Discovery of Potent and Selective Agonists for the Free Fatty Acid Receptor 1 (FFA_1/GPR40), a Potential Target for the Treatment of Type II Diabetes

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Abstract: A series of 4-phenethynyldihydrocinnamic acid agonists of the free fatty acid receptor 1 (FFA_1) has been discovered and explored. The preferred compound **20** (TUG-424, $EC_{50} = 32$ nM) significantly increased glucose-stimulated insulin secretion at 100 nM and may serve to explore the role of FFA_1 in metabolic diseases such as diabetes or obesity.

Insulin is secreted from pancreatic β-cells in response to elevated plasma glucose concentrations and restores the optimal level by eliciting glucose uptake into cells and storage as glycogen. Dysfunction in this mechanism leads to abnormal plasma glucose levels, which is the hallmark of diabetes, a disease currently afflicting 6% of the adult world population and with increasing prevalence.[1] Type II diabetes, accounting for 85−95% of cases, is linked to obesity and is characterized by improper insulin secretion and insulin resistance, often culminating in the metabolic syndrome, a cluster of diseases including diabetes, obesity, and hyperlipidaemia. Nutrients, like fatty acids, have long been known for their capacity to amplify insulin secretion, although the underlying mechanism has been unclear. Importantly, amplification of GSIS[a] by fatty acids is operative in situations of β-cell compensation for insulin resistance.[2]

The seven-transmembrane receptor FFA_1 (GPR40/FFAR1)[3] is highly expressed in pancreatic β-cells and is activated by physiological concentrations of free fatty acids.[4−6] Activation of FFA_1 enhances GSIS but does not affect insulin secretion at low glucose concentrations.[6−9] Enhancement of GSIS by FFA_1 has been confirmed in vivo,[10−13] and one study also observed sustained enhancement of GSIS after prolonged exposure to FFA_1 agonists.[12] Furthermore, two single nucleotide polymorphisms of FFA_1 significantly correlating to obesity and impaired insulin secretion further validate the link between the receptor and the disease,[14,15] although another study failed to establish a disease link for the receptor.[16]

The implication of FFA_1 in insulin secretion has attracted considerable attention to the receptor as a new potential therapeutic target for type II diabetes. Interestingly, it is still unclear if agonists or antagonists are the desired therapeutic principle. Although stimulation of FFA_1 may acutely promote GSIS, extended exposure of FFA_1 to fatty acids might mediate lipotoxicity and β-cell dysfunction. Steneberg and co-workers found support for the latter view in favor of antagonists from studies with FFA_1 deficient mice;[10] however, more recent studies have challenged these results in favor of agonist therapy.[12,13,17] Agonists and antagonists of FFA_1 are therefore required to resolve this controversy and to further validate FFA_1 as an antidiabetic target.

Drug discovery efforts have already resulted in published FFA_1 ligands. Recently, a series of agonists derived from 4-(benzylamino)dihydrocinnamic acid was described by GlaxoSmithKline,[18,19] of which compound GW9508 (4-(3-phenoxybenzylamino)phenylpropionic acid, $pEC_{50} = 7.32$) appears to be the preferred one, even though it lacked activity in primary rat or mouse islets and hence is of limited value for in vivo evaluation of the function of FFA_1.[9] Other FFA_1 agonists and a few antagonists have also been disclosed, most of which only exhibit potencies in the micromolar range.[5,12,20,21]

Most saturated and unsaturated fatty acids of 10 or more carbon atoms exhibit some degree of agonist activity on FFA_1.[4−6] The receptor thus appears to tolerantly recognize relatively large, elongated lipophilic carboxylic acids. With this in mind, conformationally constrained carboxylic acids more suitable for optimization were screened in a calcium fluorescence assay[22] using 1321N1 cells stably expressing the human FFA_1. This resulted in identification of the alkyne **1** (Table 1) as a full agonist 10-fold more potent than oleic acid. The compound and analogues were synthesized via ethyl 4-iodophenoxyacetate using the Sonogashira coupling reaction to assemble the aromatic rings around the alkyne (Scheme 1). Removing the terminal phenyl ring (**2**), moving the phenethynyl to the 3-position (**3**), or replacing the central triple bond by a double bond to give stilbene-4-oxyacetic acid (**4**) led to complete loss of activity, clearly demonstrating that **1** is in possession of specific receptor recognizing properties rather than just emulating the general structure of a fatty acid (Chart 1).

To investigate the influence of substituents on the terminal phenyl ring, analogues with methyl groups introduced in each position were synthesized (Scheme 1). Whereas a methyl substituent in the ortho position (**5**) led to a moderate loss of activity and a methyl in the para position (**6**) gave a dramatic loss of activity, the introduction of a methyl in the meta position (**7**) resulted in 2-fold increase in agonistic activity. The introduction of polar substituents on the terminal phenyl ring in all cases yielded compounds with deteriorated activity; however, the meta substitution (**8**) was again favored over para substitutions (**9**, **10**).

