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Identification of Diaryl Sulfonamides as Agonists of the Free Fatty Acid Receptor 4 (FFA4/GPR120)

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Abstract— The exploration of a diarylsulfonamide series of free fatty acid receptor 4 (FFA4/GPR120) agonists is described. This work led to the identification of selective FFA4 agonist **8** (GSK137647A) and selective FFA4 antagonist **39**. The in vitro profile of compounds **8** and **39** is presented herein.

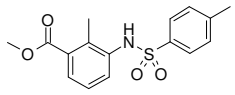
Type 2 diabetes is a major worldwide public health problem with devastating chronic complications such as cardiovascular disease, retinopathy, neuropathy, and nephropathy. Type 2 diabetes affects more than 300 million people globally and is predicted to become the seventh leading cause of death by 2030, with total deaths from diabetes projected to increase by 50% in the next 10 years.[1] The disease is polygenic in nature and characterized by hyperglycemia, defects in pancreatic insulin secretion, and insulin resistance in skeletal muscle, adipose tissue, and liver. In addition to these derangements, the rate of endogenous glucose production is significantly elevated in type 2 diabetics relative to healthy subjects.[2,3]

A variety of pharmacological strategies have been successfully employed to improve glycemic control in Type 2 diabetics which directly target the secretion and sensitization of insulin. More recently, approaches targeting the endogenous antidiabetic hormone glucagon-like peptide-1 (GLP-1) have met with success.[4] GLP-1, secreted from L-cells located in the ileum and colon, stimulates glucose-dependent insulin secretion and has been shown to inhibit glucagon release, gastric emptying, and feeding.[5] GLP-1 ($T_{1/2}$ = 2 min) is rapidly degraded by the enzyme dipeptidylpeptidase-4 (DPP-4) and agents developed as long-acting agonists of GLP-1 (exenatide and liraglutide) and inhibitors of DPP-4 (sitagliptin and vildagliptin) have successfully validated GLP-1 as an important target for the development of new antidiabetic therapies.[6]

GLP-1 is released in response to ingestion of nutrients including carbohydrates, protein, and fat.[7] Recent studies aimed at identifying the receptors involved in nutrient stimulated GLP-1 secretion have identified free fatty acid receptor 4 (FFA4/GPR120) as a potential 7TM receptor involved in long-chain fatty acid stimulated GLP-1 secretion.[8] FFA4 is highly expressed in the intestine and the intestinal endocrine cell line STC-1, additionally FFA4 mRNA has been detected in murine L-cells.[9] Elucidating the mechanism of FFA4 mediated GLP-1 secretion would be greatly aided by the identification of selective small molecule agonists and antagonists.[10] With this goal in mind, efforts directed toward the identification of small molecule FFA4 modulators culminating in a series of diaryl sulfonamide agonists are described herein.

Compound **1** was identified as a starting point for optimization following a FLIPR based medium throughput screening campaign conducted in a HEK293 cell line expressing human FFA4. The potency and efficacy of compound **1** was determined using a ten-point response curve in mammalian U2OS cells expressing human FFA4 with a FLIPR readout run in both agonist and antagonist modes.[11] The ease of synthesis of diaryl sulfonamides combined with the

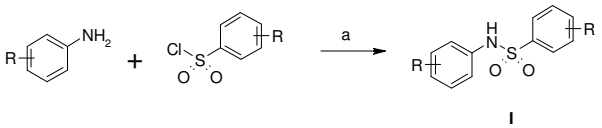
plethora of commercially available starting materials made compound **1** an attractive starting point for SAR development.



1
Human FFA4 pEC50 = 5.4 ± 0.2
% Max Resp = 109 ± 12%

Figure 1. In vitro profile of sulfonamide **1**.

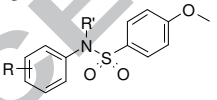
The general synthesis of the diarylsulfonamide agonists is shown in Scheme 1. Sulfonamide bond formation was typically formed by reaction of the aniline and arylsulfonyl chloride in pyridine at room temperature. In cases where electron-deficient anilines were employed, heating to elevated temperatures was required.



Scheme 1. General syntheses of the diarylsulfonamides. Reagents and conditions: (a) pyridine, RT or 50 °C

Our initial studies explored substitution about the aniline ring of the diarylsulfonamides (Table 1). Whereas monoakyl-substitution on the aniline ring resulted in compounds devoid of activity (**2**-**4**), dialkyl-substitution (**5**-**6**) provided activity equivalent to hit compound **1**. Larger alkyl groups at positions 2 and 6 of the aniline ring were not tolerated (**7**), however addition of an alkyl group at the 4-position, to provide 2,4,6-trimethyl analog **8**[12] provided a substantial increase in potency. N-substitution (**9**-**10**) was not tolerated.

