# Structure−Activity Study of Dihydrocinnamic Acids and Discovery of the Potent FFA1 (GPR40) Agonist TUG-469

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ABSTRACT The free fatty acid 1 receptor (FFA1 or GPR40), which is highly expressed on pancreatic β-cells and amplifies glucose-stimulated insulin secretion, has emerged as an attractive target for the treatment of type 2 diabetes. Several FFA1 agonists containing the *para*-substituted dihydrocinnamic acid moiety are known. We here present a structure−activity relationship study of this compound family suggesting that the central methyleneoxy linker is preferable for the smaller compounds, whereas the central methyleneamine linker gives higher potency to the larger compounds. The study resulted in the discovery of the potent and selective full FFA1 agonist TUG-469 (**29**).

TUG-469 (**29**)
EC_{50} = 19 nM

KEYWORDS Diabetes, FFA1, FFAR1, GPR40, fatty acids, drug discovery
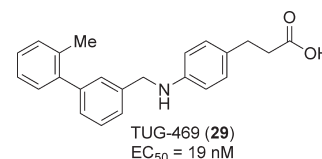
With an estimated 285 million cases worldwide and an expected 480 million cases by 2030, diabetes represents a threat to the economy and human well being, and the development of improved therapies is urgent.[1,2] Recently, the receptor GPR40, now renamed to FFA1,[3] was found to be activated by medium- and long-chain fatty acids, to be highly expressed on pancreatic β-cells, and to potentiate glucose-stimulated insulin secretion (GSIS).[4−6] FFA1 is also present in the endocrine cells of the gastrointestinal tract and has been reported to mediate free fatty acid (FFA) stimulated secretion of GLP-1 and GIP.[7] Thus, the receptor appears to act as an important nutrient sensor and metabolic regulator and to stimulate insulin secretion by direct action on the β-cells and possibly also indirectly via the incretin system. FFA1 is of considerable interest as a new target for the treatment of type 2 diabetes (T2D) since insulin secretion is promoted without the associated risk of hypoglycemia.[8,9]

FFAs have long been known to potentiate GSIS, but they are also linked to β-cell dysfunction,[10] and it is unclear at present whether FFA1 mediates one or both of these effects. Whereas some studies with FFA1-deficient mice indicate that both effects were mediated by the receptor and that FFA1 antagonists may therefore be of therapeutic interest for T2D,[11,12] other investigations confirmed the potentiation of GSIS but found no evidence for lipotoxic effects.[13,14] Tan and co-workers demonstrated that a selective FFA1 agonist (compd B, Figure 1) continued to promote GSIS with no indications of lipotoxicity after chronic administration, in contrast to fatty acids that resulted in impaired insulin secretion.[15] This outcome is also supported by several other recent studies.[16−18] Thus, although the issue is still controversial, most evidence currently supports FFA1 agonists as promising for the treatment of T2D.

Both saturated and unsaturated FFAs with more than 10 carbon atoms act as agonists on FFA1.[4−6,8,9] The ability of FFA1 to stimulate insulin secretion at elevated glucose levels attracted immediate attention, and several series of FFA1 agonists as well as some antagonists have since been described, many of these containing the *para*-substituted dihydrocinnamic acid or the corresponding thiazolidinedione moiety (Figure 1).[15,19−27] Herein, we describe structure−activity investigations around the lead compound **1** and the *para*-substituted dihydrocinnamic acids, which resulted in the identification of the potent agonist **29** (TUG-469).

It was discovered in 2003 that several thiazolidinedione PPARγ agonists, including the antidiabetic drug rosiglitazone (Figure 1), also possess FFA1 agonistic activity.[6] Presuming that thiazolidinediones and FFAs share a common binding site within FFA1, which has now been confirmed,[28] we included structural information from the thiazolidinedione agonists in the design of a small library of carboxylic acids to

