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Brief Article

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Discovery of a Potent and Selective GPR120 Agonist

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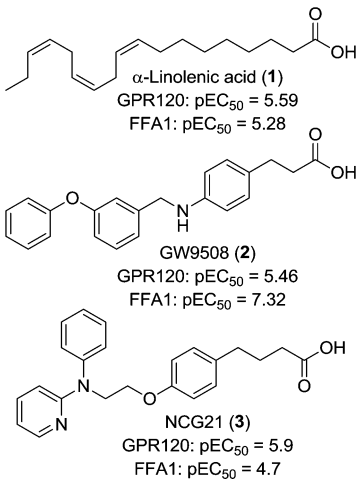
Supporting Information

ABSTRACT: GPR120 is a receptor of unsaturated long-chain fatty acids reported to mediate GLP-1 secretion, insulin sensitization, anti-inflammatory, and anti-obesity effects and is therefore emerging as a new potential target for treatment of type 2 diabetes and metabolic diseases. Further investigation is however hindered by the lack of suitable receptor modulators. Screening of FFA1 ligands provided a lead with moderate activity on GPR120 and moderate selectivity over FFA1. Optimization led to the discovery of the first potent and selective GPR120 agonist.

INTRODUCTION

Omega-3 (n-3) fatty acids are found to beneficially influence the development of metabolic syndrome and type 2 diabetes.[1] The G protein-coupled 7-transmembrane domain receptor GPR120 is abundantly expressed in intestines, lung, adipose tissue, and proinflammatory macrophages and is activated by long-chain free fatty acids (FFAs), in particular n-3 polyunsaturated FFAs such as α-linolenic acid (**1**, Chart 1), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).[2−6] The receptor has been reported to mediate FFA promoted release of glucagon-like peptide-1 (GLP-1), an incretin hormone which increases glucose-stimulated insulin secretion, insulin sensitivity, and β-cell mass and reduces appetite and gastric emptying.[2] Recently, GPR120 was also found to mediate insulin-sensitizing and anti-inflammatory effects of the n-3 fatty acids EPA and DHA.[3] It is notable in relation to this that chronic low-grade inflammation is implicated in the pathogenesis of obesity, insulin resistance, and type 2 diabetes.[7] Another study found that GPR120 is likely to mediate unsaturated fatty acid reversal of diet-induced hypothalamic inflammation, a determining factor for development of obesity.[8] Notably, dysfunctional GPR120 was recently found to lead to obesity in both mice and human.[9] As a consequence of these observations, GPR120 is currently emerging as an interesting new potential target for treatment of metabolic and inflammatory diseases such as obesity and type 2 diabetes. Further research is required to establish GPR120 as a new target; however, this research is currently hindered by the lack of potent and selective receptor modulators. Here we report the first potent and selective GPR120 agonist.

Chart 1. Current GPR120 Agonists[11,12]



The free fatty acid receptor 1 (FFA1 or GPR40) is another potential target for treatment of type 2 diabetes.[10] Although GPR120 and FFA1 are phylogenetically distant, there is a close structural resemblance between the ligands capable of recognizing and activating the two receptors. The few GPR120 agonists described hitherto, including FFAs, require micromolar concentrations and have poor or no selectivity over FFA1.[6] Briscoe and co-workers described the potent FFA1 agonist GW9508 (**2**, Chart 1), which also possesses moderate GPR120 agonist activity.[11] Suzuki and co-workers reported GPR120 agonists derived from PPARγ ligands. NCG21 (**3**) came out as the most potent and selective analogue, 16-fold selective over FFA1.[12,13] The natural product grifolic acid and its methyl ether have also been reported to act as selective partial agonists on GPR120 in concentrations above 30 μM.[14] We recently described the structure−activity relationship (SAR) studies of a dihydrocinnamic acid series on FFA1.[15] The similarity between these compounds and GW9508 sparked our interest in exploring the activity of the FFA1 series on GPR120.[15]

