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Discovery of TUG-770: A Highly Potent Free Fatty Acid Receptor 1 (FFA1/GPR40) Agonist for Treatment of Type 2 Diabetes

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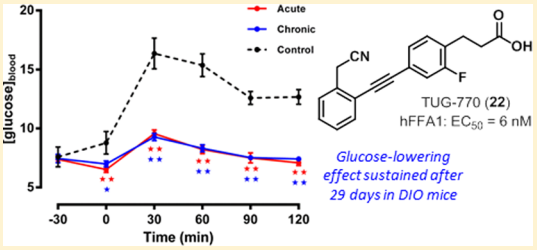
Supporting Information

ABSTRACT: Free fatty acid receptor 1 (FFA1 or GPR40) enhances glucose-stimulated insulin secretion from pancreatic β-cells and currently attracts high interest as a new target for the treatment of type 2 diabetes. We here report the discovery of a highly potent FFA1 agonist with favorable physicochemical and pharmacokinetic properties. The compound efficiently normalizes glucose tolerance in diet-induced obese mice, an effect that is fully sustained after 29 days of chronic dosing.



KEYWORDS: *Type 2 diabetes, free fatty acid receptor, TUG-770, insulin secretagogue, FFA1 agonist, GPR40 agonist*

The free fatty acid receptor 1 (FFA1, previously known as GPR40) has, since its deorphanization in 2003, received considerable attention as a new potential target for treatment of type 2 diabetes (T2D).[1−3] Activation of FFA1 increases glucose-stimulated insulin secretion but does not affect insulin secretion at low glucose levels, providing a potentially safe and efficient strategy for enhancing insulin levels in patients suffering from T2D. Accordingly, the interest in FFA1 as a new drug target has been high, and several potent agonists for the receptor have been disclosed.[4−6] Of these, TAK-875 is most advanced with highly encouraging results from phase II clinical trials.[7] Being a fatty acid receptor, FFA1 has a natural preference for relatively lipophilic compounds. This property has been reflected in the majority of the reported synthetic agonists, which mostly have been at the high end of the generally recommended lipophilicity range. We have previously reported a series of alkyne FFA1 agonists[8] and have subsequently directed our efforts toward lowering the lipophilicity of these compounds.[9,10] Herein, we report the further optimization of this compound series, leading to a highly potent FFA1 agonist with excellent physicochemical and pharmacokinetic properties and sustained glucose lowering capability in diet-induced obese (DIO) mice after acute and chronic dosing.

The alkyne ligands with either pyridine or fluoro-substituted benzene as the central ring were synthesized from the corresponding 4-bromoaldehydes (Scheme 1). Initially, a Wittig reaction with the phosphonium ylide, formed in situ from ethyl bromoacetate and triphenylphosphine, provided the corresponding cinnamic esters. The double bond was reduced by $NaBH_4$ in the presence of catalytic $CoCl_2$.[11] Subsequently, Sonogashira coupling with phenylacetylene followed by a base promoted hydrolysis provided the alkyne ligands.[12]

The 2-fluoro substituted ligands were synthesized from the central intermediate **2**, prepared from aryl bromide **1** by an initial Sonogashira coupling with trimethylsilylacetylene and subsequent removal of the TMS-group (Scheme 2). A second Sonogashira coupling of **2** with various aryl halides followed by ester hydrolysis gave the alkyne ligands in moderate to high yields.

We set out to investigate modifications in the central ring of the alkyne ligands (Table 1). Compounds were tested on the human FFA1 in a calcium mobilization assay and counterscreened on the human FFA4 (previously GPR120)[13] because of the selectivity issues frequently observed for these receptors.[14] The central benzene ring was replaced by pyridine due to its marked lipophilicity lowering effect. The 2-pyridyl

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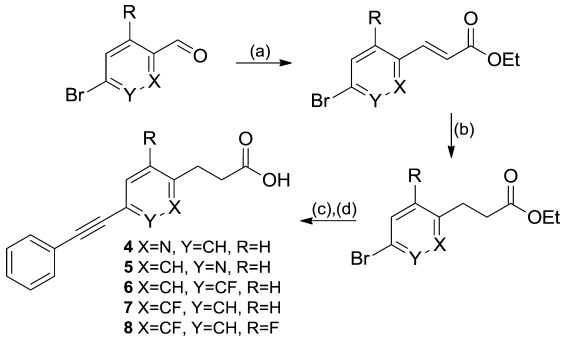
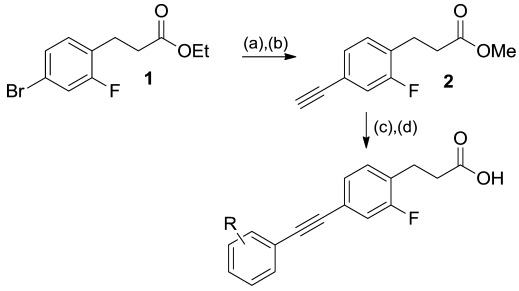
Scheme 1[a]



