Bioorganic & Medicinal Chemistry Letters 19 (2009) 2969–2973

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Substituted isoxazole analogs of farnesoid X receptor (FXR) agonist GW4064

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ARTICLE INFO

Article history:
Received 9 February 2009
Revised 13 April 2009
Accepted 14 April 2009
Available online 18 April 2009

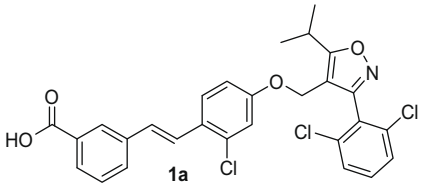
Keywords:
Farnesoid X receptor agonist
FXR
Nuclear receptor modulator
FXR X-ray co-crystal structure
GW4064
Bile acid receptor
NR1H4

ABSTRACT

Starting from the known FXR agonist GW 4064 **1a**, a series of alternately 3,5-substituted isoxazoles was prepared. Several of these analogs were potent full FXR agonists. A subset of this series, with a tether between the isoxazole ring and the 3-position aryl substituent, were equipotent FXR agonists to GW 4064 **1a**, with the 2,6-dimethyl phenol analog **1t** having greater FRET FXR potency than GW 4064 **1a**.

The farnesoid X receptor is a nuclear receptor expressed in liver, gall bladder, intestine, kidney, and adrenal glands.[1,2] Its function is critical for the regulation of bile acids as well as for lipid metabolism.[3,4] Furthermore, FXR also plays an important role in glucose homeostasis.[5] Because of the involvement of FXR in these physiologies, FXR modulators could have great potential in the treatment of cholestasis,[6–8] liver fibrosis,[9–11] and diabetes.[5] GlaxoSmithKline scientists have previously disclosed the discovery of GW4064 **1a**,[12] a potent FXR agonist. GW4064 **1a** has proven a useful tool compound to explore FXR biology, however; the stilbene moiety, contained in GW 4064 **1a**, is unstable to UV light exposure, as well as being a potentially toxic pharmacophore, lessening its potential for further development.[13]

Nonetheless, the X-ray co-crystal structure of GW 4064 **1a** with FXR revealed that the 2,6-dichlorophenyl isoxazole fragment of GW 4064 **1a** made key binding interactions with helices 10 and 12 of FXR,[13] implying that modifications in this portion of the agonist could affect co-activator/co-repressor recruitment to this nuclear receptor. Thus, employing GW4064 **1a** as a lead, GSK scientists made a series of analogs substituted at the 3- and/or 5-positions of the isoxazole ring, with the hopes that these compounds could exhibit altered modulation of FXR function. Furthermore, they might have increased potency and/or be more metabolically stable. Any interesting discoveries from these analogs might then be applied in combination with other improvements to the lead molecule to arrive at a clinical candidate.



1a

Various analogs with different functionalities at the 3- and 5-positions of the isoxazole were initially prepared employing the

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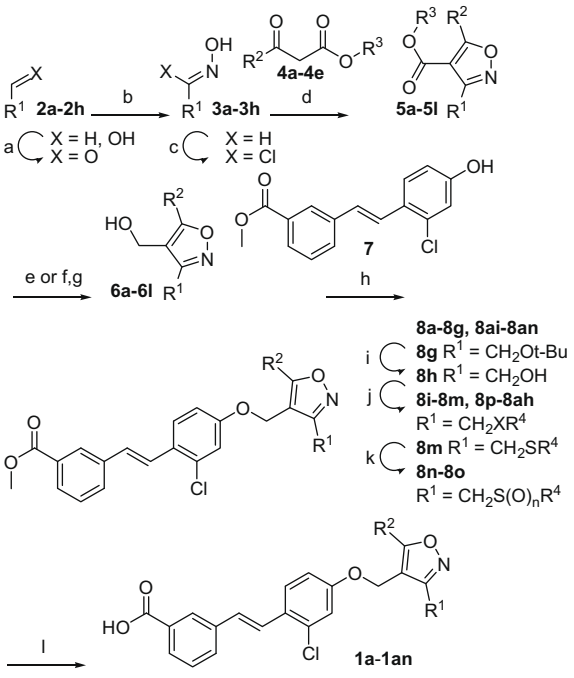
doi:10.1016/j.bmcl.2009.04.047