Table 1. In vitro FFA4 Agonism: aniline substitution

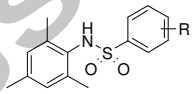


Compound	R	R'	FFA4 pEC_{50}[a]	% Max Resp (SD)
2	2-Me	H	<4.5	--
3	3-Me	H	<4.5	--
4	4-Me	H	<4.5	--
5	2,4-DiMe	H	5.6 (0.1)	81 (22)
6	2,6-DiMe	H	5.5 (0.1)	63 (9)
7	2,6-DiEt	H	<4.5	--
8 (GSK137647A)	2,4,6-TriMe	H	6.3 (0.2)	100[b]
9	2,4,6-TriMe	Me	<4.5	--
10	2,4,6-TriMe	Et	<4.5	--
Linoleic Acid	--	--	6.5	138

[a]Values are means of at least three experiments, standard deviation is given in parentheses. [b]GSK137647A (**8**) was used as a standard in the U2OS assay with the % max response set to 100, linoleic acid data is shown for comparison.

With our initial survey of the aniline SAR completed, we turned our attention toward exploration of the substitution on the arylsufonyl group (Table 2). Varying the position of a methoxy substituent revealed that *ortho*-substitution (**12**) was not tolerated while *para*-substitution (**8**) provided the most potent agonists. With this information in hand, we concentrated on *para*-substitution and identified that small nonpolar substituents were preferred, with the 4-methoxy substituent (**8**) providing the best balance of potency and efficacy. Interestingly, substitution with an electon withdrawing trifluoromethoxy group (**14**) provided nearly equal potency yet reduced efficacy (53% max response). Disubstitution at the 3- and 4-postions was well tolerated, with 5-membered fused rings (**27** vs **28**) providing optimal activity.

Table 2. In vitro FFA4 Agonism: phenyl sulfonyl substitution.

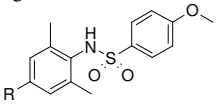


Compound	R	FFA4 pEC_{50}[a]	% Max Resp (SD)
11	H	4.9 (0.1)	86 (32)
12	2-MeO	<4.5	
13	3-MeO	4.9 (0.1)	58 (12)
8 (GSK137647A)	4-MeO	6.3 (0.2)	100[b]
14	4-CF_3O	5.9 (0.1)	53 (8)
15	4-Me	6.0 (0.1)	83 (19)
16	4-CO_2H	<4.5	--
17	4-Ac	5.1 (0)	90 (5)
18	4-F	5.2 (0.2)	113 (33)
19	4-Cl	5.5 (0.2)	104 (17)
20	4-Pr	6.4 (0.1)	17 (3)
21	4-tBu	<4.5	--
22	4-Ph	<4.5	--
23	4-OiPr	5.7 (0.1)	67 (6)
24	4-OBu	<4.5	--
25	3-MeO-4-Cl	6.1 (0.1)	73 (10)
26	3,4-DiMeO	5.00 (0.1)	42 (3)
27	3,4-Ethylenedioxy	6.5 (0.1)	78 (7)
28	3,4-Propylenedioxy	5.7 (0.2)	103 (28)

[a]Values are means of at least three experiments, standard deviation is given in parentheses. [b]GSK137647A (**8**) was used as a standard in the U2OS assay with the % max response set to 100.

Finally we explored the SAR at the 4-position of the aniline (Table 3). Incremental increases in the length of the alkyl group (**29**) maintained FFA4 activity, while further increases in the steric bulk of the alkyl group eroded activity (**30**). Consistent with the previous SAR small lipophilic groups were tolerated (**31-33**), however, attempts to introduce more polar functionality at the 4-position (**34**-**38**) were not successful.

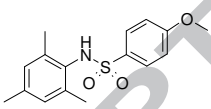
Table 3. In vitro FFA4 Agonism: aniline substitution



Compound	R	FFA4 pEC50[a] (SD)	% Max Resp (SD)
8 (GSK137647A)	Me	6.3 (0.2)	100[b]
29	Allyl	6.5 (0.1)	85 (10)
30	Cyclopropylmethyl	<4.5	--
31	F	6.0 (0.1)	75 (12)
32	Cl	6.3 (0.0)	131 (8)
33	OMe	6.4 (0.2)	54 (11)
34	OH	<4.5	--
35	CN	<4.5	--
36	CO_2H	<4.5	--
37	CO_2Me	<4.5	--
38	CH_2OH	<4.5	--

[a]Values are means of at least three experiments, standard deviation is given in parentheses. [b]GSK137647A (**8**) was used as a standard in the U2OS assay with the % max response set to 100.

