Letters

Discovery of XL335 (WAY-362450), a Highly Potent, Selective, and Orally Active Agonist of the Farnesoid X Receptor (FXR)

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Abstract: Azepino[4,5-*b*]indoles have been identified as potent agonists of the farnesoid X receptor (FXR). In vitro and in vivo optimization has led to the discovery of **6m** (XL335, WAY-362450) as a potent, selective, and orally bioavailable FXR agonist (EC_{50} = 4 nM, Eff = 149%). Oral administration of **6m** to $LDLR^{-/-}$ mice results in lowering of cholesterol and triglycerides. Chronic administration in an atherosclerosis model results in significant reduction in aortic arch lesions.

Farnesoid X receptor (FXR,[a] NR1H4), a member of the nuclear receptor (NR) subfamily, was originally identified in 1995.[1,2] Nuclear receptors are ligand-activated transcription factors that regulate the expression of specific target genes and are involved in several physiological functions including reproduction, development, and metabolism.[3] Relative to other nuclear receptors, FXR expression is relatively restricted, primarily in tissues exposed to a high concentration of bile acids such as the intestine, kidney, adrenals, and the liver.[4] Similar to some other nuclear receptors, FXR binds to specific response elements, as a heterodimer with the retinoid X receptor (RXR).

FXR, as a key sensor for bile acids, regulates multiple aspects of bile acid homeostasis and metabolism, including bile acid synthesis, conjugation, secretion, absorption, and refilling of the gall bladder. Activation of FXR results in increased hepatic expression of receptors that are involved in lipoprotein clearance (VLDL receptor and syndecan-1) and increased apoC-II that coactivates lipoprotein lipase (LPL). In addition FXR activation results in decreased expression of proteins (apoC-III and ANGPTL3)[5] that normally function as inhibitors of LPL. Studies with $Fxr^{-/-}$ mice also demonstrate differential roles for FXR in controlling lipid homeostasis.[6] Collectively these data implicate FXR with lowering plasma triglyceride levels via multiple mechanisms, repressing hepatic lipogenesis and triglyceride secretion, as well as increasing the clearance of triglyceride-rich lipoproteins from the blood. A number of recent reviews have been published that discuss the role of FXR in regulating cholesterol, lipoprotein, and glucose metabolism and its implication in liver disease.[7−9]

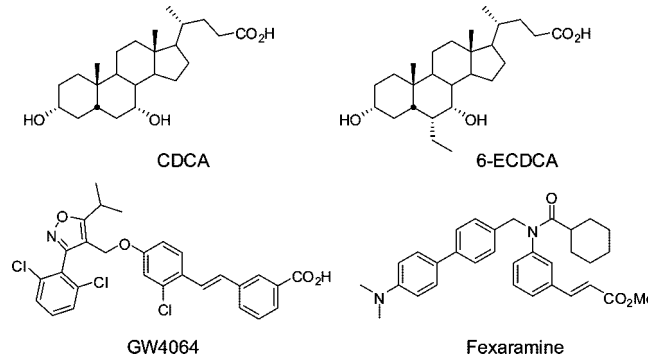

Figure 1. FXR agonists.

Bile acids such as chenodeoxycholic acid (CDCA) have been accepted as the physiologically relevant ligands for FXR[10] with data available from human and animal studies that are consistent with a key role for bile acids in controlling plasma lipids.[11] However, bile acids activate other nuclear receptors such as pregnane X receptor (PXR), vitamin D receptor (VDR), and constitutive androstane receptor (CAR). In addition, bile acids have also recently been shown to activate TGR5,[12] an intestine-expressed GPCR, further complicating the differentiation of FXR-dependent and FXR-independent pathways.