Scheme 1[a]
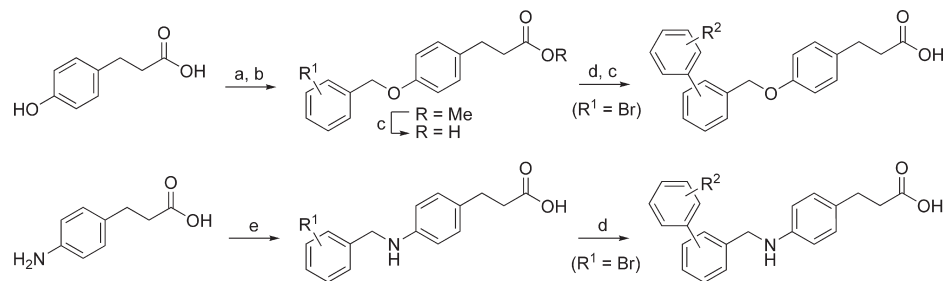
[a] Reagents and conditions: (a) MeOH, HCl (cat.). (b) Benzyl bromide, K_2CO_3, acetone. (c) LiOH, THF, H_2O. (d) ArB(OH)$_2$, Pd(OAc)$_2$, K_2CO_3, PEG-400, 45 °C, 2−24 h. (e) Benzaldehyde, NaBH(OAc)$_3$, CH_2Cl_2.
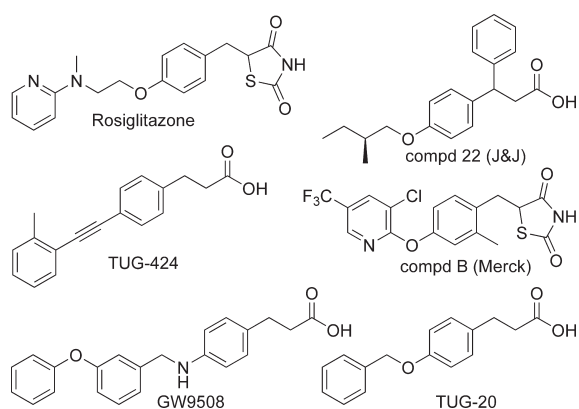
Figure 1. Thiazolidinedione and dihydrocinnamic acid FFA1 agonists.[6,15,19,22,23]

Scheme 2[a]

[a] Reagents and conditions: (a) Ag_2SO_4, I_2, CH_2Cl_2. (b) Phenylacetylene, Pd(PPh$_3$)$_4$, CuI, DIPEA, DMF, 65 °C. (c) NaOMe, THF, reflux.

Table 1. Exploration of the Central Linker and Conformational Constraints

compd	Y	X	R	hFFA1 (Ca^{2+}) pEC$_{50}$ (efficacy)[a]	LE $-\Delta g$[b]	PPARγ % at 100 μM[c]
1	CH$_2$	O	H	6.34 ± 0.02 (100)	0.46	7
2[d]	CH$_2$	NH	H	6.12 ± 0.03 (89)	0.42	7
3	O	CH$_2$	H	5.20 ± 0.03 (88)	0.38	NA
4	NH	CH$_2$	H	4.67 ± 0.01 (101)	0.34	NA
5	CH$_2$	CH$_2$	H	5.44 ± 0.04 (116)	0.39	
6	CH(Me)	O	H	5.48 ± 0.02 (106)	0.38	
7	CH$_2$	O	I	4.90 ± 0.02 (81)	0.34	42
8				5.61 ± 0.07 (92)	0.38	NA
9[e]	C≡C		H	6.70 ± 0.02 (108)	0.48	

[a] Efficacy is given as % response relative to 10 μM **1**. [b] LEs were calculated from the formula $-\Delta g = RT \ln(EC_{50})$, presuming that EC$_{50}$ approximates the dissociation constant. Values are given in kcal mol^{-1} per nonhydrogen atom.[29] [c] Activation relative to 1 μM rosiglitazone. [d] Previously reported (pEC$_{50}$ = 5.1 ± 0.4).[21] [e] Previously reported.[23]

mimic constrained fatty acids in the search for more potent and selective FFA1 agonists. The library, consisting of commercial and readily synthesized compounds, resulted in several active compounds including the two outstanding hits TUG-14, which was optimized to TUG-424 (Figure 1),[23] and TUG-20 (**1**), which served as the lead structure for the work presented here. The disclosure of GW9508 and related compounds by GSK directed our attention to the central linker unit, and we set out to investigate if the methyleneoxy or the methyleneamino linker was preferred. The initial hit **1** and analogues were synthesized as outlined in Scheme 1. Biaryl analogues were prepared by Suzuki coupling of the corresponding halobenzyloxy ester or the free halobenzylamino acid using a practical protocol with Pd(OAc)$_2$ and K_2CO_3 in PEG-400 in open air. Synthesis of the constrained benzofurane **8** is outlined in Scheme 2.