[a]Reagents and conditions: (a) ethyl bromoacetate, PPh_3, $NaHCO_3$, water, EtOAc, room temp, 18 h, 87−96%; (b) $CoCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH, 0 °C → room temp, 3 h, 59−87%; (c) PhCCH, Na_2PdCl_4, 2-(di-*tert*-butylphosphino)-1-phenylindole (PIntB), CuI, TMEDA, water, 70 → 80 °C, 0.5−4.5 h, 56−86%; (d) LiOH, THF, water, room temp, 12 h, 79−97%.

Scheme 2[a]

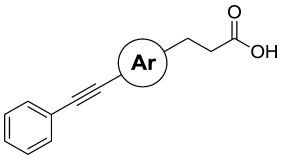


[a]Reagents and conditions: (a) trimethylsilylacetylene, Na_2PdCl_4, PIntB, CuI, TMEDA, water, 70 → 80 °C, 10 min; (b) K_2CO_3, MeOH, room temp, 2 h, 74% over two steps; (c) aryl halide, Na_2PdCl_4, PIntB, CuI, TMEDA, water, 80 °C, 1−4 h, 52−70%; (d) LiOH, THF, water, room temp, 12 h, 69−100%.

(**4**) and 3-pyridyl (**5**) analogues turned out to be twice as potent as previously reported ligands with pyridines as the terminal ring[9] but, nevertheless, resulted in >20-fold decrease in potency compared to **3**. Aromatic fluoro-substituents often result in higher metabolic stability and have been applied with success in the corresponding ring of other compound series.[15,16] Thus, we selected three mono- and difluoro-substituted analogues for synthesis and testing. The 3-fluoro analogue (**6**) showed maintained potency and only a small increase in ClogP compared to **3**. The 2-fluoro analogue (**7**) resulted in a 5-fold increased potency and the highest ligand efficiency (LE)[17] and ligand lipophilicity efficiency (LLE)[18] values and, moreover, the highest selectivity over FFA4 (>200-fold). Introduction of a second *ortho*-fluoro substituent (**8**) led to a reduction of potency back to the level of **6** and **3**.

With **7** showing high potency and LE, we decided to focus on the 2-fluoro scaffold in the exploration of the terminal ring in analogy with our previous studies (Table 2). Introduction of a corresponding 2-fluoro substituent in the lead structure TUG-424 (**9**) to give **10** resulted in increased potency but less so than for the terminally unsubstituted pair **3** and **7** (ΔpEC_{50} = 0.14 vs 0.78). Moving the methyl of the terminal ring to the *meta*-position (**11**) gave a further increase in potency. The order of potency is thus reversed relative to the analogues lacking the 2-fluoro substituent,[8] implying that previous SAR information is not directly transferrable to the 2-fluoro series.

Table 1. SAR Investigations of the Central Ring



	Ar	hFFA1[a] pEC$_{50}$ (efficacy, %)	hFFA4[b] pEC$_{50}$ (efficacy, %)	ClogP[c]	LE / LLE[d]
3		6.70 ± 0.03 (106)	5.07 ± 0.08 (91)	4.54	0.48 2.16
4		5.67 ± 0.03 (92)	n.a.	3.04	0.41 2.63
5		5.60 ± 0.03 (99)	4.04 ± 0.03 (41)	3.04	0.40 2.56
6		6.84 ± 0.02 (100)	5.24 ± 0.03 (117)	4.68	0.47 2.16
7		7.48 ± 0.05 (100)	5.10 ± 0.01 (107)	4.68	0.51 2.80
8		6.85 ± 0.02 (108)	5.08 ± 0.02 (112)	4.83	0.45 2.02

[a]Efficacy is given as % response relative to 10 μM TUG-20.[19] [b]Efficacy is given as % response relative to **9**; n.a. = no activity (pEC_{50} < 4).[14] [c]Calculated by BioByte's algorithm as implemented in ChemBioDraw Ultra 12.0 (ClogP option). [d]LE = RTln K_D, presuming that $EC_{50} \approx K_D$. Values are given in kcal mol^{-1} per non-hydrogen atom.[17] LLE = pEC_{50} − ClogP.[18]

Introduction of a cyano-substituent on **10** to give the 2-methyl-5-cyano analogue (**12**) resulted in reduced ClogP together with doubled potency and increased selectivity over FFA4. The difluoromethyl analogue (**13**) was found to be more potent than **10** but only equipotent with **12**, despite its higher lipophilicity. The 3,5-dichloro analogue (**14**) was synthesized to mimic the previously published chloro-substituted pyridine alkyne TUG-499[9] but turned out only equipotent with TUG-499, despite its high lipophilicity.