synthetic route disclosed in the original Glaxo Wellcome article,[12] starting from different commercially available or known aldehydes **2a**–**e** (X=O) or alcohols **2f**–**h** (X=H, OH)[14] as depicted in Scheme 1. This multi-step procedure, involving aldehyde oxime formation, chlorination, base promoted β-keto-ester **4a** mediated isoxazole formation, and aluminum hydride mediated ester reduction, was used to produce the alcohols **6a**, and **6c**–**h**. A single modification to this sequence was utilized to prepare alcohol **6b**. An attempt to reduce the ester **5b** with di-*iso*-butylaluminum hydride failed to give clean conversion to the desired alcohol **6b**. Therefore, an alternative approach involving hydrolysis of the ester, followed by conversion of the resulting acid to its mixed anhydride with isopropyl chloroformate, and subsequent reduction with sodium borohydride afforded the alcohol **6b**. By employing different β-ketoesters **4b**–**e**, synthesized according to literature procedures,[15,16] various alcohols **6i**–**l** with different alkyl groups at the 5-position of the isoxazole were also prepared. The alcohols **6a**–**l** were then coupled with phenol **7**[17] via a Mitsunobu reaction protocol to give the esters **8a**–**g** and **8ai**–**an**.[18] The penultimate esters were then hydrolyzed to produce the desired acids **1a**–**g** and **1ai**–**an**.[19]

Alternatively, extended heteroatom linked 3-position isoxazoles were prepared by a modified procedure, allowing more rapid synthesis of analogs as outlined below. The *tert*-butyl ether **8g** was converted to the alcohol **8h** via acid-catalyzed cleavage of the *tert*-butyl fragment. Then, this key intermediate **8h** was utilized in multiple Mitsunobu reactions with various substituted phenols **9a**–**b**, **9f**–**w**, trifluoroacetamides **9c** and **9d**, thiophenol **9e**, and thiotetrazole **9x**, to produce the esters **8i**–**m** and **8p**–**ah**. The low acidity of most anilines precludes there successful utilization in standard Mitsunobu reactions, therefore, the commercially available anilines **9c**–**d** (X=NH) were converted to their corresponding trifluoroacetamides **9c**–**d** (X=NCOCF$_3$) prior to the Mitsunobu reaction. The esters **8i**–**m** and **8p**–**ah** were then hydrolyzed to yield the acids **1i**–**m** and **1p**–**ah**, with concomitant hydrolysis of the trifluoroacetamide activating groups for **8k** and **8l**.



Scheme 1. Reagents and conditions: (a) SO_3·pyridine, DMSO, NEt_3, CH_2Cl_2, 0 °C–rt; (b) NH_2OH·HCl, NaOH, EtOH, H_2O, 31–88% for 2 steps; (c) NCS, DMF; (d) **4a**–**e**, NaOMe, THF, 21–63% for 2 steps; (e) *i*Bu_2AlH, THF, 0 °C–rt, 14–99%; (f) **6b**, NaOH, H_2O, MeOH, THF, microwave@100 °C, 81%; (g) *i*PrO(C=O)Cl, THF, NEt_3; $NaBH_4$, H_2O, 59%; (h) **7**, DIAD, PPh_3, toluene, microwave@80 °C, 21–76%; (i) TFA, CH_2Cl_2, 78%; (j) DIAD, PPh_3, phenols **9a**–**b** and **9f**–**w**, trifluoroacetamides **9c**–**d**, thiophenol **9e**, or thiotetrazole **9x**, toluene, microwave@80 °C, 22–73%; (k) *m*-CPBA, CH_2Cl_2, 0 °C–rt, 40% and 40%, respectively; (l) NaOH, MeOH, THF, H_2O, microwave@100 °C, 42–99%.