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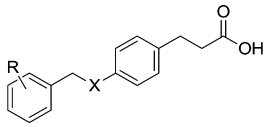
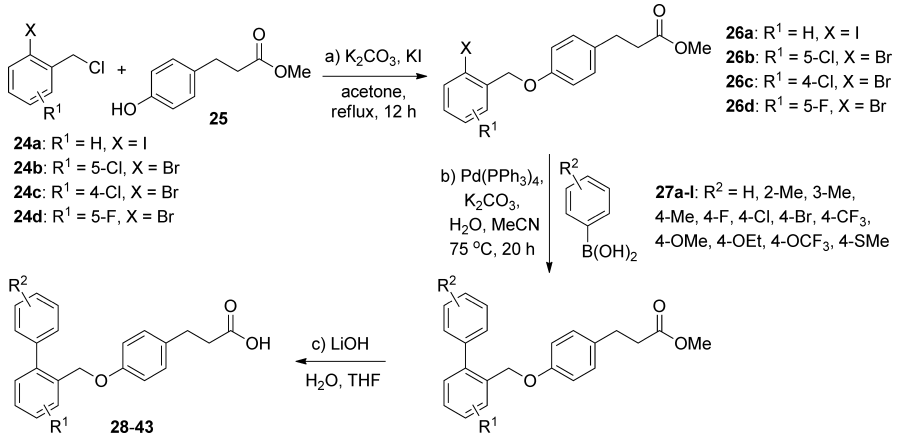
Table 1. Screening of FFA1 Agonists on GPR120



compd	R	X	GPR120 (BRET) pEC_{50} (efficacy)[a]	FFA1 (BRET) pEC_{50} (efficacy)[a]	select[b]
1	(Chart 1)		5.49 ± 0.14 (99)	5.54 ± 0.30 (88)	−0.05
2	3-OPh	NH	5.85 ± 0.05 (102)	7.55 ± 0.15 (86)	−1.70
3	(Chart 1)		5.62 ± 0.10 (96)	5.01 ± 0.23 (97)	0.61
4	(ref 14)		5.65 ± 0.05 (100)	7.52 ± 0.03 (100)	−1.87
5	H	O	4.93 ± 0.04 (105)	6.26 ± 0.09 (90)	−1.33
6	H	NH	4.24 ± 0.07 (104)	5.94 ± 0.33 (99)	−1.70
7	4-Br	O	5.27 ± 0.08 (47)	6.78 ± 0.18 (108)	−1.51
8	2-Br	O	5.76 ± 0.04 (95)	6.34 ± 0.19 (114)	−0.58
9	3-Br	O	6.32 ± 0.06 (90)	7.12 ± 0.01 (93)	−0.80
10	3-Br	NH	5.51 ± 0.03 (104)	6.62 ± 0.30 (87)	−1.11
11	3-CN	O	4.68 ± 0.03 (102)	6.13 ± 0.06 (96)	−1.45
12	3-CF_3	O	5.89 ± 0.03 (98)	7.04 ± 0.18 (109)	−1.15
13	3-CF_3	NH	5.17 ± 0.05 (98)	6.70 ± 0.07 (112)	−1.53
14	3-OPh	O	6.25 ± 0.10 (94)	7.20 ± 0.16 (90)	−0.95
15	4-OPh	O	4.78 ± 0.08 (63)	6.81 ± 0.21 (120)	−2.03
16	4-OPh	NH	5.35 ± 0.08 (44)	7.01 ± 0.10 (88)	−1.66
17	4-Ph	NH	4.23 ± 0.11 (93)	6.66 ± 0.26 (118)	−2.43
18	3-Ph	O	5.62 ± 0.04 (23)	6.17 ± 0.07 (110)	−0.52
19	3-Ph	NH	5.32 ± 0.04 (93)	7.08 ± 0.09 (112)	−1.76
20	2-Ph	O	6.43 ± 0.10 (95)	5.00 ± 0.13 (99)	1.43
21	2-Ph	NH	4.29 ± 0.09 (97)	5.32 ± 0.15 (53)	−1.03
22	3-(2-MeC_6H_4)	O	5.06 ± 0.06 (79)	7.78 ± 0.30 (86)	−2.72
23	3-(2-MeC_6H_4)	NH	5.20 ± 0.02 (53)	7.89 ± 0.06 (109)	−2.69

[a]Efficacy is relative to **4**. [b]Calculated as the difference between pEC_{50} for GPR120 and FFA1.