Propionate analogues were synthesized from dihydrocinnamic acid by iodination and coupling to the arylethynyl moiety directly or via introduction of the ethyne followed by coupling

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[a] Abbreviations: $FFA_{1/2/3}$, free fatty acid receptor 1/2/3; GSIS, glucose-stimulated insulin secretion; DMR, dynamic mass redistribution.

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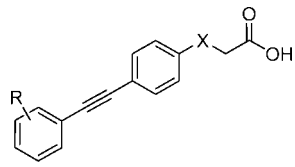
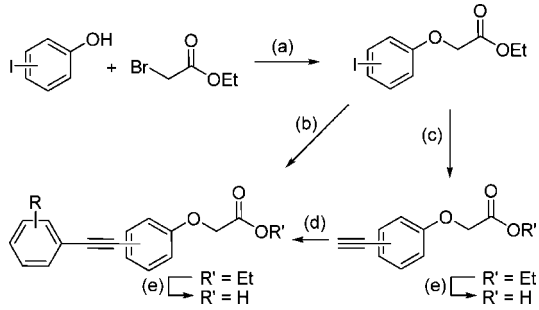
Table 1. Activity of Alkyne Carboxylic Acids at $hFFA_1$



compd	X	R	pEC_{50} (% max response)[a]
1	O	H	5.90 ± 0.04 (104)
5	O	2-Me	5.43 ± 0.03 (96)
6	O	4-Me	na[b]
7	O	3-Me	6.29 ± 0.03 (113)
8	O	3-OH	5.25 ± 0.03 (86)
9	O	4-OH	na[b]
10	O	4-CH_2OH	na[b]
11	CH_2	H	7.02 ± 0.02 (106)
12	CH_2	3-NH_2	5.96 ± 0.03 (96)
13	CH_2	3-CH_2OH	6.05 ± 0.03 (103)
14	CH_2	3-CHO	6.45 ± 0.03 (94)
15	CH_2	3-NO_2	7.23 ± 0.02 (94)
16	CH_2	3-CN	7.04 ± 0.03 (92)
17	CH_2	3-CCH	6.81 ± 0.05 (100)
18	CH_2	3-CF_3	7.05 ± 0.05 (98)
19	CH_2	3-Me	7.36 ± 0.13 (109)
20	CH_2	2-Me	7.49 ± 0.05 (105)
21	CH_2	4-Me	6.97 ± 0.03 (99)
22	CH_2	2,3-dimethyl	7.06 ± 0.03 (108)
23	CH_2	2,3-CH=CHCH=CH	7.07 ± 0.03 (100)
24	CH_2	3,5-dimethyl	7.13 ± 0.04 (101)

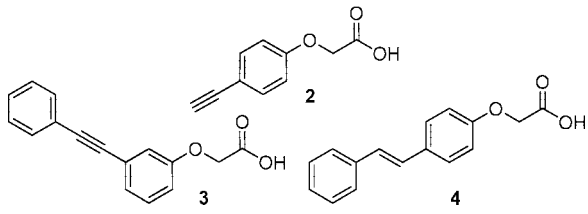
[a] Maximal response was determined relative to the full FFA_1 agonist 3-(4-(benzyloxy)phenyl)propanoic acid (TUG-20).[23] [b] na: no activity up to 31.6 μM.

Scheme 1[a]



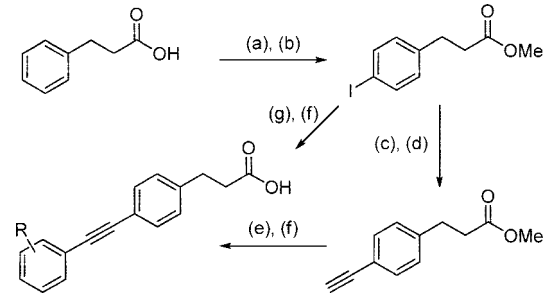
[a] Reagents and conditions: (a) K_2CO_3, acetone, room temp, 12−96 h; (b) PhCCH, $Pd(PPh_3)_4$, CuI, DIPEA, DMF, room temp, 2 h; (c) (i) Me_3SiCCH, $Pd(PPh_3)_4$, CuI, DIPEA, DMF, room temp, 2 h; (ii) TBAF, THF, room temp, 1 h; (d) aryl halide, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 50 °C, 12 h; (e) LiOH, H_2O, THF, room temp, 12 h.