Having completed our SAR studies around the diarylsulfonamide template, we chose agonist **8**, GSK137647A, for further in vitro selectivity profiling (Figure 2). Compound **8** was evaluated against a panel of 65 targets in both full curve functional and binding assays and was shown to provide at least 100-fold selectivity against the panel which included 41 G-protein-coupled receptors (GPCRs), including additional members of the free fatty acid receptors (FFA1, FFA2, & FFA3). Profiling against the rodent ortholog receptors showed compound **8** maintained activity against the mouse and rat FFA4 receptors while maintaining selectivity over the rodent FFA1, FFA2, and FFA3 receptors. While the selectivity of compound **8** is promising, the poor solubility of the compound measured in simulated intestinal fluid (FASSIF) greatly limited the utility of the compound for in vivo experiments.



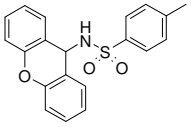
GSK137647A (**8**)

FFA4: Human pEC_{50} = 6.3 (0.17), 100% Max Response
Mouse pEC_{50} = 6.2 (0.17), 100% Max Response
Rat pEC_{50} = 6.1 (0.16), 100% Max Response
Human FFA1, FFA2, & FFA3 pEC_{50}s < 4.5
Mouse FFA1, FFA2, & FFA3 pEC_{50}s < 4.5
Rat FFA1, FFA2, & FFA3 pEC_{50}s < 4.5

FASSIF solubility = 2.9 µg/mL

Figure 2. In vitro profile of FFA4 agonist GSK137647A (**8**).

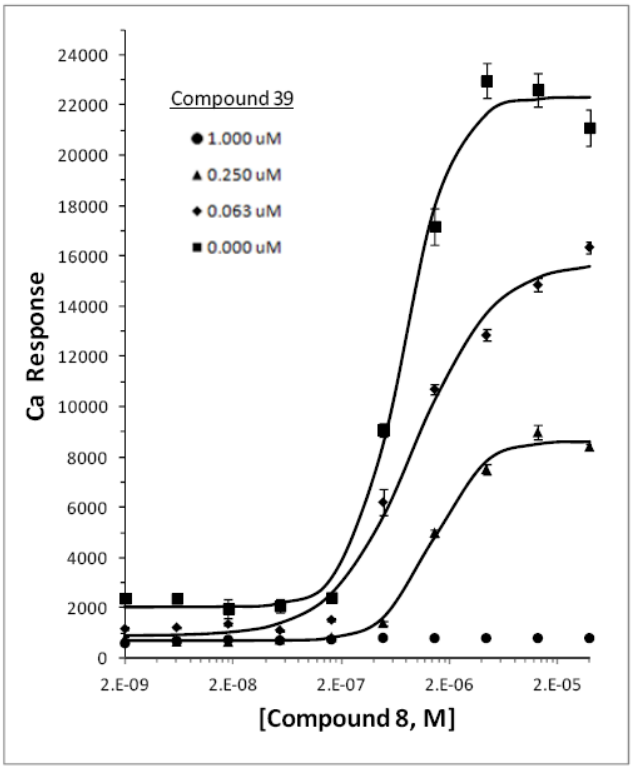
During the course of our optimization of the diarylsulfonamide chemotype as FFA4 agonists, we identified xanthene **39** as a potent and selective antagonist of FFA4 (Figure 3).[13] Xanthene **39** was able to block the agonist induced intracellular Ca^{2+} response elicited by both linoleic acid and compound **8** in competition studies run in FFA4 expressing U2OS cells (Figure 4). While competitive antagonism maybe expected given the closely related structures of agonist **8** and antagonist **39**, further studies would be needed to confirm the precise nature of antagonism due to the potential for insurmountable antagonism in these studies.[14]



39

FFA4: Human pIC_{50} = 7.1 ± 0.35
Human pEC_{50} < 4.5
Mouse pIC_{50} = 8.1
Rat pIC_{50} = 8.1
FFA1: Human pIC50 < 4.6
Human pEC50 < 4.3

Figure 3. In vitro profile of FFA4 antagonist **39**.



