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). Activation of FFA1 increases glucose-stimulated 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. 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 due to its marked lipophilicity lowering effect. The 2-pyridyl central intermediate, prepared from aryl bromide 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.

The 2-fluoro substituted ligands were synthesized from the central intermediate 2, prepared from aryl bromide 1 by an initial Sonogashira coupling with trimethylsilylacetene 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) because of the selectivity issues frequently observed for these receptors. The central benzene ring was replaced by pyridine due to its marked lipophilicity lowering effect. The 2-pyridyl...
(4) and 3-pyridyl (5) analogues turned out to be twice as potent as previously reported ligands with pyridines as the terminal ring 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 di-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 (ΔpEC50 = 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.

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-4999 but turned out only equipotent with TUG-4999, 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 ortho substituent led to 12-fold erosion of potency. Finally, homologation 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

**Table 1. SAR Investigations of the Central Ring**

| Ar          | hFFA1*   | hFFA4b | ClogP | LE / LLE^4 |
|-------------|----------|--------|-------|------------|
|            | pEC50 (efficacy, %) | pEC50 |       |            |
| 3          | 6.70 ± 0.03 (106) | 5.07 ± 0.08 (91) | 4.54 | 0.48 |
| 4          | 5.67 ± 0.03 (92) | n.a. | 3.04 | 2.16 |
| 5          | 5.60 ± 0.03 (99) | 4.04 ± 0.03 (41) | 3.04 | 2.56 |
| 6          | 6.84 ± 0.02 (100) | 5.24 ± 0.03 (117) | 4.68 | 0.47 |
| 7          | 7.48 ± 0.05 (100) | 5.10 ± 0.01 (107) | 4.68 | 0.51 |
| 8          | 6.85 ± 0.02 (108) | 5.08 ± 0.02 (112) | 4.83 | 0.45 |

*Efficacy is given as % response relative to 10 μM TUG-20.19 Efficacy is given as % response relative to 9; n.a. = no activity (pEC50 < 4).14 Calculated by BioByte’s algorithm as implemented in ChemBioDraw Ultra 12.0 (ClogP option). LE = RTln K_D, presuming that EC50 ≈ K_D. Values are given in kcal mol⁻¹ per non-hydrogen atom.17 LLE = pEC50 − ClogP.18

(4) and (2) ethyl bromoacetate, PPh₃, NaHCO₃, water, EtOAc, room temp, 18 h, 87–96%; (b) CoCl₂·6H₂O, NaBH₄, MeOH, 0 °C → room temp, 3 h, 59–87%; (c) PhCCH, Na₂PdCl₄, 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 1**

**Scheme 2**

**Reagents and conditions:** (a) trimethylsilylacetylene, Na₂PdCl₄, PIntB, CuI, TMEDA, water, 70 → 80 °C, 10 min; (b) K₂CO₃, MeOH, 0 °C → room temp, 1 h, 80% over two steps; (c) aryl halide, Na₂PdCl₄, PIntB, CuI, TMEDA, water, 80 °C, 1–4 h, 52–70%; (d) LiOH, THF, water, room temp, 12 h, 69–100%.
corresponding cyanooethyl (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\textsubscript{50} = 6 vs 14 nM), lower lipophilicity (log \text{D\textsubscript{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,\textsuperscript{10} we set out to evaluate the compound further using our previously preferred compound 21 as reference (Table 3). Compounds 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, \(p = 0.5635\)). Compounds 22 furthermore showed excellent stability toward human liver microsomes (HLM), with 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 \(\mu\)M 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, PPAR\(\gamma\), and 54 diverse receptors, transporters, and enzymes (see the Supporting Information). The compound exhibited lower potency on the rodent orthologs (mFFA1, pEC\textsubscript{50} = 6.83 ± 0.07 \((n = 3)\); rFFA1, pEC\textsubscript{50} = 6.49 ± 0.05 \((n = 2)\)). The effect of 22 was initially evaluated in vitro in mouse plasma (48 h), showing good stability (99.8% for 21 and 99.1% for 22). Pharmacokinetic properties of 21 and 22

| physicochemical properties | 21 | 22 |
|---------------------------|----|----|
| aqueous solubility (PBS, pH 7.4)\textsuperscript{a} | 196 \(\mu\)M | 197 \(\mu\)M |
| chemical stab. (PBS, 37 °C, 12 days) | 99.8% | 99.1% |
| log \(D\) (n-octanol/PBS, pH 7.4)\textsuperscript{b} | 1.28 (1.32) | 1.35 (1.44) |
| in vitro ADME properties\textsuperscript{c} | | |
| PPB (human) | >99.9% | 97.3% |
| metabolic stability (HLM) | 81% | 87% |
| CYP inhibition (10 \(\mu\)M) | | |
| CYP1A2 | −3% | −10% |
| CYP2C9 | 11% | −33% |
| CYP2C19 | −2% | −5% |
| CYP2D6 | 5% | −1% |
| CYP3A4 | 8% | −1% |
| P-gp inhibition (\% @ 30/100 \(\mu\)M) | −4.0/−1.8 | −4.4/−3.6 |
| in vivo kinetic properties\textsuperscript{d} | | |
| Pharmacokinetic properties | 21 | 22 |
| Intravenous | | |
| \(C_{\text{max}}\) (ng/mL) | 5071 | 7811 |
| \(t_{\text{max}}\) (min) | 5 | 5 |
| \(t_{1/2}\) (min) | 17 | 119 |
| AUIC\textsubscript{\text{area}} (g/mL-min) | 174 | 809 |
| \(V_{d}\) (L/kg) | 0.35 | 0.53 |
| CL\textsubscript{total} (mL/min/kg) | 14 | 3.1 |
| Oral | | |
| \(C_{\text{max}}\) (ng/mL) | 7757 | 12340 |
| \(t_{\text{max}}\) (min) | 30 | 15 |
| \(t_{1/2}\) (min) | 50 | 355 |
| AUIC\textsubscript{\text{area}} (g/mL-min) | 732 | 4388 |
| \(F\) (%) | 105 | 136 |

\textsuperscript{a}The maximum concentration of the assay is 200 \(\mu\)M. \textsuperscript{b}Determined by shake-flask method. \textsuperscript{c}The values given in parentheses were determined at Cerep Inc. \textsuperscript{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.
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 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 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.

**ACKNOWLEDGMENTS**

We thank Lone Overgaard Storm for excellent technical assistance and the Danish Council for Independent Research| Technology and Production (grant 09-070364), the Danish Council of Strategic Research (grant 11-116196), and the Canadian Institutes of Health Research (fellowship to B.D.H.) for financial support.

**ABBREVIATIONS**

BRET, bioluminescence resonance transfer; FFA1, free fatty acid receptor 1 (GPR40); IPGTT, intraperitoneal glucose
tolerance test; LE, ligand efficiency; LLE, ligand lipophilicity efficiency; OGTT, oral glucose tolerance test

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