Extension of the *ortho*- and *meta*-methyl with the hydrophilic mesyl group was explored (**15** and **16**) and resulted in significantly reduced ClogP values and improved LLE but unfortunately also markedly reduced potency. Methoxymethyl substituents on the terminal ring have previously shown good potency and significantly reduced lipophilicity in the alkyne series.[10] When adding larger substituents on the terminal ring of the alkyne ligands, the *meta*-substituted compounds (**18** and **20**) were found to be favored over the *ortho* analogues (**17** and **19**). Although all four analogues exhibited high selectivity over FFA4, the potency was found to be rather low (EC_{50} = 0.3−0.7 μM).

We then directed our attention to the cyanomethyl alkyne TUG-488 (**21**).[10] The corresponding 2-fluoro analogue **22** (TUG-770) showed a pronounced increase in potency on FFA1 (ΔpEC_{50} = 0.51) with EC_{50} = 6 nM and 150-fold selectivity over FFA4. Moving the cyanomethyl to the *meta*-position (**23**), which had been beneficial for the methyl analogue (**11**), led to 12-fold erosion of potency. Finally, homologation to the

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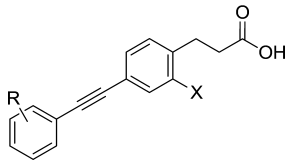
Table 2. Structure–Activity Investigations of the 2-Fluoro Alkyne Agonists



			pEC$_{50}$ (efficacy, %)				
compd	R^1	X	hFFA1, calcium[a]	hFFA4, BRET[b]	ClogP[c]	LE[d]	LLE[e]
9	2-Me	H	7.34 ± 0.07 (103)	5.84 ± 0.01 (103)	5.04	0.50	2.30
10	2-Me	F	7.48 ± 0.03 (107)	5.80 ± 0.03 (98)	5.18	0.49	2.30
11	3-Me	F	7.65 ± 0.03 (100)	5.41 ± 0.07 (124)	5.18	0.50	2.47
12	2-Me, 5-CN	F	7.77 ± 0.03 (104)	5.02 ± 0.04 (123)	4.62	0.46	3.15
13	2-CF_2H, 5-F	F	7.74 ± 0.04 (97)	5.93 ± 0.04 (117)	5.02	0.44	2.72
14	3,5-Cl	F	7.42 ± 0.07 (99)	5.50 ± 0.25 (90)	6.11	0.46	1.31
15	2-CH_2Ms	F	5.84 ± 0.02 (97)	n.a.	2.67	0.33	3.17
16	3-CH_2Ms	F	5.71 ± 0.02 (104)	4.19 ± 0.06 (26)	2.67	0.33	3.04
17	2-$CH_2O(CH_2)_2$Ms	F	6.21 ± 0.03 (94)	n.a.	3.33	0.31	2.88
18	3-$CH_2O(CH_2)_2$Ms	F	6.44 ± 0.03 (93)	n.a.	3.33	0.33	3.11
19	2-$CH_2O(CH_2)_3$Ms	F	6.14 ± 0.04 (92)	n.a.	3.59	0.30	2.55
20	3-$CH_2O(CH_2)_3$Ms	F	6.43 ± 0.04 (83)	n.a.	3.59	0.31	2.84
21	2-CH_2CN	H	7.70 ± 0.04 (103)	6.11 ± 0.06 (99)	3.96	0.48	3.76
22	2-CH_2CN	F	8.21 ± 0.03 (102)	6.03 ± 0.06 (98)	4.11	0.49	4.10
23	3-CH_2CN	F	7.13 ± 0.03 (104)	5.41 ± 0.07 (115)	4.11	0.42	3.02
24	2-CH_2CH_2CN	F	7.74 ± 0.04 (97)	5.86 ± 0.00 (114)	4.25	0.44	3.50

[a]Efficacy is given as % response relative to 10 μM TUG-20.[19] [b]Efficacy is given as % response relative to **9**; n.a. = no activity (pEC$_{50}$ < 4).[14] [c]Calculated by BioByte's algorithm as implemented in ChemBioDraw Ultra 12.0 (ClogP option). [d]LE = RTln K_D, presuming that EC$_{50}$ ≈ K_D. Values are given in kcal mol^{-1} per non-hydrogen atom.[17] [e]LLE = pEC$_{50}$ − ClogP.[18]

corresponding cyanoethyl (**24**) resulted in good potency but the compound could not compete with **22**.