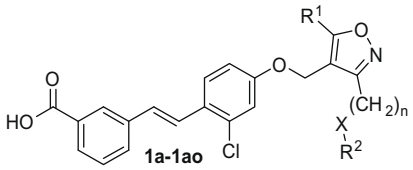
The thiophenol **8m** was oxidized with approximately 2 equiv of 3-chloroperoxybenzoic acid to yield a mixture of the sulfoxide **8n** and the sulfone **8o**, which were separated by flash column chromatography. The esters **8n** and **8o** were then hydrolyzed to yield the acids **1n** and **1o**.

The structure–activity relationships of the isoxazole analogs **1a**–**an** are depicted in Table 1. A X-ray co-crystal structure of GW 4064 **1a** revealed little room for further substitution of the terminal aryl ring.[13] Moreover, since *ortho*, *ortho* disubstitution of the terminal phenyl ring is important to conformationally twist the aryl ring out of plane of the isoxazole ring, a dichloropyridine analog **1b** was prepared with the hope of increasing water solubility and possibly forming a hydrogen bond interaction with the receptor. This substitution had little effect on potency or efficacy in either the fluorescent resonance energy transfer (FRET) assay for recruitment of an SRC-1 peptide (EC_{50} = 150 nM, %Max = 97) or the transient transfection (TT) assay (EC_{50} = 52 nM, %Max = 88), despite the higher desolvation penalty to strip hydrogen bonded water molecules from the pyridine of analog **1b** relative to phenyl moiety of lead **1a** (TT EC_{50} = 65 nM), implying that the pyridine nitrogen might be forming a weak interaction with the nuclear receptor.

The X-ray co-crystal structure of GW 4064 **1a**, with the FXR ligand binding domain, further revealed some open space adjacent to the terminal aryl ring, that might be exploited to enhance the potency or efficacy of the lead **1a**, by employing a tether to allow more flexibility in the ligand's ability to interact with the receptor. Thus, several extended phenyl analogs were prepared to explore this hypothesis. The naked benzyl compound **1c** (TT EC_{50} = 230 nM) was less potent than the lead **1a**. This potency drop is likely the sum of two factors. A one carbon tether possibly places the terminal phenyl at the wrong angle to properly fit in the receptor subpocket. A second reason for the decreased potency could be the lack of optimal substitution on the phenyl ring. The more flexible and longer phenethyl derivative **1d** (TT EC_{50} = 93 nM) was more potent than the one atom tether **1c**, despite a higher entropic penalty to bind in a single conformation than its less flexible analog **1c**. This flexibility likely allows the two atom tether of **1d** to kink around, placing its phenyl group in an acceptable position in the protein. In contrast, the phenpropyl derivative **1e** (TT, EC_{50} = 1200 nM) is 10-fold less potent than its shorter analog **1d**. It is also a partial agonist. This decrease in activity could reflect the higher entropic penalty to freeze out the appropriate binding conformer of the longer tether **1e**. Yet, more likely, the phenpropyl derivative **1e** is too large to fit into the receptor subpocket and distorts the active receptor conformation, altering its ability to recruit co-activators and resulting in lower efficacy (FRET%Max = 44, TT%Max = 68).