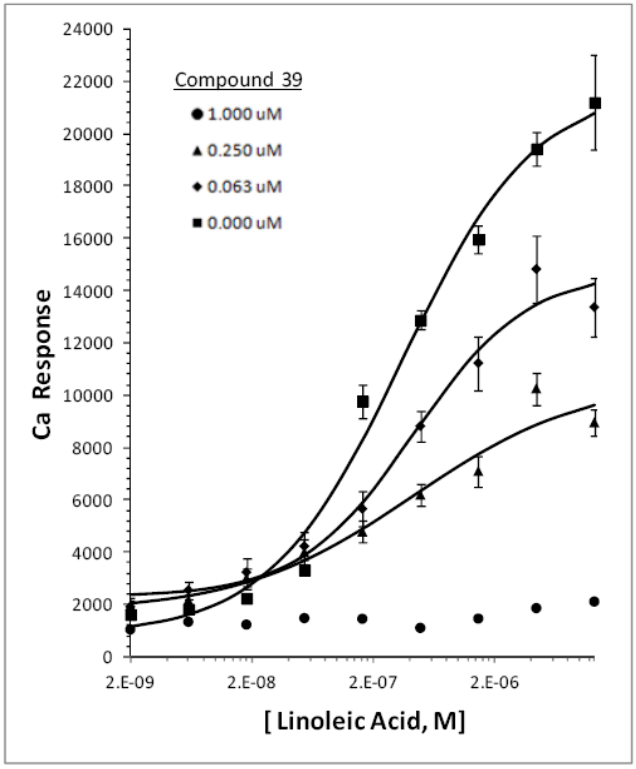


Figure 4. Inhibition of linoleic acid and GSK137647A (**8**) induced intracellular Ca^{2+} increase by compound **39**, a selective FFA4 receptor antagonist.

Pharmacological profiling of the responses of the selective FFA4 agonist and antagonist in both murine and human cell lines was next explored. Previous studies have shown that free fatty acids such as linoleic acid can produce a glucose-dependent increase in insulin secretion from the mouse insulinoma cell line MIN6.[15] As our data demonstrate the presence of FFA4 gene expression in the MIN6 cell line, consistent with findings reported in the literature, we chose to examine the effects of compound **8** on glucose-stimulated insulin secretion (GSIS) in MIN6 cells.[8,16,17]

Compound **8** produced a concentration-dependent increase in glucose stimulated insulin secretion (Figure 5) under high glucose conditions (25 mM). The magnitude of the potentiation of insulin secretion observed under conditions of high glucose is noteworthy and is similar to that demonstrated in our laboratories with other insulin secretagogues including glibenclamide, linoleic acid, and FFA1 agonists.[18] The enhancement in glucose-stimulated insulin secretion by **8** was abolished in the presence of the selective FFA4 antagonist **39**.

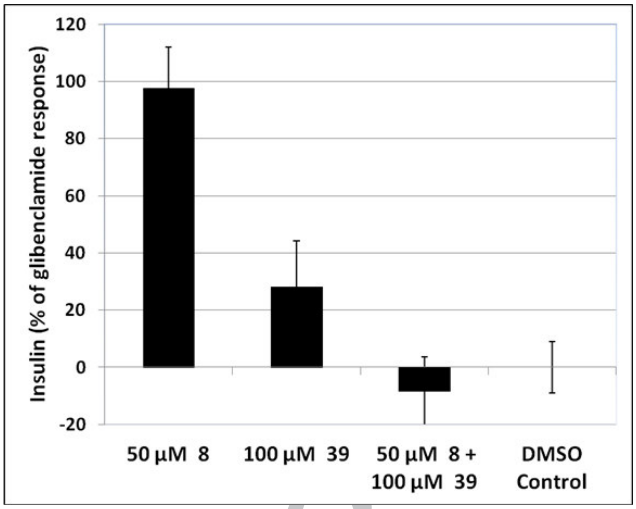


Figure 5. Effect of agonist **8** and antagonist **39** on insulin secretion from the MIN6 mouse insulinoma cell line in the presence of 25 mM glucose.

Finally, effects on GLP-1 secretion utilizing compounds **8** and **39** were studied in the human intestinal cell line NCI-H716 (Figure 6).[19] While literature reports vary on the levels of FFA4 expression in the NCI-H716 cell line,[8,20] our in-house data was supportive of the presence of the receptor.[17] Treatment with agonist agonist **8** provided a modest increase in GLP-1 secretion which could be blocked with the addition of the antagonist **39**.