Several potent and selective FXR agonists have been reported (Figure 1) such as GW4064,[13] fexaramine,[14] and 6α-ethyl-chenodeoxycholic acid (6-ECDCA).[15] While these compounds have served as useful tools to investigate the role of FXR biology, the nonsteroidal analogues have suboptimal in vitro and in vivo characteristics, thus limiting their utility as small-molecule drugs for treating FXR-mediated metabolic diseases. 6-ECDCA is currently reported to be in phase II clinical trials.[15b]

Herein, we describe the identification and SAR of a novel series of azepino[4,5-*b*]indoles as potent FXR agonists leading up to the identification of the clinical candidate **6m** (XL335, WAY-362450, FXR-450).[16]

Our efforts on the discovery of FXR agonists were initiated by the high-throughput screening of the Exelixis compound library and the identification of the azepino[4,5-*b*]indole **1** (Figure 2) as a FXR agonist lead compound. This discovery also represented a novel class of nonsteroidal, selective FXR agonists.[17] Compound **1** was identified by screening our

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[a] Abbreviations: FXR, farnesoid X receptor; LDLR, low-density lipoprotein receptor; NR, nuclear receptor; RXR, retinoid X receptor; VLDL, very-low-density lipoprotein; apoC, apolipoprotein C; LPL, lipoprotein lipase; ANGPTL3, angiopoietin-like 3; CDCA, chenodeoxycholic acid; GPCR, G-protein-coupled receptor; LBD, ligand-binding domain; NHR, nuclear hormone receptor; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; SXR, steroid and xenobiotic receptor; ER, estrogen receptor; GR, glucocorticoid receptor; AR, androgen receptor; MR, mineralocorticoid receptor; PR, progesterone receptor; CYP7A1, cholesterol 7α-hydroxylase; SHP, small heterodimer partner; IBABP, intestinal bile acid binding protein; BSEP, bile salt excretory pump.

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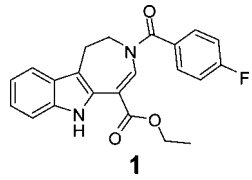

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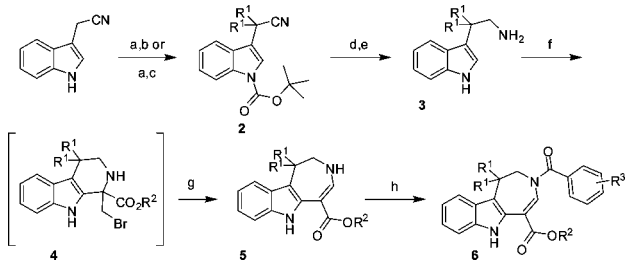
Figure 2. Azepino[4,5-*b*]indole **1**.

Scheme 1. Synthesis of Azepino[4,5-*b*]indoles **6**[a]



[a] Reagents: (a) di-*tert*-butyl dicarbonate, Et_3N, DMAP, DCM, 84%; (b) (i) LiHMDS (1.0 equiv), THF, −78 °C; (ii) MeI, −78 to 25 °C, 71%; (c) MeI (2.5 equiv), LiHMDS (2.5 equiv), THF, −78 to 25 °C, 84%; (d) TFA, DCM, 99%; (e) $LiAlH_4$, THF, reflux, 82%; (f) HCl, ethyl or isopropyl bromopyruvate, EtOH, reflux, 16 h; (g) Et_3N or pyridine, 100 °C, 40% (two steps); (h) ArCOCl, DIPEA, DCE, 70 °C, 78−85%.

Table 1. SAR of Azepino[4,5-*b*]indole Analogues **6**

compd	R^1	R^2	R^3	EC_{50}[a,c] (nM)	Eff[b,c] (%)
1	H, H	Et	4-F	600	100
6a	H, H	*i*-Pr	4-F	500	120
6b	H, H	*n*-Pr	4-F	500	130
6c	H, H	*n*-Bu	4-F	1100	109

[a] Compounds were screened in a transient transfection assay using hFXR on an EcREx7-TK-Luc construct in CV-1 cells. [b] Maximum efficacy relative to 100 μM CDCA. [c] All values are the mean of $n \geq 3$, and standard deviations are ≤30% of the mean values.

compounds in a 384-well format using a single point cell-based h-FXR cotransfection luciferase gene reporter assay. Resynthesis and full dose−response provided an in vitro potency of 0.6 μM.