Replacing the central oxygen of **1** by a nitrogen (**2**) produced a compound with somewhat reduced activity in the calcium mobilization assay (Table 1). We were interested to probe if the −CH$_2$X− linker functioned only as a flexible spacer between the central and the terminal benzene rings; thus, compounds **3** and **4** with inverted linkers were synthesized and evaluated. Both compounds turned out more than an order of magnitude less potent than the corresponding **1** and **2**, indicating a key role of the linker. Replacing the central heteroatom by a methylene group (**5**) produces a pure hydrocarbon tail more closely resembling the endogenous

FFA ligands. Despite increased lipophilicity, the compound turned out to be significantly less potent than **1** and **2**, indicating that the methyleneoxy and the methyleneamino linkers

play specific roles in the recognition of the receptor binding site by the ligands. A methyl substituent on the methyleneoxy linker (**6**) resulted in significant loss of activity, as did an iodo substituent on the central ring vicinal to the benzyloxy (**7**).

The specific effect of the central linkers might be due to hydrogen bond interactions, conformational effects, or overall effects on the dipolar momentum. Conformational analysis revealed that the methyleneoxy (**1**) and in particular the methyleneamino (**2**) are preferentially oriented in the plane of the central benzene ring, whereas the inverted linkers (**3** and **4**) and the ethylene linker (**5**) are preferentially oriented orthogonally to the central benzene. The benzofurane derivative **8** (Scheme 2), designed as a constrained planar analogue of **1**, turned out almost an order of magnitude less active but still demonstrates that the receptor binding pocket can accommodate the conformation with the phenyl group extended in the plane of the central ring. Notably, replacing the flexible methyleneoxy or methyleneamino with an alkyne (**9**) results in a significant increase in potency up to pEC$_{50}$ = 6.70 and confirms that the binding pocket can accommodate the fully extended molecule.[23]

An initial screen of the terminal ring with bromo substituents (**10**−**13**) demonstrated that substitution is tolerated in all positions and preferred in the *meta*-position, whereas the polar 3-nitrile analogue (**14**) indicated that the *meta*-substituent should be nonpolar to increase potency (Table 2). The nonpolar 3-CF$_3$ indeed produced a significant improvement in analogues **15** and **16**.

GW9508 is central in the series and was included to enable direct comparison under the same conditions.[19−21] We also synthesized the potent thiazolidinedione FFA1 agonist disclosed by Merck (compd B) to directly compare our compounds to one with established efficacy in animal models.[15,25]

In contrast to compounds with smaller benzyl substituents for which the methyleneoxy analogues were more potent (**1/2**, **12/13**, and **16/15**), the methyleneoxy analogue of GW9508 (**17**), carrying a larger *meta*-phenoxy substituent, turned out less potent than GW9508. As expected, the corresponding *para*-phenoxy analogues **18** and **19** exhibited lower potency. All N/O pairs of the *ortho*-, *meta*-, and *para*-biphenyl (**20**−**25**) compounds were synthesized and tested, confirming the *meta*-position as the generally preferred and the methyleneamino linker as preferred for the larger aromatic substituents, which is advantageous as the methyleneamino linker produces less lipophilic compounds, on average with calculated log P 0.4 units lower than the corresponding methyleneoxy compounds. A 2-methyl substituent on the *meta*-phenyl (**28** and **29**) resulted in significant further increase of activity for both linkers, whereas the corresponding 4-methyl substituent (**26** and **27**) rather decreased activity. The 2-methoxy compounds **30** and **31** exhibit activities very close to the unsubstituted **22** and **23**, suggesting that the increased activity of **28** and **29** might be a consequence of increased lipophilicity and a close fit around the 2-methyl in the binding site.

