Mice[a]

	3	40
Intravenous		
C_{max} (ng/mL)	2284	5071
t_{max} (min)	5	5
$t_{1/2}$ (min)	10	17
$AUC_{0-\infty}$ (μg/mL·min)	47	174
V_d (L/kg)	0.80	0.35
CL_{total} (mL/min/kg)	53	14
Oral		
C_{max} (ng/mL)	2591	7757
t_{max} (min)	30	30
$t_{1/2}$ (min)	48	50
$AUC_{0-\infty}$ (μg/mL·min)	205	732
F (%)	109	105

[a]Data are mean concentrations in mouse plasma (n = 3) following a single 2.5 mg/kg intravenous dose or 10 mg/kg oral dose.

studied. **3** showed a significant improvement in glucose tolerance at a 50 mg/kg dose (Figure 3), similar to the effect of the oral antihyperglycemic DPP-4 inhibitor sitagliptin (10 mg/kg), a compound on the market for treatment of T2D. **40** exhibited a dose-dependent response with a significant effect similar to that of sitagliptin at 10 mg/kg and a maximal effect reached at 50 mg/kg, sustained at 250 mg/kg (Figure 3). Compound **40** is confirmed to be a full agonist also of the murine FFA1, but with an order of magnitude lower potency (pEC_{50} = 6.40 on mFFA1) than on the human orthologue. The considerable difference in efficacy and to some degree potency between the two compounds in the glucose tolerance test can thus be ascribed mainly to the improved pharmacokinetic properties of **40**.

CONCLUSION

In the continued SAR exploration of the alkyne series of FFA1 agonists represented by **3**, we focused especially on lowering the lipophilicity of the compounds. Introduction of polar substituents on the terminal phenyl ring led to the discovery of **40**, a compound with significantly improved potency and reduced lipophilicity relative to **3** (EC_{50} = 20 vs 46 nM, log $D_{7.4}$ 1.3 vs 2.4). Compound **40** exhibited high selectivity over a panel of enzymes, receptors, and transporters, and in vitro ADME-tox studies indicated high absorption, good metabolic stability, and no inhibition of enzymes implicated in drug−drug interactions. Pharmacokinetic evaluation in mice indicated complete bioavailability and >3-fold higher exposure of **40** compared to **3**. The effect of **40** in a glucose tolerance test in mice indicated an effect comparable to sitagliptin at 10 mg/kg after oral administration and maximal effect reached at 50 mg/kg. Altogether, **40** appears as a promising candidate for more advanced animal studies and further development of improved therapeutics for T2D.

EXPERIMENTAL SECTION

All commercial starting materials and solvents were used without further purification, unless otherwise stated. THF was freshly distilled from sodium/benzophenone. Purification by flash chromatography was carried out using silica gel 60 (0.040−0.063 mm, Merck). TLC analysis was performed on silica gel 60 F_{254} plates. ^{1}H and ^{13}C NMR spectra were calibrated relative to TMS internal standard or residual solvent peak. High-resolution mass spectra (HRMS) were obtained on Thermo Finnigan TSQ 700 using electrospray ionization (ESI) or