With **22** being the clearly superior agonist in terms of potency and LLE, as well as displaying significantly higher potency (EC$_{50}$ = 6 vs 14 nM), lower lipophilicity (log $D_{7.4}$ = 1.41 vs 2.24) and higher ligand efficiency (LE = 0.49 vs 0.29) compared to the most advanced compound in the field TAK-875,[20] we set out to evaluate the compound further using our previously preferred compound **21** as reference (Table 3). Compound **22** displayed excellent physicochemical and in vitro ADME properties, with good aqueous solubility, good chemical stability, low lipophilicity, and decreased plasma protein binding (PPB). In support of the lower PPB, **21** showed significantly decreased activity on hFFA1 in a BRET assay in the presence of 0.1% BSA (from 7.16 ± 0.09 to 6.62 ± 0.05, p = 0.0024), whereas the corresponding reduction of activity for **22** was insignificant (from 7.64 ± 0.09 to 7.58 ± 0.06, p = 0.5635). Compound **22** furthermore showed excellent stability toward human liver microsomes (HLM), no inhibition of selected CYP-enzymes implicated in drug–drug interactions, no P-glycoprotein (P-gp) inhibition, and good permeability in the Caco-2 cell assay. Pharmacokinetic studies in mice showed a fast oral absorption, higher plasma concentration, a longer half-life, lower clearance, and increased bioavailability, overall giving a markedly improved pharmacokinetic profile compared to **21**. No cytotoxicity was observed in vitro in up to 100 μM concentration (see the Supporting Information), and no adverse effects were seen in mice after four weeks of daily oral treatment of 20 mg/kg and acute treatment in doses up to 250 mg/kg.

In addition to the counterscreen on FFA4, **22** showed a high selectivity over FFA2, FFA3, PPARγ, and 54 diverse receptors, transporters, and enzymes (see the Supporting Information). The compound exhibited lower potency on the rodent orthologs (mFFA1, pEC$_{50}$ = 6.83 ± 0.07 (n = 3); rFFA1, pEC$_{50}$ = 6.49 ± 0.05 (n = 2)). The effect of **22** was initially evaluated in vitro in

Table 3. Physicochemical Properties, in Vitro ADME, and Pharmacokinetics of 21 and 22

physicochemical properties	**21**	**22**
aqueous solubility (PBS, pH 7.4)[a]	196 μM	197 μM
chemical stab. (PBS, 37 °C, 12 days)	99.8%	99.1%
log D (n-octanol/PBS, pH 7.4)[b]	1.28 (1.32)	1.35 (1.44)
in vitro ADME properties[c]		
PPB (human)	>99.9%	97.3%
metabolic stability (HLM)	81%	87%
CYP inhibition (10 μM)		
CYP1A2	−3%	−10%
CYP2C9	11%	−33%
CYP2C19	−2%	−5%
CYP2D6	5%	−1%
CYP3A4	8%	−1%
P-gp inhibition (% @ 30/100 μM)	−4.0/−1.8	−4.4/−3.6
Caco-2 (A to B, TC7, pH 6.5/7.4)	91 × 10^{-6} cm/s	72 × 10^{-6} cm/s
pharmacokinetic properties[d]	**21**	**22**
Intravenous		
C_{max} (ng/mL)	5071	7811
t_{max} (min)	5	5
$t_{1/2}$ (min)	17	119
AUC$_{0-\infty}$ (μg/mL·min)	174	809
V_d (L/kg)	0.35	0.53
CL_{total} (mL/min/kg)	14	3.1
Oral		
C_{max}(ng/mL)	7757	12340
t_{max} (min)	30	15
$t_{1/2}$ (min)	50	355
AUC$_{0-\infty}$ (μg/mL·min)	732	4388
F (%)	105	136

[a]The maximum concentration of the assay is 200 μM. [b]Determined by shake-flask method.[16] The values given in parentheses were determined at Cerep Inc. [c]Determined at Cerep Inc. [d]Data are mean concentrations in mouse plasma (n = 3) following a single 2.5 mg/kg intravenous dose or 10 mg/kg oral dose.

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the rat INS-1E cell line, performed as previously reported,[9] where the compound caused significantly increased insulin secretion (10.75 ± 0.74% of total content with 10 μM **22** vs 8.74 ± 0.54 with vehicle, $p < 0.05$) at high glucose concentration (12.4 mM) and, as expected, no effect (4.14 ± 0.15% of total content with 10 μM **22** vs 4.02 ± 0.08 with vehicle) at low glucose concentration (2.8 mM).