Ligand efficiency (LE) reflects the contribution of each nonhydrogen atom to the activity of a compound and has become a widely accepted measure to avoid oversized molecules from the optimization process.[29] Although the LE dropped

Table 2. Substituted 4-Benzyloxy and 4-Benzylamino Dihydrocinnamic Acids
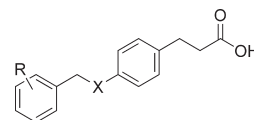
compd	R^2	X	hFFA1 (Ca^{2+}) pEC$_{50}$ (efficacy)^a	LE −Δg^b	PPARγ % at 100 μM^c
GW9508^d	3-OPh	N	7.54 ± 0.04 (103)	0.40	54
compd B^e			6.75 ± 0.04 (103)	0.34	
10	4-Br	O	6.26 ± 0.03 (98)	0.43	
11	2-Br	O	6.35 ± 0.04 (99)	0.43	
12	3-Br	O	7.05 ± 0.02 (99)	0.49	19
13	3-Br	N	6.76 ± 0.04 (110)	0.46	27
14	3-CN	O	6.28 ± 0.02 (102)	0.41	
15	3-CF$_3$	N	6.73 ± 0.05 (102)	0.40	
16	3-CF$_3$	O	7.08 ± 0.03 (103)	0.42	
17	3-OPh	O	7.24 ± 0.03 (98)	0.38	51
18	4-OPh	O	6.69 ± 0.03 (98)	0.35	23
19^f	4-OPh	N	6.74 ± 0.03 (100)	0.35	34
20^g	4-Ph	O	6.04 ± 0.05 (97)	0.33	NA
21^h	4-Ph	N	6.38 ± 0.02 (92)	0.34	23
22	3-Ph	O	6.87 ± 0.05 (105)	0.38	25
23	3-Ph	N	7.27 ± 0.02 (101)	0.40	33
24	2-Ph	O	5.72 ± 0.04 (97)	0.31	
25	2-Ph	N	5.97 ± 0.02 (97)	0.33	--^{i}
26	3-(4-MeC$_6$H$_4$)	O	6.31 ± 0.03 (86)	0.33	15
27	3-(4-MeC$_6$H$_4$)	N	6.90 ± 0.04 (92)	0.36	31
28	3-(2-MeC$_6$H$_4$)	O	7.30 ± 0.05 (112)	0.39	37
29	3-(2-MeC$_6$H$_4$)	N	7.73 ± 0.04 (114)	0.41	86
30	3-(2-MeOC$_6$H$_4$)	O	6.92 ± 0.04 (105)	0.35	37
31	3-(2-MeOC$_6$H$_4$)	N	7.16 ± 0.03 (109)	0.36	51

^{a} Efficacy is given as % response relative to 10 μM **1**. The maximal response of **1** was determined to 123% relative to linoleic acid. ^{b} LEs were calculated from the formula −Δg = RT ln(EC$_{50}$), presuming that EC$_{50}$ approximates the dissociation constant. Values are given in kcal mol$^{−1}$ per nonhydrogen atom.[29] ^{c} Activation relative to 1 μM rosiglitazone. ^{d} Reported previously (pEC$_{50}$ = 7.32 ± 0.03, 7.19 ± 0.54, and 7.1 ± 0.2).[19−21] ^{e} Reported previously (EC$_{50}$ = 88 and 10 nM).[15,25] ^{f} Reported previously (pEC$_{50}$ = 6.71).[21] ^{g} Tested with 0.05% BSA because of poor solubility. ^{h} Reported previously (pEC$_{50}$ = 6.6 ± 0.2).[21] ^{i} Toxic at 100 μM and 15% activation at 10 μM.

from 0.46 for **1** to 0.41 for **29** (Tables 1 and 2), maintenance of a value > 0.4 is satisfactory.

The lead compound **1**, GW9508, and the most potent methyleneoxy analogue **28** and methyleneamine analogue **29** were tested in a dynamic mass redistribution (DMR) assay to obtain integrated cellular response profiles of FFA1 transfected cells.[30] Dose−response curves from DMR assays (Figure 2) give pEC$_{50}$ values that correlate well with the calcium data (Table 2).