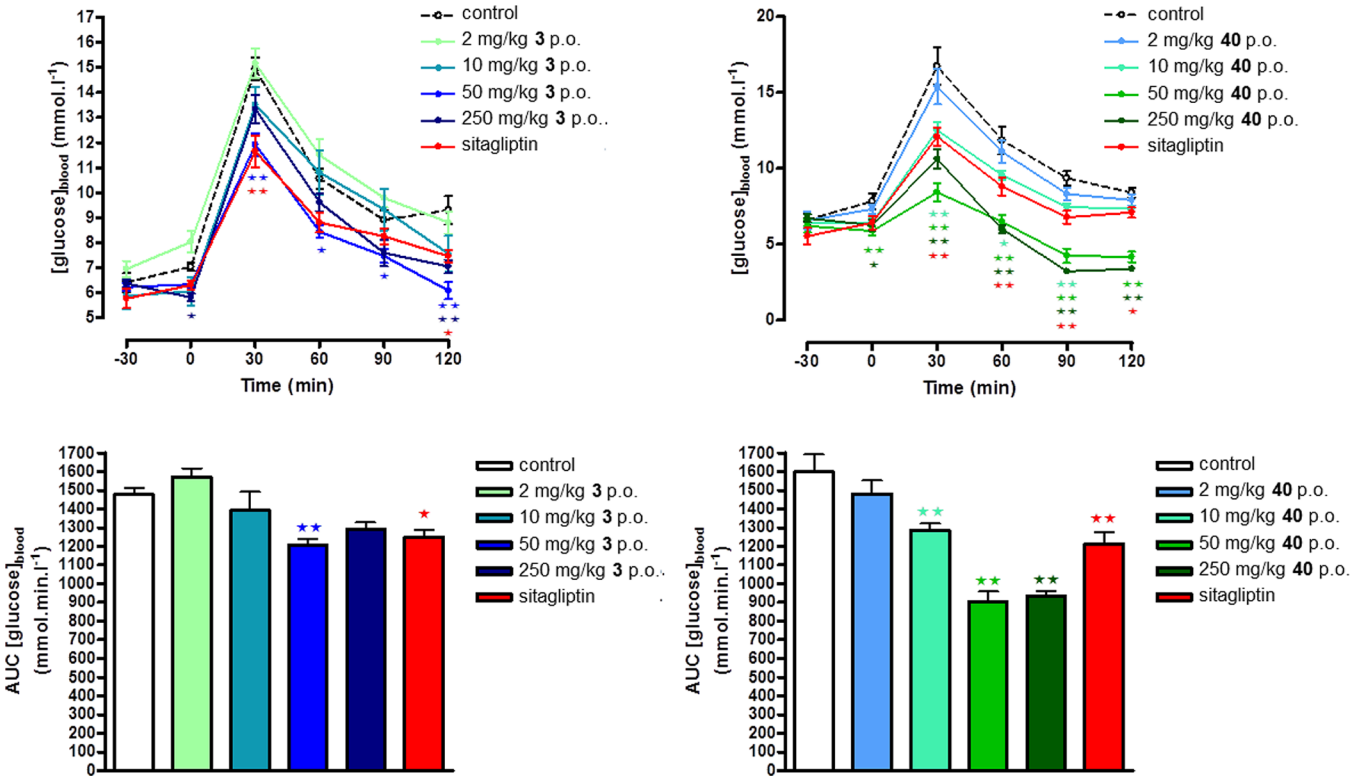

Figure 3. Effect of **3** and **40** on glucose tolerance in normal mice. Male C57Bl/6 mice were dosed po with test compound, vehicle, or positive control (sitagliptin, 10 mg/kg) 30 min prior to a 2 g/kg glucose challenge. Top panels show plasma glucose concentration curves as a function of time, and bottom panels show areas under the curves. Means ± standard errors (n = 6) are shown (*, $p < 0.05$; **, $p < 0.01$).

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Bruker micrOTOF-Q II (ESI). Purity was determined by HPLC and confirmed by inspection of NMR spectra. HPLC analysis was performed using a Dionex 120 C18 column (5 μm, 4.6 × 150 mm) with 10% acetonitrile in water (0−1 min), 10−100% acetonitrile in water (1−10 min), 100% acetonitrile (11−15 min), with both solvents containing 0.05% TFA as modifier; a flow of 1 mL/min; and UV detection at 230 and 254 nm. All test compounds were of ≥95% purity unless otherwise stated.

General Procedure I: Sonogashira Coupling. A Schlenk flask charged with Na_2PdCl_4 (1 mol %), 2-(di-*tert*-butylphosphino)-*N*-phenylindole (PIntB, 2 mol %), CuI (2 mol %), alkyne (1 equiv), aryl halide (1.1−1.5 equiv), H_2O (0.2 mL/mmol), and TMEDA (1.8 mL/mmol) was evacuated and back-filled with argon three times and then heated to 80 °C. After consumption of the alkyne, the reaction was cooled to room temperature, water was added, and the mixture was extracted with EtOAc (×3). The organic phases were combined, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by flash chromatography and dried under vacuum to give the desired product.

General Procedure II: Ester Hydrolysis. A solution of $LiOH·H_2O$ (2−3 equiv) in H_2O (~2 mL/mmol ester) was added to the ester dissolved in THF (~5 mL/mmol ester). The reaction was stirred at room temperature until complete consumption of the starting material as indicated by TLC, typically after 1−12 h. The reaction had water added, was acidified with 3% HCl until pH <1, and was extracted with EtOAc (×3). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated under vacuum.

*3-(4-((3-(Methoxymethyl)phenyl)ethynyl)phenyl)propanoic Acid (**35**). Step 1.* **35a** was prepared from **1**[34] (93 mg, 0.49 mmol) and 1-bromo-3-(methoxymethyl)benzene (99 mg, 0.49 mmol) according to the general procedure I to give 96 mg (64%) of a clear oily product after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:10): R_f = 0.22 (EtOAc:petroleum ether, 1:4); ^{1}H NMR ($CDCl_3$) δ 7.51 (s, 1H), 7.47−7.42 (m, 3H), 7.36−7.27 (m, 2H), 7.21−7.16 (m, 2H), 4.45 (s, 2H), 3.67 (s, 3H), 3.40 (s, 3H), 2.96 (t, J = 7.7 Hz, 2H), 2.64 (t, J = 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 173.1, 140.9, 138.5, 131.8, 130.8, 128.44, 128.36, 127.5, 123.5, 121.2, 89.4, 89.0, 74.2, 58.2, 51.7, 35.4, 30.8.

Step 2. **35** was prepared from **35a** (76 mg, 0.25 mmol) according to the general procedure II to give 61 mg (84%) of a white solid (t_R = 11.96, purity 98.7% by HPLC); ^{1}H NMR (acetone-d_6) δ 7.55−7.43 (m, 4H), 7.42−7.29 (m, 4H), 4.46 (s, 2H), 3.36 (s, 3H), 2.95 (t, J = 7.6 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H); ^{13}C NMR (acetone-d_6) δ 173.8, 142.9, 140.3, 132.4, 131.19, 131.15, 129.6, 129.4, 128.3, 124.1, 121.7, 90.0, 89.6, 74.3, 58.2, 35.5, 31.4; ESI-HRMS calcd for $C_{19}H_{18}O_3Na$ (M + Na^+) 317.1148, found 317.1159.

