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Conformationally constrained farnesoid X receptor (FXR) agonists: Naphthoic acid-based analogs of GW 4064

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ABSTRACT

Starting from the known FXR agonist GW 4064 **1a**, a series of stilbene replacements were prepared. The 6-substituted 1-naphthoic acid **1b** was an equipotent FXR agonist with improved developability parameters relative to **1a**. Analog **1b** also reduced the severity of cholestasis in the ANIT acute cholestatic rat model.

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The human nuclear receptor superfamily consists of 48 members which regulate gene transcription.[1] In addition to the classical steroid hormone receptors with known ligands such as the estrogen receptor, the so-called orphan receptors, identified by gene homology studies, have received considerable research focus with many of these orphans becoming adopted with the discovery of their natural ligands. The farnesoid X receptor (FXR, NR1H4, bile acid receptor (BAR)) is one such member. Bile acids, such as chenodeoxycholic acid (CDCA), are its natural ligands, inducing gene transcription typically through heterodimerization with the retinoid X receptors.[2,3]

FXR is expressed in liver, gall bladder, intestine, kidney, and adrenal glands,[4,5] where it serves as a key controller of bile acid homeostasis through its regulation of bile acid synthesis, conjugation, secretion, and absorption.[6,7] FXR also plays a role in lipid regulation, influencing triglyceride synthesis,[8,9] as well as lipoprotein metabolism and clearance.[10] Furthermore, FXR helps maintain glucose homeostasis, via its effects on gluconeogenesis,[11] insulin sensitization,[12] and glycogen synthesis.[13] Moreover, FXR helps prevent bacterial overgrowth in the intestine[14–16] and assists in regeneration of damaged liver.[17] With these diverse functions, FXR modulators could have utility in cholestasis,[18–20] liver fibrosis,[21–23] liver cancer,[24,25] atherosclerosis,[26,27] diabetes,[11] obesity, metabolic syndrome, cholesterol gallstone disease,[28] and inflammatory bowel disease.[14,15]

GlaxoSmithKline scientists have previously disclosed the discovery of the potent FXR agonist GW 4064 **1a**.[8] This compound, along with other FXR ligands like 6-ethyl-CDCA[29] and fexaramine,[30] has played a key role in helping define the physiological

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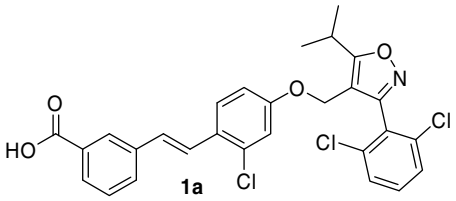
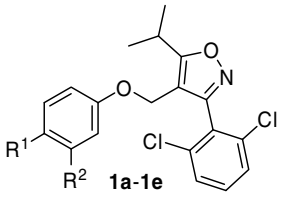
functions of FXR. Although a useful tool compound, GW 4064 is rapidly cleared in rats after oral administration, exhibiting a short terminal half-life and limited oral exposure ($F < 10\%$). Furthermore, its stilbene functionality is a potentially toxic pharmacophore.[31,32] Moreover, this functionality is responsible for its UV light instability (*vide infra*). To address these issues, GSK researchers sought to replace the stilbene group. Since the stilbene fragment provides considerable conformational rigidity to the agonist, a simple reduction of the double bond would likely lead to a decrease in transcriptional activity. Therefore, the exploration of stilbene replacements that maintained this limited flexibility was undertaken. Since the carboxylic acid on the stilbene likely resides in only one of its two predominant solution conformations in the FXR ligand binding domain, a design strategy that converted the stilbene to a naphthalene via cyclization from the benzoic acid aryl ring onto the distal carbon of the alkene would provide two potential synthetic targets **1b** and **1d** as shown in Table 1. Since the placement of the carboxylic acid group in GW 4064 **1a** may not be optimal, it was decided to prepare all four possible naphthalene derivatives **1b**–**1e** with the acidic moiety distal to the middle aryl ring. For synthetic ease, the analogs were prepared in the deschloro series.