Azepino[4,5-*b*]indoles were prepared via the general synthetic route shown in Scheme 1. 2-(1*H*-indol-3-yl)acetonitrile was converted to the *tert*-butyl carbamate followed by mono- or dialkylation to afford the respective analogues **2**. Preparation of dialkylated indoles **2** were obtained when excess reagents are used. Subsequent deprotection of **2** with TFA and reduction with $LiAlH_4$ in THF at reflux afforded the corresponding tryptamines **3**. Condensation of **3** with a bromopyruvate in the presence of HCl provided the intermediate carbolines **4** (not isolated) via the Pictet−Spengler reaction. When the sample was heated in pyridine, **4** were converted to azepino[4,5-*b*]indoles **5**, presumably via rearrangement of an intermediate aziridine.[18] Finally azepino[4,5-*b*]indoles **5** were reacted with the desired aroyl chlorides to afford the acylated products **6**.

Lead optimization was initiated with exploration of the ester functionality of the HTS lead **1**, and the data are shown in Table 1. A large number of alkyl esters (linear and branched) were explored with only a select set shown in Table 1. Activity was maintained with the isopropyl and *n*-propyl esters (**6a**, **6b**), while reduced activity was observed for longer alkyl chains (**6c**).

We then proceeded to explore the alkylation of the 1-position starting initially with the monomethyl analogues (Table 2). The monoalkylated compound 1-methyl azepino[4,5-*b*]indole **6d** was found to increase the FXR potency ~10-fold when compared to the HTS lead **1**. With the monoalkylated azepinoindole, we explored the SAR around the benzamide functionality, and

Table 2. SAR of Azepino[4,5-*b*]indole Analogues **6**

compd	R^1	R^2	R^3	EC_{50}[a,c] (nM)	Eff[b,c] (%)
6d	Me,H	Et	4-F	57	160
6e	Me,H	Et	4-Cl	73	155
6f	Me,H	Et	4-OMe	60	145
6g	Me,H	Et	3,4-F_2	32	170

[a] Compounds were screened in a transient transfection assay using hFXR on an EcREx7-TK-Luc construct in CV-1 cells. [b] Maximum efficacy relative to 100 μM CDCA. [c] All values are the mean of $n \geq 3$, and standard deviations are ≤30% of the mean values.

Table 3. SAR of Azepino[4,5-*b*]indole Analogues **6**

compd	R^1	R^2	R^3	EC_{50}[a,c] (nM)	Eff[b,c] (%)
6h	Me, Me	Et	4-F	22	148
6i	Me, Me	Et	3,4-F_2	12	144
6j	Me, Me	Et	4-Cl	25	190
6k	Me, Me	Et	4-OMe	29	139
6l	Me, Me	*i*-Pr	4-F	8	146
6m	Me, Me	*i*-Pr	3,4-F_2	4	149

[a] Compounds were screened in a transient transfection assay using hFXR on an EcREx7-TK-Luc construct in CV-1 cells. [b] Maximum efficacy relative to 100 μM CDCA. [c] All values are the mean of $n \geq 3$, and standard deviations are ≤30% of the mean values.

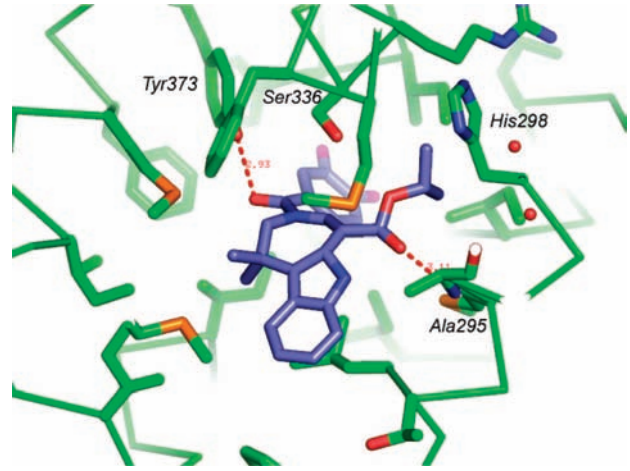


Figure 3. X-ray crystal structure of **6m** in hFXR LBD.

selected substitution options are presented in Table 2. 4-Substitution with F, Cl, and OMe was activity neutral (**6d**−**f**), while the 3,4-difluoro analogue **6g** showed a nominal 2-fold improvement.