Scheme 1. Synthesis of *ortho*-Biphenyl Ligands



RESULTS AND DISCUSSION

Compounds were screened on GPR120 transfected HEK cells in a β-arrestin 2 interaction bioluminescence resonance energy transfer (BRET) assay and counterscreened on FFA1 in an equivalent assay. α-Linolenic acid (**1**), GW9508 (**2**), and NCG21 (**3**) exhibited values in the BRET assay of both GPR120 and FFA1, in agreement with those reported previously in calcium assays (Chart 1).[2,11,12]

A diverse selection of 4-benzyloxy- and 4-benzylaminodihydrocinnamic acids and the alkyne FFA1 agonist 4-(2-toluylethynyl)dihydrocinnamic acid (TUG-424, **4**)[16] were included in the screen. **4** exhibited full agonist activity on GPR120 in the micromolar range and a selectivity for FFA1 of close to two orders of magnitude. All 4-benzyloxy- and 4-benzylaminodihydrocinnamic acids turned out to also activate GPR120 (Table 1). The SAR on GPR120 of compounds with no or small substituents on the terminal phenyl followed the trends previously observed for FFA1: a central O-linker is preferable over an NH-linker (**5**/**6**, **9**/**10**, **12**/**13**) and *meta*-substituents on the terminal ring appeared to be preferred (**7**−**9**), with nonpolar substituents performing better (**9** and **12** vs **11**). In contrast to FFA1, a general preference of the O-linker compounds for GPR120 was still observed with larger substituents on the terminal ring, i.e., biphenyl and phenoxyphenyl systems (e.g., **14** vs **2**, **18** vs **19**), although the trend was less consistent. Several compounds behaved as

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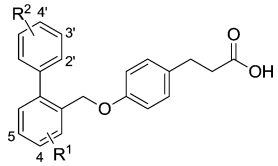
Table 2. Optimization of the *ortho*-Biphenyl Ligands



compd	R^1	R^2	GPR120 (BRET) pEC_{50} (efficacy)[a]	FFA1 (BRET) pEC_{50} (efficacy)[a]	select[b]
28	H	2′-Me	5.15 ± 0.11(86)	6.22 ± 0.15 (60)	−1.06
29	H	3′-Me	5.71 ± 0.05 (97)	6.28 ± 0.09 (93)	−0.56
30	H	4′-Me	6.75 ± 0.05 (96)	6.20 ± 0.14 (107)	0.54
31	H	4′-F	6.23 ± 0.15 (100)	6.03 ± 0.20 (116)	0.20
32	H	4′-Cl	6.51 ± 0.03 (103)	6.29 ± 0.18 (99)	0.21
33	H	4′-Br	6.53 ± 0.07 (98)	6.07 ± 0.19 (103)	0.46
34	H	4′-CF_3	5.69 ± 0.04 (97)	5.94 ± 0.21 (97)	−0.25
35	H	4′-OMe	6.72 ± 0.11 (100)	5.69 ± 0.03 (101)	1.02
36	H	4′-OEt	5.66 ± 0.06 (83)	5.35 ± 0.37 (59)	0.31
37	H	4′-OCF_3	5.36 ± 0.04 (80)	6.20 ± 0.28 (53)	−0.85
38	H	4′-SMe	6.63 ± 0.22 (88)	5.45 ± 0.20 (97)	1.18
39	4-Cl	H	6.62 ± 0.13 (98)	6.22 ± 0.12 (91)	0.40
40	5-Cl	H	6.04 ± 0.03 (105)	6.96 ± 0.25 (51)	−0.92
41	4-Cl	4′-Me	6.29 ± 0.05 (102)	6.39 ± 0.16 (88)	−0.09
42	4-F	H	6.96 ± 0.03 (99)	6.02 ± 0.22 (58)	0.94
43	4-F	4′-Me	7.36 ± 0.15 (104)	4.19 ± 0.97	3.17

[a]Efficacy is relative to **4**. [b]Calculated as the difference between pEC_{50} for GPR120 and FFA1.