Appropriate substitution of the two atom tether phenyl ring could enhance interactions with the receptor, leading to improved efficacy and/or potency. The 2,6-dichlorophenyl analog **1f** (TT, EC_{50} = 48 nM) was twice as potent as the naked phenyl compound **1d** and equipotent to the starting lead GW 4064 **1a**. A heteroatom was introduced into the tether between the isoxazole and the terminal phenyl ring to facilitate synthesis of substituted terminal aryl analogs. The naked phenyl ether **1i** (TT, EC_{50} = 1200 nM) was substantially less potent than the all carbon linker **1d**, possibly because of a higher enthalpic penalty to strip away water molecules upon receptor binding. Analog **1i** serves as a baseline for the effect of phenyl ring substitution. Interestingly, the *tert*-butyl ether synthetic intermediate **1g** (TT, EC_{50} = 600 nM) is slightly more potent than the phenyl ether **1i**. In contrast, the alcohol **1h** (TT,

Table 1
Activation of human FXR



#	R^1	*n*	X	R^2	FXR FRET EC_{50}[a] (nM)	%Max[b]	FXR TT EC_{50}[c] (nM)	%Max[b]
1a	*i*-Pr	0	—	2,6-Dichlorophenyl	59	100	65	100
1b	*i*-Pr	0	—	2,6-Dichloro-4-aza-phenyl	150	97	52	88
1c	*i*-Pr	1	—	Phenyl	550	67	230	120
1d	*i*-Pr	2	—	Phenyl	320	96	93	110
1e	*i*-Pr	3	—	Phenyl	4400	44	1200	68
1f	*i*-Pr	2	—	2,6-Dichlorophenyl	150	105	48	104
1g	*i*-Pr	1	O	*t*-Bu	660	77	600	132
1h	*i*-Pr	1	O	H	7800	22	>10,000	—
1i	*i*-Pr	1	O	Phenyl	1100	55	1200	27
1j	*i*-Pr	1	O	2,6-Dichlorophenyl	78	102	89	89
1k	*i*-Pr	1	NH	2,6-Dichlorophenyl	72	104	140	70
1l	*i*-Pr	1	NH	2,6-Dichloro-4-aza-phenyl	400	68	420	31
1m	*i*-Pr	1	S	2,6-Dichlorophenyl	51	110	100	86
1n	*i*-Pr	1	SO	2,6-Dichlorophenyl	28	110	72	100
1o	*i*-Pr	1	SO_2	2,6-Dichlorophenyl	150	100	390	68
1p	*i*-Pr	1	O	2-Chlorophenyl	460	82	82[e]	71
1q	*i*-Pr	1	O	3-Chlorophenyl	310	51[d]	2000[e]	42
1r	*i*-Pr	1	O	4-Chlorophenyl	450	58	1300	53
1s	*i*-Pr	1	O	2-Chloro-6-methylphenyl	48	106	150	111
1t	*i*-Pr	1	O	2,6-Dimethylphenyl	17	110	56	118
1u	*i*-Pr	1	O	2-Chloro-6-fluorophenyl	74	103	290	121
1v	*i*-Pr	1	O	2,6-Difluorophenyl	2200	100	720	112
1w	*i*-Pr	1	O	2,3-Dichlorophenyl	480	82	630	38
1x	*i*-Pr	1	O	2,4-Dichlorophenyl	620	72	690	39
1y	*i*-Pr	1	O	2,5-Dichlorophenyl	1000	83	900	37
1z	*i*-Pr	1	O	3,4-Dichlorophenyl	1100	57	>10,000[e]	—
1aa	*i*-Pr	1	O	3,5-Dichlorophenyl	1100	40	>10,000[e]	—
1ab	*i*-Pr	1	O	2,6-Dichloro-4-fluorophenyl	87	112	540[e]	56
1ac	*i*-Pr	1	O	2,4,6-Trifluorophenyl	110	106	300	95
1ad	*i*-Pr	1	O	2,4,6-Trichlorophenyl	58	101	200	68
1ae	*i*-Pr	1	O	2,6-Dimethyl-4-chlorophenyl	130	99	110	66
1af	*i*-Pr	1	O	2-Naphthyl	930	65	2300	27
1ag	*i*-Pr	1	O	1-Naphthyl	960	79	1200	34
1ah	*i*-Pr	1	S	1-Methyltetrazole	930	59	2100	45
1ai	*i*-Pr	0	*t*-*c*-Pr	2,6-Dichlorophenyl	2000	38	>10,000[e]	—
1aj	*c*-Bu	0	—	2,6-Dichlorophenyl	410	67	140	100
1ak	*c*-Pentyl	0	—	2,6-Dichlorophenyl	560	81	240	75
1al	3-Pentyl	0	—	2,6-Dichlorophenyl	2200	82	280[e]	71
1am	(*S*)-*s*-Bu	0	—	2,6-Dichlorophenyl	190	96	290	99
1an	(*R*)-*s*-Bu	0	—	2,6-Dichlorophenyl	105	100	93	105