Results of dialkylation of the azepineindole on the 1-position with the chemistry described in Scheme 1 are shown in Table 3. In general, a 2−3× improvement in potency was observed for various benzamides explored, with the 3,4-difluoro analogue **6i** showing the highest potency within the ethyl esters, similar to that observed for the monomethyl analogue **6g** (Table 2).

Incorporation of an isopropyl ester (**6l** and **6m**) yielded another 3-fold potency improvement over the ethyl ester analogues. Overall, **6m** was identified as the most potent FXR agonist within our series (EC_{50} = 4 nM, Eff = 149%) and was selected for further profiling (in vitro and in vivo).

The crystal structure of the complex of **6m** bound to the ligand-binding domain (LBD) of human FXR was determined to a resolution of 2.0 Å (Figure 3). The ligand resides in a predominately hydrophobic pocket with only a few polar atoms making contact with the ligand. Among these are a 3.1 Å hydrogen bond from the peptide amide of A295 (α3) to the carbonyl oxygen of the isopropyl ester, along with a 2.9 Å hydrogen bond from the hydroxyl of Y373 (α7) to the amide oxygen attached to the azepine ring. Similar to that observed

Table 4. PK Profile of **6m** in Rat at 3 mg/kg

route	iv[a]	po[b]
C_{max} (μM)	30.5	0.5
T_{max} (h)	0.02	10.0
AUC_{0-t} (μM h)	19.1	7.0
Cl ((mL/h)/kg)	341	
V_{ss} (L/kg)	3.3	
$t_{1/2}$ (h)	40.1	24.6
f (%)		37.6

[a] iv: NMP/Solutol/PEG400/H_2O (10:10:40:40). [b] po: corn oil.

in the structure of other FXR agonists, the C-terminal AF2 motif (α12) adopts the "agonist" conformation, although no direct contact with the ligand is observed. Nevertheless, residues F465 and W478 (α12) are positioned within 4.5 Å of the indole ring of **6m** and form a lid presumably sequestering the ligand.

The selectivity of **6m** was evaluated using a panel of nuclear receptor expression plasmids transiently transfected in CV-1 cells together with NHR responsive luciferase reporters. The panel tested consisted of LXRα, LXRβ, PPARα, PPARγ, PPARδ, RXRα, RARγ, VDR, SXR, ERα, ERβ, GR, AR, MR, and PR. Compound **6m** is highly selective, as no significant cross-reactivity with these receptors was observed at concentrations up to 10 μM.

FXR has been shown to regulate several target genes associated with bile acid synthesis and transport.[19] Activation of FXR by bile acids or synthetic agonists results in transcriptional repression of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the bile acid biosynthesis pathway,[20] induction of the small heterodimer partner (SHP), a transcriptional repressor found in the liver and intestine,[21] and induction of genes encoding for some bile acid transport proteins, such as intestinal bile acid binding protein (IBABP)[22] and bile salt excretory pump (BSEP).[23] In promoter assays, utilizing reporter constructs under control of the human BSEP, human SHP, and mouse IBABP genes were up-regulated by **6m** with EC_{50} of 17, 230, and 33 nM, respectively. In addition, **6m** significantly induced mRNAs encoding for BSEP, SHP, and IBABP in human cell cultures at 1 μM (13-, 2-, and 20-fold, respectively). As anticipated, the observed induction of BSEP, SHP, and IBABP promoters by our FXR agonists is consistent with these genes being direct targets of FXR.