partial agonists on GPR120 with efficacy down to 23% in the case of **18**. No clear structural determinants of partial agonism were observed except that all carried substituents in the *meta*- or *para*-position of the benzyl group. The potent and selective FFA1 agonist **23** (TUG-469) turned out to be a micromolar partial agonist on GPR120, slightly more potent than the corresponding O-linked analogue **22** but still 500-fold selective for FFA1.[15] All compounds were 10−1000-fold less potent on GPR120 than on FFA1 with one notable exception: the *ortho*-biphenyl analogue **20** exhibited pEC_{50} = 6.43 for GPR120 with 27-fold selectivity over FFA1. The corresponding NH-linked **21** was >100-fold less active. Thus, **20** was selected for further optimization.

Analogues of the *ortho*-biphenyl ligand **20** were prepared by coupling of **24a−d** with **25** to form 2-bromo- or 2-iodobenzyl intermediates **26a−d**, followed by Suzuki−Miyaura cross-coupling and ester hydrolysis to provide the target compounds (Scheme 1). Introduction of methyl groups in the 2′- or 3′-position of the terminal phenyl ring (**28**, **29**) resulted in reduced potency, whereas a methyl substituent in the 4′-position (**30**) doubled the potency (Table 2). Focusing on the 4′-position, various small substituents (**31**−**38**), including halogens and alkoxys, were investigated. Most substituents produced GPR120 agonists with pEC_{50} > 6, but the larger substituents (**36**, **37**) tended to give somewhat lower potency. Although the 4′-OMe substituent (**35**) produced an equipotent compound, none were more potent than **30**. Most compounds exhibited a moderate selectivity for GPR120.

We then turned our attention to the proximal ring of the biphenyl system. Introduction of a 4-chloro substituent (**39**) boosted potency, whereas a 5-chloro (**40**) produced a moderate drop relative to **20**. The combination of 4-chloro and 4′-methyl (**41**) gave a compound with lower potency than both parent compounds and the unsubstituted **20**, demonstrating that the effects of substituents in different parts of the biphenyl system are not necessarily additive. A 4-fluoro substituent (**42**) produced a considerable increase in potency. Gratifyingly, when the 4-fluoro was combined with 4′-methyl (**43**), a significant further increase in potency up to pEC_{50} = 7.36 was observed. Equally notable was the low activity on FFA1, resulting in excellent selectivity for GPR120 over FFA1. Potent full agonist activity on GPR120 and appreciable selectivity over FFA1 was confirmed in the calcium assay (GPR120: pEC_{50} = 7.02 ± 0.09, 114%. FFA1: pEC_{50} = 5.30 ± 0.04, 121%.). Critically in light of the importance of rodent models and cell-lines, **43** was also found to be highly potent on murine GPR120 (pEC_{50} = 7.77 ± 0.09, 75% efficacy). The compound was confirmed to be devoid of activity on the short-chain FFA receptors FFA2 and FFA3.

A model of GPR120 in complex with **43** was constructed using the crystal structure of a nanobody-stabilized active state of the β_2-adrenoceptor (PDB code 3P0G)[19] as template (Figure 1). The model shows that the carboxylic acid residue is

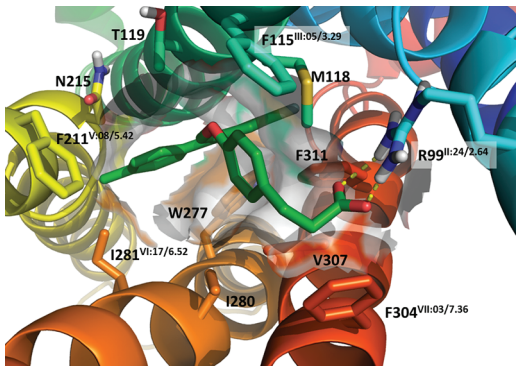


Figure 1. Model of **43** in complex with hGPR120. The shown surface is defined by M118, T119, G122, N215, W277, I280, I281, V307, and F311. Residues are labeled with sequence number and with Schwartz−Baldwin[17] and Ballesteros−Weinstein[18] notations given as superscript.