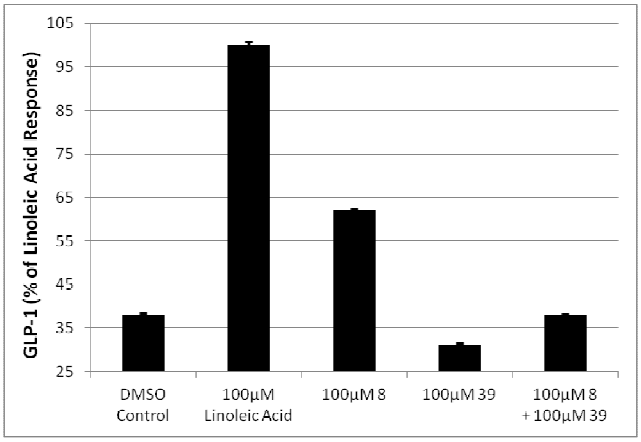


Figure 6. Effect of agonist **8** and antagonist **39** on GLP-1 secretion from the NCI-H716 cells.

In summary, the SAR of a series of diaryl sulfonamide FFA4 agonists was explored which resulted in the identification of a selective FFA4 agonist **8** (GSK137647A) and a selective FFA4 antagonist **39**. While the tool compounds did not have appropriate properties for utilization in an in vivo setting, experiments in secretory cell lines detail the utility of this highly synthetically accessible class of low molecular weight diaryl sulfonamides for the in vitro exploration of FFA4-mediated biology. Further

improvements in physiochemical properties for this series will need to be realized in order to access tool compounds suitable for in vivo experimentation.

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11. (a) Assay Conditions: U2OS (human osteosarcoma ATCC HTB-96; ATCC, USA) cells were grown in Dulbecco's modified Eagle's medium (DMEM)/F-12 supplemented with 10% fetal bovine serum and 2mM L-glutamine. Recombinant FFA4 (NM_001195755) expressing cells were generated by transducing U2OS cells with BacMam viruses encoding the respective receptor and the chimeric G-protein Gα16 according to established protocols. In brief, cells were plate to a density of $2x10^5$ cells/mL in cell culture medium containing human FFA4 (0.25%, v/v), mouse FFA4 (0.5%, v/v) or rat FFA4 (0.8%, v/v) BacMam virus. Gα16 BacMam virus (0.12%, v/v) was also transduced in preparation of recombinant human FFA4 cells to allow efficient coupling of human FFA4 receptor to phospholipase C pathway. This solution of cells/virus mixture was then plated at a density of 10^4 cells/well and cultured at 37°C, 5% CO_2, 95% humidity for 24h. Functional EC_{50} Studies cells were performed in cells incubated with Hank's buffered salt solution (HBSS) containing the cytoplasmic calcium indicator, Fluo-4 dye in the acetylmethyl form (4mM), 2.5mM probenecid and 250μM Brilliant Black at 37 °C for 60 min. Compound plates were generated containing 3% dimethyl sulfoxide in dye loading buffer. Compounds (i.e. FFA4 agonist GSK137647A or Histamine for host untransduced U2OS cells) were added to the cells at a 1:3 dilution and calcium mobilization was measured using a Fluorescence image plate reader (FLIPR, Molecular Devices). Data were converted into normalized responses with respect to assay standards GSK137647A (for FFA4) or Histamine (for Host U2OS cells). Data were further analyzed using a 4-parameter fit to calculate EC_{50} values. (b) Condreay, J. P.; Witherspoon, S. M.; Clay, W. C.; Kost. T. A. 1999. *Proc. Natl. Acad. Sci.* **1999**, *96*, 127.
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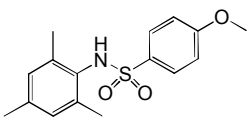


8 (GSK137647A)

FFA4: Human pEC_{50} = 6.3

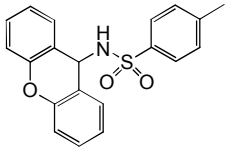
Mouse pEC_{50} = 6.2

Rat pEC_{50} = 6.1

Human FFA1, FFA2, & FFA3 pEC_{50}s < 4.5

Mouse FFA1, FFA2, & FFA3 pEC_{50}s < 4.5

Rat FFA1, FFA2, & FFA3 pEC_{50}s < 4.5



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FFA4: Human pIC_{50} = 7.1 ± 0.35

Human pEC_{50} < 4.5

Mouse pIC_{50} = 8.1

Rat pIC_{50} = 8.1

FFA1: Human pIC_{50} < 4.6

Human pEC_{50} < 4.3