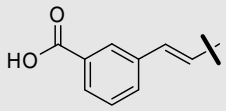
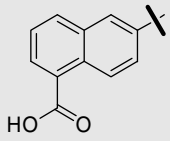
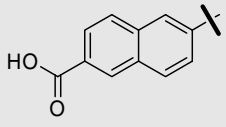
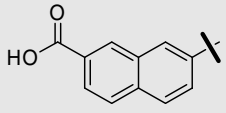
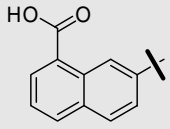
1a

The naphthalene carboxylic acid analogs **1b**–**1e** were prepared as depicted in Scheme 1.[33] The commercially available 6-hydroxy-1-naphthoic acid **2a** (X = OH) was converted to its methyl ester **3a** via acid catalysis. Then, the prepared phenol **3a** and the known phenols methyl 7-hydroxy-2-naphthalenecarboxylate **3c** (X = OH)[34,35] and methyl 7-hydroxy-1-naphthalenecarboxylate **3d**[36,37] (X = OH) were reacted with trifluoromethanesulfonic anhydride to produce the corresponding triflates. Palladium-mediated Suzuki coupling of the triflates **3a**, **3c**–**3d** (X = OTf), or the commercially available methyl 6-bromo-2-naphthoate **3b** (X = Br) with boron derivatives **4a** or **4b** gave the phenols **5a**–**5d**. Then, alkylation of the phenols **5a**–**5d** with the chloride **6b**, derived from the known alcohol **6a**,[8]

Table 1
Activation of human FXR



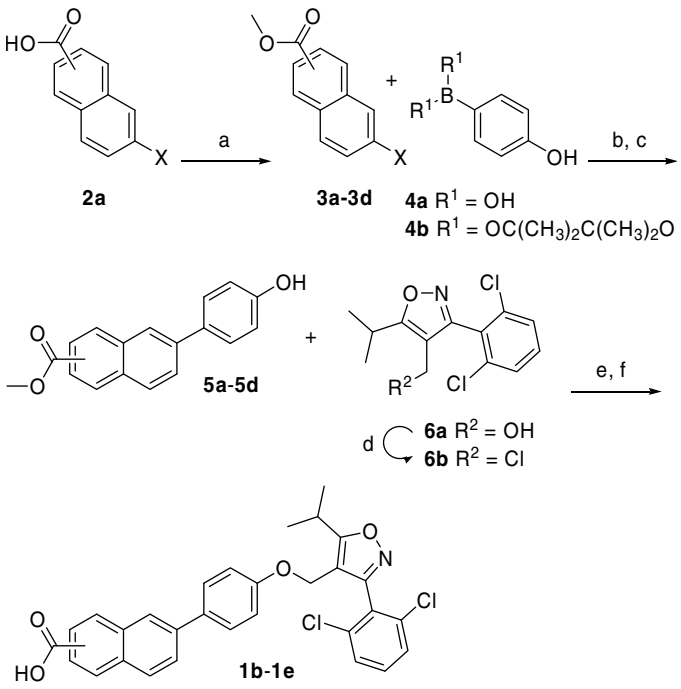
1a-1e

#	R^1	R^2	FXR FRET EC_{50}nM[a]	%Max[b]	FXR TT EC_{50} nM[c]	%Max[b]
1a		Cl	59	100	65	100
1b		H	87	134	68	104
1c		H	100	90	45	87
1d		H	420	69	500	90
1e		H	2300	52	3300	27

[a] FXR ligand-seeking assay measuring ligand-mediated interaction of the SRC-1 peptide (B-CPSSHSSLTERHKILHRLLQEGSPS-$CONH_2$) with the FXR $^{237-472}$LBD, using 5 nM biotinylated FXR LBD coupled to 5 nM allophycocyanin-labeled streptavidin and 10 nM biotinylated SRC-1 coupled to 5 nM Europium-labeled streptavidin as reagents in 10 mM DTT, 0.1 g/L BSA, 50 mM NaF, 50 mM MOPS, 1 mM EDTA, and 50 μM CHAPS, at pH 7.5. The EC_{50} values are the mean of at least two assays.