[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 luminescence 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.

[d] Entry represents the result of one assay.

[e] The FRET assay is a cell free assay measuring recruitment of a SRC-1 co-activator peptide. The TT assay is a reporter assay employing African green monkey CV-1 kidney cells containing a mixture of nuclear receptor co-activator and co-repressor proteins. Differences between FRET and TT values could arise due to recruitment of different co-activators/co-repressors resident in the CV-1 kidney cell versus the cell free FRET assay.

EC_{50} = >10,000 nM) is a poor agonist, implying that this receptor subpocket appears to prefer hydrophobic ligands. In contrast to the drop in activity of the phenyl ether **1i** relative to the naked phenyl **1d**, the dichloroaryl ether analog **1j** (TT, EC_{50} = 89 nM) exhibited similar potency to the 2,6-dichlorophenyl derivative **1f** and GW 4064 **1a**. Possibly, analog **1j** pays a lower desolvation penalty upon receptor binding, as the *ortho*, *ortho* disubstitution in **1j** could shield the ether oxygen from hydrogen bonding to bulk water. Furthermore, substitution of the ether oxygen of compound **1j** with nitrogen or sulfur is well tolerated as the aniline **1k** (TT, EC_{50} = 140 nM) and the thioether **1m** (TT, EC_{50} = 100 nM) have similar activity to the ether **1j**.

At physiological pH, the pyridine **1l** (TT, EC_{50} = 420 nM) likely bears at least a partial positive charge and the tetrazole **1ah** (TT, EC_{50} = 2100 nM) is quite hydrophilic. Reinforcing the hypothesis that this receptor subpocket prefers hydrophobic ligands, these charged analogs are weak, partial agonists of FXR. In contrast, the tether region tolerates some hydrophilicity, as the racemic sulfoxide **1n** (TT, EC_{50} = 72 nM) and the sulfone **1o** (TT, EC_{50} = 390 nM) are potent, full agonists of FXR.

In an effort to discover the optimal substitution for the two atom tethered aryl ring, a systematic exploration of the receptor subpocket was undertaken. The *ortho*-chlorophenyl analog **1p** (TT, EC_{50} = 82 nM) was a potent full FXR agonist. In contrast, the *meta*-chloro- and *para*-chlorophenyl analogs **1q** (TT, EC_{50} = 2000 nM) and **1r** (TT, EC_{50} = 1300 nM) were less potent and only partial agonists. Furthermore, the 2,3-, 2,4-, 2,5-, 3,4-, and 3,5-dichlorophenyl analogs **1w**–**aa** are also less potent agonists than the 2,6-derivative **1j**, implying that the protein cannot accommodate large *meta* or *para* substituents in this region. The 2-naphthyl- and 1-naphthyl derivatives **1af** (TT, EC_{50} = 2300 nM) and **1ag** (TT, EC_{50} = 1200 nM) give further support to this conclusion.