In vivo examination of **22** in an acute intraperitoneal glucose tolerance test (IPGTT) in normal mice revealed a good dose dependent response with maximal reduction in glucose level reached at 50 mg/kg (Figure 1). The study was followed up by

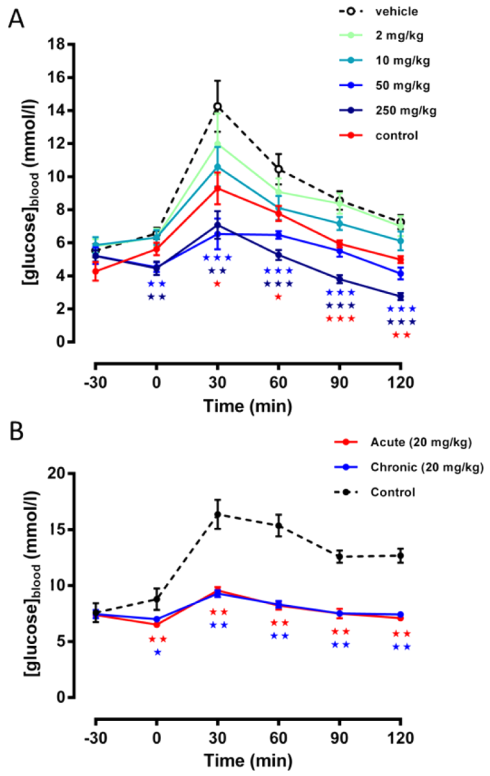

Figure 1. In vivo evaluation of **22** in mice on glucose tolerance. (A) Effect of **22** on acute IPGTT in normal mice. Mice were dosed ip with **22**, vehicle, or control (sitagliptin, 10 mg/kg). (B) Effect of **22** on OGTT in a chronic study in DIO mice: acute (4 weeks vehicle prior to treatment with **22**), chronic (4 weeks treatment with **22**), and control (vehicle). Means ± standard errors ($n = 6$) are shown (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

a chronic oral glucose tolerance test (OGTT) study in DIO mice, which showed that **22** was more effective than **21** (see the Supporting Information) and that the effect of **22** was fully sustained after 29 days of daily oral treatment. Additional evaluation of **22** in rats confirmed a significant glucose lowering effect for the high doses already after 10 min and for all doses after 30 min (Figure 2). This was in agreement with an observed increase in plasma insulin concentration, with maximum concentration 15 min after glucose challenge. With an approximately 30-fold higher potency on human than on rodent receptors, it appears reasonable to expect that the effective dose would be correspondingly lower in humans.

In conclusion, optimization of the FFA1 alkyne agonists has resulted in the discovery of **22**, a highly potent FFA1 agonist with excellent physicochemical and pharmacokinetic properties.

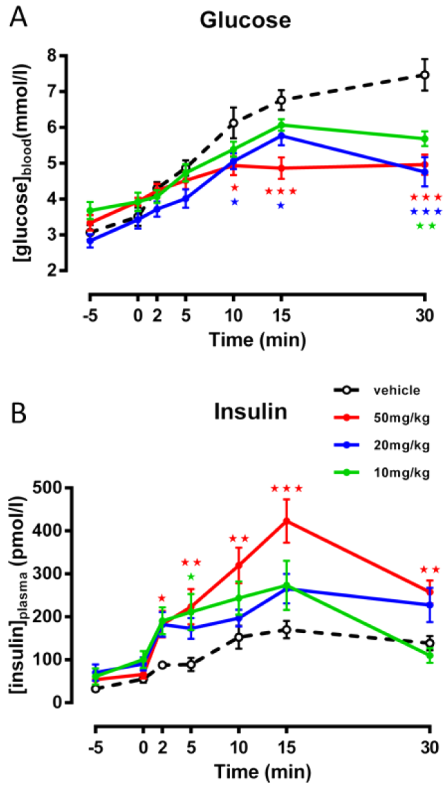

Figure 2. In vivo evaluation of **22** in Sprague–Dawley rats on glucose tolerance after oral dosing. (A) Effect on plasma glucose levels. (B) Effect on plasma insulin levels. Means ± standard errors ($n = 6$) are shown (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

The compound demonstrated a potent effect on glucose tolerance in DIO mice, a situation that was sustained after 29 days of chronic dosing. The compound all together appears as a promising candidate for development of improved T2D therapeutics.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedure, compound characterization, 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

BRET, bioluminescence resonance transfer; FFA1, free fatty acid receptor 1 (GPR40); IPGTT, intraperetoneal glucose