[b] Maximum percent efficacy of the test compound relative to FXR activation via GW 4064 **1a**.

[c] FXR transient transfection assay measuring the ligand-mediated luminescense resulting from FXR-induced transcription of a luciferase reporter. FXR and the luciferase reporter genes are transfected into African green monkey CV-1 kidney cells, then treated with test compound. The EC_{50} values are the mean of at least two assays.



Scheme 1. Reagents and conditions: (a) X=OH, $MeSO_3H$, MeOH, ↓↑, 96%; (b) X=OH, Tf_2O, pyridine, CH_2Cl_2, 0 °C to rt, or X=OH, Tf_2O, K_3PO_4, PhMe, H_2O, 46–93%; (c) X=OTf, **4a**, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 65–70 °C, 78–95%; or X=Br, **4b**, $PdCl_2(dppf)$, Na_2CO_3, DME, H_2O, 65–70 °C, 35%; (d) $SOCl_2$, hydroxybenzotriazole, CH_2Cl_2, 99%; (e) **6b**, Cs_2CO_3 or K_2CO_3, DMF, 65–70 °C, 68–75%; (f) NaOH, THF, MeOH or EtOH, H_2O, 60 °C or microwave at 100 °C, 74–94%.

produced the ethers. Subsequent base-catalyzed hydrolysis of the methyl esters afforded the naphthalene carboxylic acids **1b**–**1e**.

The structure–activity relationships of the naphalene carboxylic acid analogs are depicted in Table 1. The 1,6-disubstituted naphthalene derivative **1b** and the 2,7-disubstituted naphthalene **1d** each mimic one of the two predominant solution conformations of GW 4064 **1a**. The 6-substituted 1-naphthoic acid **1b** is a full agonist (%Max = 134% of GW 4064 **1a**) of FXR in the fluorescent resonance energy transfer (FRET) assay[8] for recruitment of the coactivator peptide of SRC-1 with an EC_{50} = 87 nM, and is essentially equipotent to GW 4064 **1a**. It is also a full agonist (%Max = 104%) of FXR in the transient transfection (TT) assay[8] with an EC_{50} = 68 nM, and likely approximates the active conformation of GW 4064 **1a**. In contrast, the 7-substituted 2-naphthoic acid **1d** is an approximately 5-fold less potent, partial agonist in the FRET assay (EC_{50} = 420 nM, %Max = 69%). Although still a full agonist (%Max = 90%) in the TT assay, it is still substantially less potent than **1b**.

Since it was not known if the orientation of the carboxylic acid of GW 4064 **1a** was optimal, the 2,6-disubstituted naphthalene derivative **1c** and the 1,7-disubstituted naphthalene **1e** were also prepared. The 6-substituted 2-naphthoic acid **1c** is also a full agonist in both the FRET (EC_{50} = 100 nM, %Max = 90%) and TT (EC_{50} = 45 nM, %Max = 87%) assays with similar potency to GW 4064 **1a**. Not surprisingly, based on the results from the other three naphthalene derivatives **1b**-**1d**, the 7-substituted 1-naphthoic acid **1e** was a weak, partial agonist of FXR (FRET EC_{50} = 2,300 nM, %Max = 52%; TT EC_{50} = 3,300 nM, %Max = 27%).

X-ray co-crystal structures of GW 4064 **1a** and naphthoic acid **1b** with FXR were obtained and are depicted in Figs. 1 and 2, respectively.[38] They shed further light on the agonist recognition elements of the FXR ligand binding domain as well as on the binding modes of GW 4064 derivatives. In both structures, the carboxylic acid groups form electrostatic interactions with ^{331}Arg in helix