In addition to exploring the optimal substitution pattern of the terminal aryl ring, the nature of the substituents was also altered to ascertain the best complementarity between the receptor and ligand. Thus, the 2-chloro-6-methylphenyl-, the 2,6-dimethyl phenyl-, the 2-chloro-6-fluorophenyl-, and the difluorophenyl analogs **1s**–**v**, respectively, were prepared. The methyl substituted analogs **1s** (EC_{50} = 150 nM) and **1t** (EC_{50} = 56 nM) maintain potency, while there is a diminishment in potency as chlorines are sequentially replaced by fluorines as in analogs **1u** (EC_{50} = 290 nM) and **1v** (EC_{50} = 720 nM). From these results, it can be inferred that the electronic nature of the 2- and 6-substituents are less important than their steric size for receptor potency, as the more electronegativity, but smaller fluorine group is less potent than the larger, but more electropositive methyl fragment, in addition to being less active than the large, electronegative chlorine moiety. The 2,4,6-trisubstituted analogs **1ab**–**ae** were also prepared with the goal of shielding a potential oxidatively soft site from metabolism. Unfortunately, trisubstitution did not enhance potency.

Since the two atom tether likely exists in only one of its three lowest energy conformers when bound to the receptor, biasing the ligand by rotamer constraint should lower the entropic cost of binding, increasing analog potency. A *trans*-cyclopropyl tether analog **1ai** was synthesized as an *anti*-rotamer mimic to try to take advantage of this possibility, but this analog was a poor agonist. Thus, the preferred rotamer of **1f** is either not the anti-rotamer or the constraining scaffolding is not accommodated by the receptor.

The X-ray co-crystal structure of GW 4064 **1a** with the FXR ligand binding domain revealed a small hydrophobic space near the *iso*-propyl moiety of the isoxazole. Thus, several small 5-alkylisoxazole analogs were prepared with the hope of enhancing the interaction of the ligand with the receptor. Furthermore, these analogs might be sterically less susceptible to oxidative metabolism, in contrast to the methine CH of the *iso*-propyl group. Both the cyclobutyl **1aj** (TT, EC_{50} = 140 nM) and cyclopentyl **1ak** (TT, EC_{50} = 240 nM) were slightly less potent than the lead **1a**. Of the two *sec*-butyl derivatives **1am** (TT, EC_{50} = 290 nM) and **1an** (TT, EC_{50} = 93 nM), the *R*-isomer was slightly more potent than the *S*-isomer and equipotent to GW 4064 **1a**.[20] It was hoped that the 3-pentyl analog **1al**, being slightly bigger that the *sec*-butyl analog **1an**, would have the advantage of completely filling the receptor subpocket, yet be achiral. Unfortunately, **1al** (TT, EC_{50} = 280 nM) although a full agonist, was less potent than GW 4064 **1a**. Thus, although attempts were made to maximize the interaction of the 5-isoxazole substituent with the FXR ligand binding domain, there was no enhancement in activity realized by these analogs.

A X-ray co-crystal structure of sulfoxide **1n** with the ligand binding domain of FXR and a SRC-1 co-activator peptide was solved. It is depicted in Figure 1, overlayed with the X-ray co-crystal structure of GW 4064 **1a**,[13] and these structures provide insight into the structure–activity relationships of these analogs. The vast majority of the binding sites are very similar between GW 4064 **1a** and sulfoxide **1n**. The carboxylic acids of the compounds both