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engaged in an electrostatic and double hydrogen bond interaction with Arg99. The phenylpropionate moiety interacts with Phe304 on the upper side and Phe311 on the bottom side. The narrow lipophilic pocket around the biphenyl moiety is defined by Met118, Thr119, Gly122, Phe211, Asn215, Ile280, Ile281, and Trp277. The model thus provides a rationale for the preference of GPR120 for the *ortho*-biphenyl moiety and explains the restricted tolerability for substitution around this moiety such as the lower activity observed with the slightly longer substituted biphenyl system of **41** compared to **43**. The interaction with Arg99 is confirmed by the complete lack of β-arrestin recruitment upon addition of **43** to cells expressing the GPR120 R99Q mutant.

CONCLUSION

In conclusion, by screening representatives from our collection of FFA1 agonists on GPR120 and optimizing the most promising hit, we have identified the first potent and selective GPR120 agonist (**43**). The compound has high potency on both the human and murine receptor and will enable studies to further explore GPR120 as a target for the treatment of type 2 diabetes, obesity, and other metabolic diseases.

EXPERIMENTAL SECTION

Chemistry. All commercial starting materials and solvents were used without further purification unless otherwise stated. THF was freshly distilled from sodium/benzophenone. Acetone and *N*,*N*-diisopropylethyl amine (DIPEA) were dried over 4 Å sieves. Purification by flash chromatography was carried out using silica gel 60 (0.040−0.063 mm, Merck). ^{1}H and ^{13}C NMR spectra were calibrated relative to TMS internal standard or residual solvent peak. Reactions were routinely monitored by TLC using Merck silica gel F_{254} plates. HPLC analysis was performed using a Dionex 120 C18 column (5 μ, 4.6 mm × 150 mm); flow, 1 mL/min; 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; UV detection at 254 nm. High-resolution mass spectra (HRMS) were obtained on a Bruker micrOTOF-Q II (ESI). Melting points were measured on a Büchi melting point apparatus and are uncorrected. Purity was determined by HPLC and confirmed by NMR. All test compounds were of >95% purity.

General Procedure A (Williamson Ether Synthesis). A mixture of benzyl chloride (1 equiv), phenol (1.1 equiv), potassium carbonate (2.0 equiv), potassium iodide (0.5 equiv), and anhydrous acetone (5 mL/mmol) was heated at reflux. After consumption of the benzyl chloride, the reaction mixture was cooled, filtered, and concentrated, and the residue was purified by flash chromatography.

General Procedure B (Suzuki Cross-Coupling). A flask charged with aryl halide (1.0 equiv), boronic acid (1.1 equiv), potassium carbonate (1.2 equiv), acetonitrile (6.4 mL/mmol), water (2.1 mL/mmol), and $Pd(PPh_3)_4$ (5 mol %) was evacuated, backfilled with argon three times, and heated at 75 °C until consumption of the aryl halide. The reaction mixture was concentrated, diluted with water, and extracted with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4, concentrated, and purified by flash chromatography.

General Procedure C (Ester Hydrolysis). To the ester (1 equiv) dissolved in THF (5 mL/mmol ester) was added LiOH (2−3 equiv) in H_2O (2 mL/mmol ester). The reaction was stirred at room temperature until complete consumption of the starting material as indicated by TLC, typically after 2−4 h. The reaction was acidified with 1 M HCl until pH <1 and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated.