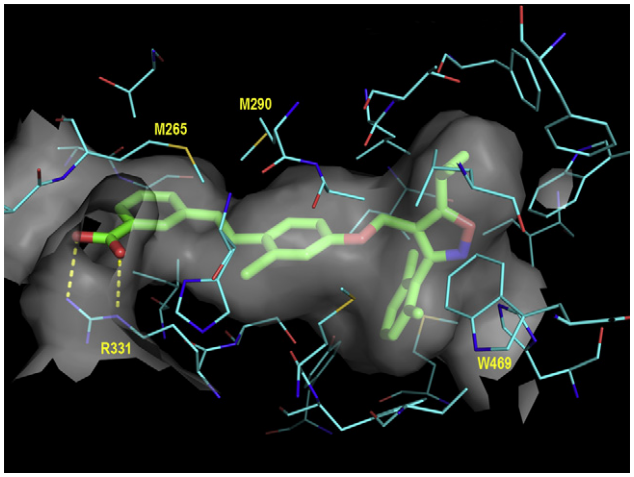


Figure 1. Ligand binding domain of the X-ray co-crystal structure of GW 4064 **1a** complexed with FXR. The FXR carbons are colored cyan with agonist **1a** carbons colored green. The semi-transparent gray surface represents the molecular surface, while hydrogen bonds are depicted as yellow dashed lines. The coordinates have been deposited in the Brookhaven Protein Data Bank (PDB code 3DCT). This figure was generated using PYMOL version 1.0 (Delano Scientific, www.pymol.org).

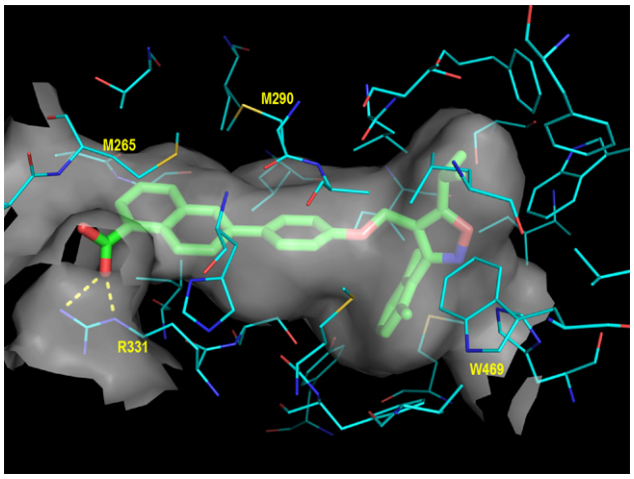


Figure 2. Ligand binding domain of the X-ray co-crystal structure of naphthoic acid **1b** complexed with FXR. The FXR carbons are colored cyan with agonist **1b** carbons colored green. The semi-transparent gray surface represents the molecular surface, while hydrogen bonds are depicted as yellow dashed lines. The coordinates have been deposited in the Brookhaven Protein Data Bank (PDB code 3DCU). This figure was generated using PYMOL version 1.00 (Delano Scientific, www.pymol.org).

5. This electrostatic interaction echoes the binding mode of the carboxylic acids of the bile acid natural ligands.[39] The carboxylic acid of GW 4064 **1a** is co-planar with its phenyl ring, with the two oxygen atoms of the carboxylate coordinating with one NH_2 and the ε-NH of the guanidine group of ^{331}Arg. In contrast, the C-8 hydrogen of the naphthalene of **1b** causes the carboxylic acid to twist out-of-the plane of the aryl ring, forcing a single oxygen of the carboxylate to coordinate to the same NH_2 and the ε-NH of the guanidine group of ^{331}Arg. However, this single oxygen interaction for **1b** is more geometrically favored than the dual interaction of **1a**. Furthermore, the 3-phenyl isoxazole moiety rests up against ^{454}Trp and ^{447}His on the C-terminal side of helix 10, with the isoxazole making an edge to face stacking interaction with ^{469}Trp located on helix 12 (AF2). The 2,6-dichloro substitution of the phenyl ring causes an out of plane twist relative to the isoxazole ring, biasing the ligand conformation to a rotamer population that better fits the active binding site of the ligand binding domain of FXR. In addition, the *iso*-propyl

group occupies a pocket formed by ^{284}Phe, ^{287}Leu, ^{454}Trp, and ^{461}Phe, leading to further stabilization of a protein conformer capable of recruiting co-activator proteins for gene transcription. Although the scaffoldings in between these two end fragments likely pick up some hydrophobic binding interactions, they mostly serve to appropriately display the terminal pharmacophores for optimal binding interactions. All of these interactions help stabilize the hydrophobic core of the receptor, which leads to an active conformation of the receptor capable of shedding co-repressors and recruiting co-activators to induce gene transcription.