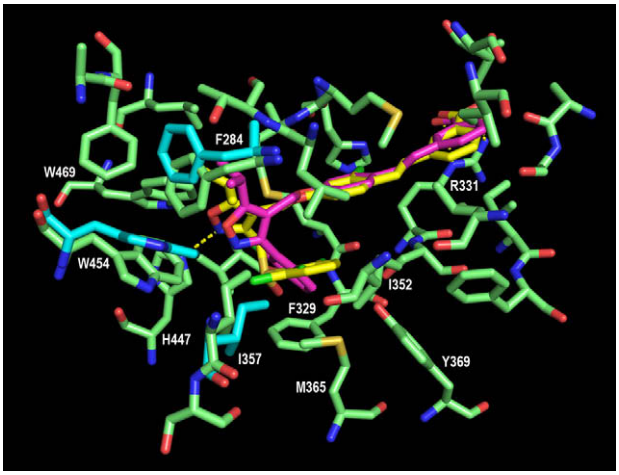


Figure 1. Superimposed ligand binding domains of the X-ray co-crystal structures of agonist **1n** (ligand **1n** carbons colored yellow) and GW 4064 **1a** (ligand **1a** carbons colored magenta) complexed with FXR. The FXR carbons from the co-crystal structure with agonist **1n** are colored green, with the FXR 'shifted' residues from the co-crystal structure with GW4064 **1a** colored cyan. The hydrogen bonds are depicted as yellow dashed lines. The coordinates have been deposited in the Brookhaven Protein Data Bank (**1a** PDB code 3DCT, **1n** PDB code 3GD2). This figure was generated using PYMOL version 1.00 (Delano Scientific, www.pymol.org).

interact with the guanidine of ^{331}Arg, while the stilbenoid moieties also have similar interactions with ^{335}Ile, ^{348}Leu, ^{287}Leu, ^{328}Met, and ^{290}Met. Only a few side chain residues of amino acids exhibit significantly different conformations (rotomers) between the two structures, while there are no major changes in the amino acid backbone conformations of the two complexes. The altered residues are ^{284}Phe, ^{357}Ile, and ^{454}Trp, which rotate to accommodate the larger ligand. The conformation of the thiophenol oxide ring of **1n** is significantly different from the phenyl ring of **1a**, with the dichlorophenyl ring of analog **1n** in a more 'stacked' arrangement to the isoxazole compared to the orthogonal disposition of the aryl ring in lead **1a**. However, despite this different arrangement, the dichlorophenyl group of sulfoxide **1n** occupies similar space in the binding site of FXR as the aryl ring of lead **1a**, interacting with ^{365}Met, ^{369}Tyr, ^{329}Phe, ^{352}Ile, ^{357}Ile, and ^{454}Trp. Most of these residues show little movement between the two co-crystal structures, but ^{357}Ile and ^{454}Trp do shift in the sulfoxide **1n** structure relative to the GW 4064 **1a** structure. The positional change of ^{454}Trp cascades to ^{284}Phe, which moves to maintain a face to edge aromatic interaction with the indole of ^{454}Trp. In order to accommodate the larger linker, the plane of the isoxazole has shifted slightly, but the *iso*-propyl of analog **1n** remains in a similar location. The shift of the isoxazole, places it in position to accept a hydrogen bond from the imidazole NH of ^{447}His. This hydrogen bond of the isoxazole nitrogen of analog **1n** appears stronger than the potential hydrogen bond of the isoxazole moiety in the GW 4064 **1a** complex, but this change also results in a weaker interaction between the isoxazole of compound **1n** and the indole of ^{454}Trp. Both ^{447}His and ^{469}Trp (on the AF2 helix) remain in a face to edge stacking arrangement, believed to be important for coactivator recruitment, like in the GW 4064 **1a** structure.

Most of the analogs shown in Table 1 were tested in a panel of nuclear receptor assays. In general, the compounds were at least 100-fold selective for FXR versus the other nuclear receptors. The series did have some activity in the PXR assay, where several compounds were weak full agonists. The activities of analog **1n** are shown in Table 2 as an example.

Several compounds of the series were selected for pharmacokinetic studies in rats. As shown in Table 3, all of the analogs profiled

Table 2
Nuclear receptor selectivity of FXR agonist **1n**

NR	Assay type[a]	**1n** XC_{50} (nM)	**1n** %Max[b]
LRH	FRET	4200	46
LXRα	FRET	1400	33
LXRβ	SPA	2500	101
PXR	SPA	960	100
RORα	FRET	>10,000	—

[a] FRET, ligand seeking assay measuring ligand-mediated interaction of the NR LBD with a coactivator peptide; SPA, scintillation proximity 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 agonists in rat