Chart 1. Structures Devoid of FFA_1 Activity at 10 μM



to aryl halides (Scheme 2). The unsubstituted 4-phenethynylcinnamic acid **11** exhibited a 14-fold increase in potency to give a full agonist with an EC_{50} of 96 nM. Assuming structure−activity relationships analogous to those of the phenoxyacetic acid compounds, the initial focus was directed toward the meta position of the terminal benzene ring. Introduction of polar groups in general led to significant loss of potency (**12**−**14**), whereas the more lipophilic 3-nitro analogue **15** was twice as

Scheme 2[a]



[a] Reagents and conditions: (a) I_2, KIO_3, H_2SO_4, AcOH, H_2O, reflux, 5 h; (b) MeOH, HCl (cat.), room temp, 2 h (33% over two steps after recrystalization); (c) Me_3SiCCH, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 90 °C, 12 h; (d) K_2CO_3, MeOH, room temp, $2^1/_2$ h (90% over two steps); (e) aryl halide, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 50 °C, 12 h; (f) LiOH, H_2O, dioxane, room temp, 12 h; (g) ArCCH, CuI, Et_3N, DMF, 50 °C, 12 h.

potent as the unsubstituted **11**. Interestingly, the 3-nitrile substituent (**16**) was tolerated with no change in potency, whereas the more lipophilic 3-ethyne analogue **17** was somewhat less potent. Likewise, the hydrophobic and electron withdrawing 3-trifluoromethyl substituent **18** displayed conserved potency relative to **11**, whereas the electron donating 3-methyl substituent **19** resulted in doubled potency. After exclusion of the sterically slightly more demanding **17** and **18**, an almost perfect correlation between potency and lipophilicity of the meta substituted compounds was observed.[24]

Moving the methyl to the ortho position (**20**) surprisingly resulted in even higher potency (EC_{50} = 32 nM), indicating that structure−activity relationships are not directly transferable from the phenoxyacetates to the dihydrocinnamates. The 4-methyl substituent led to a compound (**21**) equipotent with **11**, compared with abolishment of activity for the corresponding phenoxyacetate **6**. Although the 2- and 3-methyl substituents separately resulted in substantially increased potency, combining both substituents in the same molecule (**22**) led to a compound only equipotent with **11**. The same was true for the sterically similar 2-naphthyl compound (**23**), whereas the 3,5-dimethyl modification (**24**) exhibited a modestly increased potency. Altogether, the dihydrocinnamates appeared not only generally more potent than the phenoxyacetates but also more tolerant to diverse substituents on the terminal benzene ring. None of the compounds exhibited any activity on the related receptors FFA_2 (GPR43) and FFA_3 (GPR41) or on nontransfected 1321N1 cells (data not shown).

Compound **20** was studied in a dynamic mass redistribution (DMR) assay. Notably, this technology is capable of visualizing all major G protein pathways in a single assay platform in real time, yielding signaling pathway-specific optical signatures.[25,26] In agreement with its reported coupling profile, the positive DMR signals are well compatible with G_i- and G_q-signaling pathway activation by FFA_1.[4,6] Biosensor recordings show a clear concentration−response relationship with a significant response occurring already at 1 nM (Figure 1). Treatment of nontransfected cells with 10 μM **20** produced no response, demonstrating that no signaling pathways or endogenously expressed receptors are affected by the compound.

The insulinotropic effect of **20** was tested in the rat insulin secreting cell line INS-1E.[8,27] Increasing concentrations (100 nM to 10 μM) of **20** enhanced glucose-stimulated insulin secretion significantly already at 100 nM and with a maximal effect at 3 μM (Figure 2, top). The approximately 2-fold stimulation of secretion by **20** in the presence of 12 mM glucose is comparable to that induced by palmitate (400 μM in 0.4%

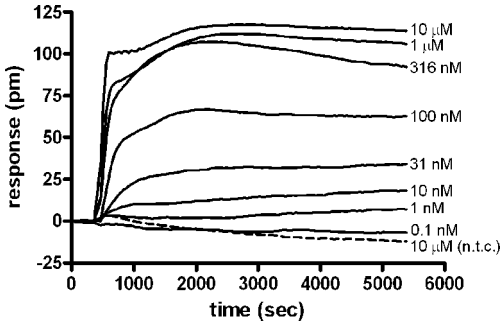


Figure 1. Sensograms of **20** from DMR assay. A pEC_{50} of 7.04 ± 0.07 was derived from the optical recordings. ntc denotes nontransfected cells.