*3-(4-((4-Fluoro-4′-methyl-[1,1′-biphenyl]-2-yl)methoxy)phenyl)-propanoic Acid (**43**).* Step 1: Following general procedure B, **26d** (97 mg, 0.26 mmol) was coupled with *p*-tolylboronic acid (43 mg, 0.32 mmol) to give 83 mg (83%) of a colorless oil after flash chromatography (SiO_2, EtOAc:petroleum ether, 1:9); R_f = 0.43 (SiO_2, EtOAc:petroleum ether, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (dd, J = 9.7, 2.7 Hz, 1H), 7.26 (dd, J = 8.3, 5.9 Hz, 1H), 7.21 (s, 4H), 7.10−7.02 (m, 3H), 6.80−6.73 (m, 2H), 4.89 (s, 2H), 3.66 (s, 3H), 2.87 (t, J = 7.8 Hz, 2H), 2.58 (t, J = 7.8 Hz, 2H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 163.4, 161.0, 156.8, 137.2, 137.2, 136.7, 136.68, 136.5, 133.0, 131.6, 131.5, 129.2, 129.1, 129.0, 115.3, 115.1, 114.9, 114.8, 114.6, 67.6, 51.6, 35.9, 30.1, 21.1. HRMS calcd for $C_{22}H_{24}FO_3$ (M^+), 379.1704; found, 379.1683. Step 2: Following general procedure C, the methyl ester from step 1 (75 mg, 0.20 mmol) was hydrolyzed to give 68 mg (94%) of a thick syrup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (dd, J = 9.7, 2.7 Hz, 1H), 7.28−7.24 (m, 1H), 7.21 (s, 4H), 7.11−7.02 (m, 3H), 6.78 (d, J = 8.6 Hz, 2H), 4.89 (s, 2H), 2.88 (t, J = 7.7 Hz, 2H), 2.63 (t, J = 7.7 Hz, 2H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.0, 163.5, 161.0, 156.9, 137.2, 137.2, 136.7, 136.6, 136.6, 132.7, 131.6, 131.5, 129.2, 129.1, 129.0, 115.3, 115.1, 114.9, 114.8, 114.6, 67.6, 35.6, 29.7, 21.1. HRMS calcd for $C_{23}H_{21}FO_3Na$ (M + Na^+), 387.1367; found, 387.1349.

Biological Assays. *BRET β-Qrrestin 2 Interaction Assay.* The BRET assay was performed as described previously.[20] Briefly, plasmids encoding either GPR120 or FFA1 fused at their C-terminal to enhanced yellow fluorescent protein were cotransfected into HEK 293 cells with a plasmid encoding β-arrestin 2 fused to Renilla luciferase. Cells were distributed into white 96-well plates 24 h post-transfection and then maintained in culture for another 24 h prior to their use. To conduct the assay, cells were first washed in Hank's Balanced Salt Solution and then the Renilla luciferase substrate coelenterazine h (5 μM), and the ligand of interest were added. Cells were incubated at 37 °C for either 5 min (GPR120) or 30 min (FFA1) before luminescence at 535 and 475 nm was measured using a Pherastar FS. The ratio of luminescence at 535/475 nm was then used to calculate the BRET response. To test the activity of **43** on GPR120 R99Q in the BRET assay, this point mutation was incorporated into the wild-type GPR120-enhanced yellow flourescent protein construct using the Quickchange method (Stratagene).

Calcium Asssay. Calcium assays were carried out on Flp-In T-REx293 cell lines, generated to inducibly express either GPR120 or FFA1 upon treatment with doxycycline. One day prior to conducting the experiment, cells were seeded at 50000/well in black clear-bottom 96-well microplates. Cells were allowed to adhere for 3−4 h before the addition of 100 ng/mL doxycycline to induce receptor expression. The following day, cells were incubated in culture medium containing the calcium sensitive dye Fura2-AM (3 μM) for 45 min. Cells were then washed three times and then allowed to equilibrate for 15 min in Hank's Balanced Salt Solution prior to conducting the assay. Fura2 fluorescent emission was measured at 510 nm following excitation at both 340 and 380 nm during the course of the experiment using a Flexstation plate reader (Molecular Devices). Calcium responses were then measured as the difference between 340/380 ratios before and after the addition of test compounds.

Counterscreen on FFA2 and FFA3. The activity of **43** was tested on FFA2 and FFA3 in the [^{35}S]GTPγS assay as described previously.[21]

ASSOCIATED CONTENT

Supporting Information

Synthesis and compound characterization, procedures for biological testing and molecular modeling. 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

BRET, bioluminescence resonance energy transfer; DHA, *all-cis*-4,7,10,13,16,19-docosahexaenoic acid; DIPEA, diisopropylethylamine; EPA, *all-cis*-5,8,11,14,17-eicosapentaenoic acid; FFA, free fatty acid; FFA1, free fatty acid receptor 1 (GPR40); FFA2, free fatty acid receptor 2 (GPR43); FFA3, free fatty acid receptor 3 (GPR41); GLP-1, glucagon-like peptide-1

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