#	$t_{1/2}$[a] (min)	C_l[b] (mL/min/kg)	V_{SS}[c] (mL/kg)	F[d] (%)
1b	52	48	1100	7.8
1j	130	20	1200	9.2
1k	140	16	2100	2.6
1ab	140	15	1400	1.9

[a] $t_{1/2}$ is the iv terminal half-life dosed as a solution. All in vivo pharmacokinetic values are the mean of two experiments.
[b] C_l is the iv total clearance.
[c] V_{SS} is the iv steady state volume of distribution.
[d] F is the oral bioavailability.

exhibited medium volumes of distribution (V_{SS} = 1100–2100 mL/kg), with the three two atom tether analogs (**1j**, **1k**, and **1ab**) having moderate clearances (C_l = 15–20 mL/min/kg) of less than one third of hepatic blood flow in the rat, translating into terminal half-lives ($t_{1/2}$ = 130–140 min) of greater than 2 h. Unfortunately, all of the analogs had poor oral exposure (F = 1.9–9.2%). These clearances were significantly lower than the starting lead GW 4064 **1a** (C_l = 36 mL/min/kg). Furthermore, despite similar clearances between phenol **1j** and aniline **1k**, the potentially more soluble aniline **1k** (F = 2.6%) had a lower oral bioavailability than the phenol **1j** (F = 9.2%). Possibly, the stilbene moiety that is present in all of the analogs is detrimental to oral exposure and overrides any changes elsewhere in the agonists.

In summary, a series of analogs of GW 4064 **1a** were synthesized as potential modulators of FXR. Incorporation of a two atom tether between the isoxazole and the 2,6-dichlorophenyl ring produced a number of highly efficacious FXR agonists with comparable efficacy and potency to the original lead **1a**. Introduction of a heteroatom into the linker allowed the rapid production of terminal ring analogs. Several of the compounds with a two atom tether had lower in vivo clearances than GW 4064 **1a**. Structure–activity relationships identified from this work should prove useful in the design of improved FXR modulators based on GW 4064 **1a**.

References and notes

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14. The alcohol **2h** was prepared from commercially available (*E*)-2,6-dichlorocinnamic acid in three synthetic steps. First, the acid was esterified employing the Fischer method with catalytic sulfuric acid in methanol (94% yield). Then, the resulting unsaturated methyl ester was converted to the methyl 2-(2,6-dichlorophenyl)cyclopropanecarboxylate via its addition to the in situ generated sulfur ylide prepared by adding sodium hydride to trimethylsulfoxonium iodide in dimethyl sulfoxide (26% yield). Finally, reduction of the ester with di-*iso*-butylaluminum hydride in tetrahydrofuran afforded the desired [2-(2,6-dichlorophenyl)cyclopropyl]methanol **2h** (78% yield).
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18. Mitsunobu reactions in this Letter were performed under the following conditions: a mixture of 1.0 equiv each of the alcohol, the phenol, di-*iso*-propyl diazodicarboxylate, and triphenylphosphine were placed in a microwave reaction vessel and toluene was added to make a 0.1 M mixture. Then, the vessel was sealed and heated in a microwave reactor between 85 and 100 °C for between 600 and 1000 s. The resulting mixture was then concentrated and the residue was purified by silica gel chromatography with gradients of ethyl acetate in hexanes.
19. Saponification reactions in this Letter were performed under the following conditions: The ester was placed in a microwave reaction vessel and a 2:1 mixture of tetrahydrofuran and methanol was added, followed by 1.5 equiv of 1 M sodium hydroxide. Then, the vessel was sealed and heated in a microwave reactor between 100 and 120 °C for 500 s. The resulting mixture was neutralized with 1 M hydrochloric acid and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and concentrated.
20. The stereoisomers of the *sec*-butyl group were separated at the alcohol **6l** stage and carried through the rest of the synthesis separately to give analogs **1am** and **1an**.