Compound **6m** was dosed in rat at 3 mg/kg (po and iv) and shows good oral bioavailability (38%) (Table 4). There is a protracted half-life of 25 h, modest volume of distribution, and low clearance (3.3 L/kg, ~10% of hepatic blood flow). Additional pharmacokinetic studies in mice and higher species have been completed, and the data will be reported elsewhere.

To evaluate the pharmacokinetic and pharmacodynamic effects of **6m** on lipid profiles, we administered **6m** (10 mg/kg, po) to normal C57bl/6 mice for a period of 7 days and sampled plasma 3 h after the final dose. Compound **6m** significantly lowered triglycerides (82.3 ± 2.9 and 62.0 ± 6.4 mg/dL for vehicle [80% PEG/20% Tween] and **6m**, respectively) and total cholesterol (99.8 ± 4.0 and 78.1 ± 5.0 mg/dL for vehicle and **6m**, respectively). To further evaluate the efficacy of **6m**, we evaluated it in $LDLR^{-/-}$ mice that had been fed a Western diet (Purina 21551) for 8 weeks at the beginning of the study. After 6 weeks of daily oral treatment, **6m** lowered both triglycerides (by 19% and 39% at 1 and 3 mg/kg, respectively) and total cholesterol (by 23% and 50% at 1 and 3 mg/kg respectively; see Figure 4).

In parallel with the reduction in plasma lipids, preatherosclerotic lesion area, measured by en face analysis after lipid staining, was observed in the aortic arch. Treatment with **6m**

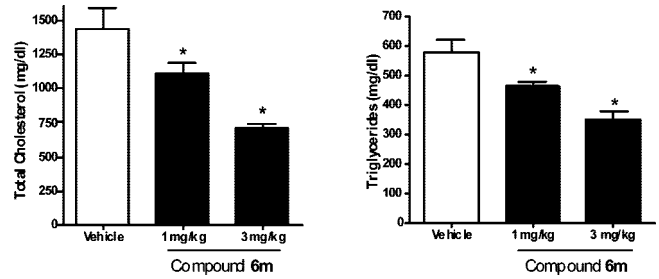


Figure 4. Effect of **6m** on total cholesterol and plasma triglycerides in $LDLR^{-/-}$ mice fed a high-fat diet: (*) $P < 0.05$ versus vehicle control, one way ANOVA followed by Bonferroni post hoc test.

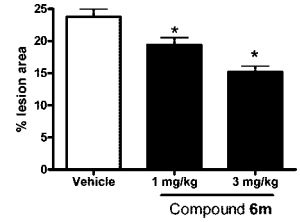


Figure 5. Effect of **6m** on reduction of aortic arch lesion by en face analysis: (*) $P < 0.05$ versus vehicle control, one way ANOVA followed by Bonferroni post hoc test.

inhibited lesion formation by 18% and 36% at doses of 1 and 3 mg/kg, respectively (see Figure 5).

In summary, we have identified a series of novel azepino[4,5-*b*]indole analogues from HTS that act as novel and selective FXR agonists. Our lead optimization efforts have led to the discovery of **6m** as a potent, selective, and orally bioavailable FXR agonist. We have successfully solved the cocrystal structure of **6m** and related analogues, gaining a strong understanding of the SAR around the azepino[4,5-*b*]indole core. Compound **6m** displays potent agonist activity in the FXR reporter gene assays and on FXR target genes in cell-based assays. Compound **6m** also showed potent effects on cholesterol and triglyceride lowering in $LDLR^{-/-}$ mice and antiatherogenic activity with respect to reduction of aortic arch lesions. On the basis of these data and other in vitro and in vivo characterization, **6m** has been selected for further clinical evaluation.[16]

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Note Added after ASAP Publication. This paper was published ASAP on January 21, 2009 with an error in Figure 1. The revised version was published on January 30, 2009.

Supporting Information Available: Details of the synthesis and characterization of azepino[4,5-*b*]indole **6m** and analogues; protocols for in vitro and in vivo experiments; X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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