None of the compounds have significant activity on the related receptors FFA2 (GPR43) or FFA3 (GPR41). Because the antidiabetic target PPARγ, like FFA1, is activated by fatty
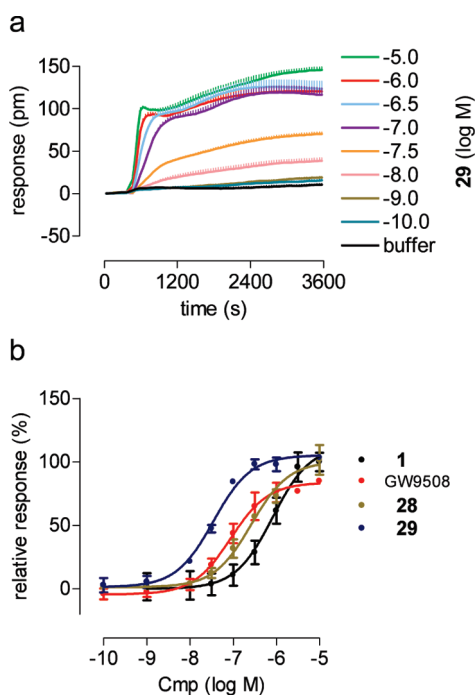
Figure 2. DMR analysis of FFA1 agonists. (a) hFFA1 transfected 1321N1 cells were challenged with the indicated concentrations of **29** ($n > 2$), and the wavelength shift (pm) was monitored over time as a measure of receptor activation. (b) Dose–response curves: GW9508 $pEC_{50} = 7.06 \pm 0.11$ (red line); **1** $pEC_{50} = 6.08 \pm 0.15$ (black line); **28** $pEC_{50} = 6.58 \pm 0.09$ (gold line); and **29** $pEC_{50} = 7.46 \pm 0.06$ (blue line).

Figure 3. Effect of **29** on insulin secretion from the pancreatic rat INS-1E β-cells at basal and stimulatory glucose concentrations. Shown are mean values $\pm$ SEMs ($n = 3$). The asterisk indicates significance to 12 mM glucose ($p < 0.001$).

selectivity over relevant receptors, and the confirmed ability to increase GSIS in a pancreatic β-cell line indicate that **29** has potential for further development and will be a useful tool in future studies of FFA1.

SUPPORTING INFORMATION AVAILABLE Synthetic procedures and compound characterization, procedures for calcium assay, DMR assay, PPARγ assay, and insulin secretion assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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acids and thiazolidinediones, it is reasonable to expect that FFA1 ligands may also possess PPARγ activity. Most of the compounds turned out to exhibit PPARγ agonist activity at 100 μM (Tables 1 and 2), but none show significant activity at 1 μM, and all compounds are > 100-fold selective for FFA1.

Being carboxylic acids and related to FFAs, it is natural to expect that the compounds might bind strongly to serum albumin. The activities of **29** and **28** as FFA1 agonists were, however, only moderately affected by increasing amounts of BSA, with pEC_{50} values of 7.83 ± 0.04 and 7.09 ± 0.04, respectively, in the presence of 0.05% BSA and 7.35 ± 0.03 and 6.25 ± 0.03 with 0.5% BSA. The effect of **29** on insulin secretion was examined in the rat β-cell line INS-1E. Glucose (12 mM) stimulated insulin secretion 2.7-fold, and **29** was competent to amplify GSIS by 55% but did not affect insulin secretion at low glucose concentrations (Figure 3).

In conclusion, structure–activity relationships of *para*-substituted dihydrocinnamic acid FFA1 agonists have been explored with focus on the central linker and terminal aromatic ring. The results indicate that the central methyleneoxy linker is preferred for compounds with small substituents on the terminal phenyl, whereas the methyleneamino linker appears to be preferred for compounds with larger aromatic substituents. The effort converted the initial lead compound **1** into the potent and selective agonist **29**, a compound closely related to GW9508, while maintaining LE > 0.4 kcal/mol per nonhydrogen atom. A high activity in the label-free DMR assay, satisfactory tolerance to BSA, high
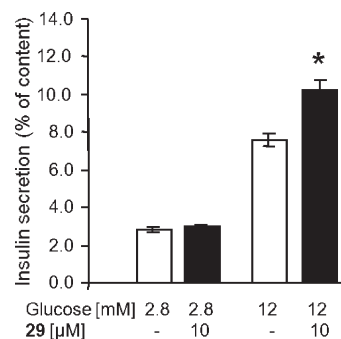
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