It can be deduced from these structures that the flexible side chain guanidine of ^{331}Arg can move to best interact with the negative charge of acid moieties on agonists like **1c**. In contrast, as the acidic group is displayed farther from the vicinity of the positively charged guanidine, the electrostatic interaction with the negatively charged acid moiety will decrease, reducing the overall binding energy of the ligands like **1d** and **1e**.

Like GW 4064 **1a**, the naphthoic acid **1b** was quite selective for FXR versus other closely homologous nuclear receptors as shown in Table 2. It did exhibit modest interaction with the pregnane X receptor (PXR) with a selectivity ratio of 35–50 for FXR versus PXR.

Since, the naphthoic acid **1b** exhibited comparable efficacy and selectivity to GW 4064 **1a**, it was selected for further study. In contrast to GW 4064 **1a** which degrades the equivalent of ~10%/day as a solid in sunlight, the naphthoic acid **1b** exhibited minimal degradation under similar exposure conditions. Furthermore, its Cyp 450 inhibition profile in pooled human liver microsomal assays was acceptable (1A2 IC_{50} = 23,000 nM, 2C19 IC_{50} = 17,000 nM, 3A4 [midazolam] IC_{50} = 7200 nM, 3A4 [atorvastatin] IC_{50} = 4600 nM, 3A4 [nifedipine] IC_{50} = 8800 nM). In addition, it was also highly permeable in the Madin-Darby canine kidney cell (MDCK) absorption assay with an apparent permeability factor P_{APP} = 190 nm/s. In contrast to these encouraging developability parameters, **1b** was poorly soluble in fasted state-simulated intestinal fluid (FaS-SIF) at pH 6.5 (Sol. = 1000 ng/mL). Consequently, if dissolution after oral dosing was slow, the oral absorption might be poor.

The naphthoic acid **1b** was profiled in four species to ascertain its pharmacokinetic parameters. The agonist had low to moderate steady state volume of distributions in all species as shown in Table 3. The clearances in mouse (C_l = 7.0 mL/min/kg), dog (C_l = 2.0 mL/min/kg), and monkey (C_l = 5.5 mL/min/kg) were low, being less than one third of hepatic blood flow for each species, respectively. In contrast, the rat clearance was very high (C_l = 57 mL/min/kg), approaching hepatic blood flow for the rat. This clearance coupled with the moderate volume (V_{SS} = 1000 mL/kg) resulted in a very short terminal half-life in the rat ($t_{1/2}$ = 24 min). In contrast, the terminal half-life in the dog was greater than 4 h, while the mouse and monkey half-lives were in between (mouse $t_{1/2}$ = 96 min, monkey $t_{1/2}$ = 90 min) those of the dog and rat.

Table 2
Nuclear receptor selectivity of naphthoic acid FXR agonist **1b**

NR	Assay type[a]	**1b** XC_{50} nM	**1b** %Max[b]
ERα	FP	>20,000	—
ERβ	FP	>20,000	—
GR	FP	>20,000	—
LRH	FRET	>10,000	—
LXRα	FRET	>20,000	—
LXRβ	TT	>20,000	—
PPARα	SPA	>10,000	—
PPARγ	SPA	>10,000	—
PPARδ	TT	>10,000	—
PXR	SPA	3100	63
RORα	FRET	>10,000	—
TRβ	FRET	>20,000	—

[a] FP, fluorescence polarization assay; FRET, ligand-seeking assay measuring ligand-mediated interaction of the NR LBD with a coactivator peptide; SPA, scintillation proximity assay; TT, transient transfection assay. The XC_{50} values are the mean of at least two assays.
[b] Maximum percent efficacy of the test compound relative to activation via a standard.