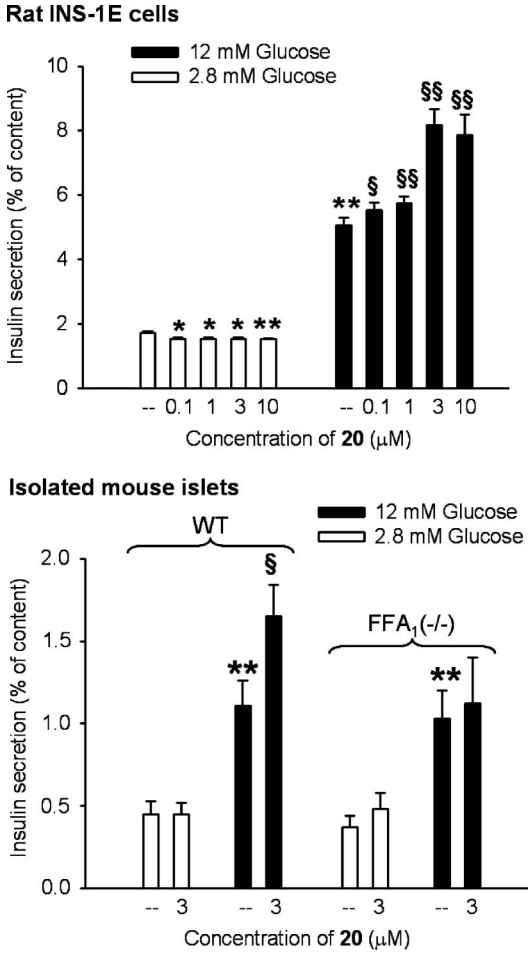


Figure 2. Activity of **20** in glucose-stimulated insulin secretion from rat INS-1E cells (top) and islets isolated from wild-type or $FFA_1(-/-)$ mice (bottom). Shown are mean values ± SEM of at least three independent experiments: (∗, ∗∗) significant effect to 2.8 mM glucose; (§, §§) significant effect to 12 mM glucose; (∗, §) $p < 0.05$; (∗∗, §§) $p < 0.005$.

BSA) in the presence of the same glucose concentration (data not shown). Interestingly, basal insulin secretion at 2.8 mM glucose was slightly but significantly reduced by **20**. Compound **20** was furthermore evaluated on islets isolated from wild-type mice and exhibited significant enhancement of GSIS here also, whereas lack of effect in islets isolated from their FFA_1-deleted litter mates demonstrates that the effect is mediated by FFA_1 (Figure 2, bottom).

The compounds described herein share the 4-substituted dihydrocinnamic acid fragment with the FFA_1 agonist series

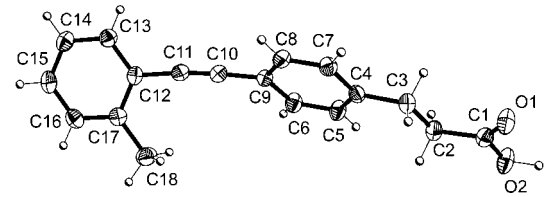


Figure 3. Crystal structure of **20**.

represented by GW9508,[9,18] and overlapping receptor interactions at least for this part of the compound series may be hypothesized. Using computational modeling and site-directed mutagenesis, Tikhonova and co-workers suggested a model of the interactions of GW9508 with FFA_1.[28] It is, however, not possible to fit more rigid diphenylethynes described here in this receptor model, which requires a folded conformation of GW9508. As the binding mode of GW9508 has been validated by site-directed mutagenesis, it appears natural to hypothesize a distinct binding mode for **20**, although it still seems reasonable to presume a similar interaction for the carboxylic acid. The crystal structure of **20** was obtained and shows a 50° twist between planes of the two aromatic rings and the propionic acid extended in the plane of the central ring (Figure 3). Other conformations accessible by rotating around the alkyne and the three rotatable bonds of the propionic acid may be as likely to represent the receptor bound conformation as the one of the crystal structure.

In conclusion, we have identified a new series of 4-phenethynyldihydrocinnamic acid agonists of FFA_1 with no activity on related receptors. The compound series exhibited high tolerance to substituents around the terminal phenyl ring. Introduction of a 2-methyl substituent on this ring produced the full agonist **20**, which exhibited an EC_{50} of 32 nM. The compound enhanced glucose-stimulated insulin secretion in a rat β-cell line already at 100 nM and from isolated mouse islets through FFA_1 and thus compares favorably to FFA_1 agonists described hitherto. This compound is expected to be useful in the further exploration of FFA_1 and may also be valuable as a lead structure for new potential antidiabetic therapeutics.

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Supporting Information Available: Synthetic procedures and compound characterization data, purity data, procedures for calcium assay, sensogram measurements, islet isolation, and insulin secretion assays. This material is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data for **20** have been deposited at the Cambridge Crystallographic Data Centre (CCDC 698298).

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