*3-(4-((5-Cyano-2-methylphenyl)ethynyl)phenyl)propanoic Acid (**39**). Step 1.* **39a** was prepared from **1**[34] (98 mg, 0.52 mmol) and 3-iodo-4-methylbenzonitrile (136 mg, 0.56 mmol) according to the general procedure I to give 104 mg (68%) of a clear oily product after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:10): R_f = 0.20 (EtOAc:petroleum ether, 1:5); ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J = 1.7 Hz, 1H), 7.49 (d, J = 1.7 Hz, 1H), 7.48−7.44 (m, 2H), 7.33 (d, J = 8.0 Hz, 1H), 7.24−7.18 (m, 2H), 3.68 (s, 3H), 2.98 (t, J = 7.7 Hz, 2H), 2.65 (t, J = 7.7 Hz, 2H), 2.56 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 173.0, 145.5, 141.7, 135.1, 131.8, 131.2, 130.3, 128.5, 124.8, 120.5, 118.4, 110.0, 95.6, 85.7, 51.7, 35.3, 30.9, 21.2; ESI-MS m/z 326.1 (M + Na^+).

Step 2. **39** was prepared from **39a** (91 mg, 0.30 mmol) according to the general procedure II to give 83 mg (96%) of a white solid (t_R = 12.08, purity: 99% by HPLC); ^{1}H NMR ($CDCl_3$) δ 11.20 (s, 1H), 7.75 (d, J = 1.7 Hz, 1H), 7.52−7.44 (m, 3H), 7.33 (d, J = 8.0 Hz, 1H), 7.23 (d, J = 8.3 Hz, 2H), 2.99 (t, J = 7.6 Hz, 2H), 2.71 (t, J = 7.7 Hz, 2H), 2.56 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 178.3, 145.5, 141.3, 135.1, 131.9, 131.2, 130.4, 128.5, 124.8, 120.6, 118.4, 110.0, 95.5, 85.8, 35.2, 30.5, 21.2; ESI-HRMS calcd for $C_{19}H_{15}NO_2Na$ (M + Na^+) 312.0996, found 312.0983.

*3-(4-((2-(Cyanomethyl)phenyl)ethynyl)phenyl)propanoic Acid (**40**). Step 1.* **40a** was prepared from **1**[34] (250 mg, 1.33 mmol) and 2-(2-iodophenyl)acetonitrile (354 mg, 1.45 mmol) according to the general procedure I to give 312 mg (77%) of a white solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:4): R_f = 0.08 (EtOAc:petroleum ether, 1:4); ^{1}H NMR ($CDCl_3$) δ 7.55−7.46 (m, 4H), 7.37−7.34 (m, 2H), 7.21 (d, J = 8.4 Hz, 2H), 3.96 (s, 2H), 3.67 (s, 3H), 2.97 (t, J = 7.7 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 173.0, 141.6, 132.3, 131.7, 131.6, 128.9, 128.5, 128.1, 122.9, 120.4, 117.4, 95.6, 85.7, 51.7, 35.3, 30.8, 22.7; ESI-MS m/z 326.1 (M + Na^+).

Step 2. **40** was prepared from **40a** (292 mg, 0.96 mmol) according to the general procedure II to give 216 mg (77%) of a white solid (t_R = 11.44, purity: 99.9% by HPLC) after purification by flash chromatography [SiO_2, EtOAc (with 1% AcOH):petroleum ether, 1:2]; ^{1}H NMR (DMSO-d_6) δ 11.87 (br s, OH), 7.31−7.21 (m, 4H), 7.16−7.12 (m, 2H), 7.03−7.00 (m, 2H), 3.87 (s, 2H), 2.57 (t, J = 7.5 Hz, 2H), 2.27 (t, J = 7.5 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 173.6, 142.3, 132.8, 132.0, 131.4, 129.3, 128.9, 128.7, 128.3, 122.2, 119.6, 118.4, 95.3, 85.9, 34.8, 30.3, 22.1; ESI-HRMS calcd for $C_{19}H_{15}NO_2Na$ (M + Na^+) 312.0996, found 312.1002.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures and compound characterization, evaluation of log *P* calculation methods, and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DMR, dynamic mass redistribution; DPP-4, dipeptidyl peptidase 4; FFA1, free fatty acid receptor 1 (GPR40); FFA2, free fatty acid receptor 2 (GPR43); FFA3, free fatty acid receptor 3 (GPR41); GSIS, glucose-stimulated insulin secretion; HLM, human liver microsomes; LLE, ligand lipophilicity efficiency; PIntB, 2-(di-*tert*-butylphosphino)-1-phenylindole; SPhos, 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl; T2D, type 2 diabetes.

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