Table 3
Pharmacokinetics of FXR agonist **1b**

Species	Dose[a] mg/kg	$t_{1/2}$[b] min	C_l[c] mL/min/kg	V_{SS}[d] mL/kg	F[e] %
Mouse	30	96	7.0	400	3.9
Rat	30	24	57	1000	7.7
Beagle Dog	5	250	2.0	480	56
Cynomolgus Monkey	5	90	5.5	350	34

[a] Dose is the amount of **1b** dissolved into the formulation solution for oral administration.
[b] $t_{1/2}$ is the i.v. terminal half-life dosed as a solution. All in vivo pharmacokinetic values are the mean of two experiments.
[c] C_l is the i.v. total clearance.
[d] V_{SS} is the i.v. steady state volume of distribution.
[e] F is the oral bioavailability.

The rodent oral bioavailabilities of **1b** were poor (mouse F = 3.9%, rat F = 7.7%). In contrast, in higher mammals, **1b** exhibited good oral exposure (dog F = 56%, monkey F = 34%). It is possible that the increase in acidity when **1b** reaches the stomach causes precipitation of **1b** as its free acid. With its even poorer solubility at acidic pH (solubility in simulated gastric fluid at pH 1 SGF = <1 ng/mL) than as the carboxylate at neutral pH, a slow dissolution rate might explain the low oral bioavailability, despite its high permeability. In higher mammals, a longer GI transit time could account for the much improved oral absorption.

The liver, intestine, and kidneys can be key tissues for metabolizing xenobiotics, and orally administered drugs can obtain significant concentrations in these tissues. Since FXR is expressed in liver, intestine, and kidney, significant systemic exposure of FXR modulators may not be necessary to realize a pharmacodynamic response. Furthermore, since activated FXR can amplify its signal via gene transcription, continuous exposure may not be necessary for the realization of a therapeutic effect. Therefore, despite its limited pharmacokinetics in rat, naphthoic acid **1b** was profiled in the α-naphthyl-isothiocyanate (ANIT) chemically induced rat acute cholestasis model.[18] Rats were dosed with ANIT (50 mg/kg p.o.), then sacrificed 48 h later for analysis. The rats received vehicle or the sodium salt of **1b** bid p.o. for 4 days, starting 2 days prior to ANIT administration. ANIT treatment significantly elevated the liver function serum markers alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), total cholesterol (TC), HDL-C, triglycerides (TG), and bile acids (BA), and caused peribiliary inflammation, edema, bile duct necrosis, and portal disorganization. FXR agonist **1b**, at doses of 100 and 300 mg/kg, significantly reduced AP, ALT, AST, GGT, HDL-C, BA, and direct bilirubin (DBIL). Furthermore, histological exams revealed that **1b** reduced peribiliary inflammation, edema, bile duct necrosis, and portal disorganization.

In summary, a series of conformationally constrained GW 4064 **1a** analogs were synthesized as potential modulators of FXR. The 6-substituted 1-naphthoic acid GSK8062 **1b** and the 6-substituted 2-naphthoic acid **1c** were equipotent full FXR agonists. Agonist **1b** was quite selective versus related nuclear receptors. It was also well absorbed in dog and monkey. Despite poor oral exposure in rodents, naphthoic acid **1b** reduced the severity of cholestasis in the ANIT acute cholestatic rat model. Information gained from these studies should prove to be useful in the design of other FXR modulators. Furthermore, these tools may prove useful in further defining the physiological roles of FXR.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.073.

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35. Although methyl 7-hydroxy-2-naphthoate **3c** is known in the literature, it was prepared from commercially available 7-methoxy-2-naphthol in three synthetic steps. First, 7-methoxy-2-naphthol was converted into the triflate with trifluoromethanesulfonic anhydride. Then, palladium-mediated carbonylation of the triflate under a carbon monoxide atmosphere in methanol and *N*,*N*-dimethylformamide provided methyl 7-methoxy-2-naphthoate. Finally, cleavage of the methyl ether with boron tribromide gave the desired methyl 7-hydroxy-2-naphthoate **3c**.
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