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Pyrrole[2,3-*d*]azepino compounds as agonists of the farnesoid X receptor (FXR)

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ABSTRACT

Pyrrole[2,3-*d*]azepines have been identified as potent agonists of the farnesoid X receptor (FXR). Based on the planar X-ray crystal structure of WAY-362450 **1** in the ligand binding domain and molecular modeling studies, non-planar reduced compounds were designed which led to agonists that exhibit high aqueous solubility and retain moderate in vitro potency.

The farnesoid X receptor (FXR) is a nuclear hormone receptor expressed in the liver, kidney and intestine that is activated by bile acids.[1,2] Upon activation, FXR binds to DNA as a heterodimer with the retinoid X receptor (RXR).[3] This binding event regulates the expression of a number of genes and proteins involved in bile acid and cholesterol homeostasis (CYP7A1, SHP, IBABP, BSEP, and Apo A-I)[4], triglyceride synthesis[5], and lipogenesis (SREBP-1c and Apo C-III).[6] Early FXR agonists reported in the literature include GW4064,[7] fexaramine, fexarene, and fexarine.[8] Although these compounds were shown to activate FXR, they suffered from poor ADME properties. These reports demonstrated that agonism of FXR lowers triglyceride levels in animals[9] and the mechanism of action suggests a potential to reduce total cholesterol levels as well.[10]

Recently, WAY-362450 **1** (EC_{50} = 5 nM in a transient transfection assay using hFXR on an ECREx7-TK-Luc construct in CV-1 cells), which contains a novel azepino[4,5-*b*]indole core structure was disclosed as a potent and selective FXR agonist that advanced to clinical trials (Fig. 1).[11] When **1** was dosed in LDL receptor knockout (LDLR KO) mice, statistically significant reductions in both cholesterol and triglyceride levels were observed; however, the poor aqueous solubility of **1** necessitated the use of non-aqueous vehicles such as corn oil for oral dosing. The limited solubility of **1** could be attributed, in part, to its high lipophilicity (*c*Log *P* = 5.30). In addition, a small molecule crystal structure of **1** revealed the exceedingly planar nature of the molecule that may result in a high degree of crystal packing, additionally evidenced by its high melting point of 174–175 °C, and may therefore contribute to its poor aqueous solubility.

To potentially improve the solubility of **1**, we examined compounds in which the indole ring was replaced with a 2-cyanopyrrole moiety. The use of a pyrrole[2,3-*d*]azepino core would decrease both the *c*Log *P* and the molecular weight of the **1** analogs. In addition, to disrupt the planarity of the core, we investigated analogs in which the azepine olefin was reduced, therefore diminishing the planarity of the molecule and the potential for tight crystal packing.

The synthesis of the pyrrole[2,3-*d*]azepino analogs began with the formylation of 1*H*-pyrrole-2-carboxylic acid methyl ester **2** using dichloromethoxymethane and aluminum chloride to yield compound **3**. Treatment of aldehyde **3** with dimethyl amine and sodium triacetoxyborohydride followed by treatment with methyl iodide provided the quaternary ammonium salt **4** which was reacted with sodium cyanide to give the nitrile **5.** After Boc protection of the pyrrole nitrogen, the acetonitrile was dimethylated, and the protecting group was removed to afford intermediate **6**. Reduction of the nitrile using Raney Nickel provided the primary amine which was Boc protected prior to hydrolysis of the methyl

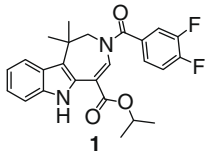

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Figure 1. The azepino[4,5-*b*]indole WAY-362450 (**1**).

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ester with sodium hydroxide to form compound **7**. Conversion of the carboxylic acid to the nitrile was accomplished over two steps, namely, treatment with thionyl chloride followed by trifluoroacetic anhydride. Removal of the Boc group with 6 N HCl provided hydrochloride salt **8** which underwent a one-pot Pictet–Spengler cyclization-rearrangement reaction to provide the desired pyrrole[2,3-*b*]azepino core **9**. Subsequent acylation of the azepine nitrogen with various acyl chlorides furnished targets **10a–h** (Scheme 1).

The compounds were evaluated using an FXR functional assay that measured their ability to activate the gal4-hFXR-LBD fusion protein in HEK293 cells. Maximal efficacy was reported as a percentage of the maximal efficacy observed in this assay with GW4064. FXR functional potency and efficacy data for this series of pyrrole[2,3-*b*]azepine analogs are shown in Table 1. All eight of the pyrrole[2,3-*d*]azepino analogs were either equipotent to **1** or exhibited an improvement in potency. Indeed, the direct analogue of **1**, **10a** (EC_{50} = 6.2 nM) was three times more potent than the indoleazepine lead and the 3-fluorophenyl compound **10b** (EC_{50} = 2.5 nM) was, at the time, the most potent FXR agonist prepared in our laboratories. Unfortunately, despite the significantly reduced *c*log *P*, none of the analogues exhibited solubility in aqueous pH 7.4 buffer.[12]

To gain insight into the pharmacokinetic parameters of these analogs, compound **10a** was orally dosed to C57 mice (30 mg/kg in 2% Tween80/0.5% methylcellulose, an aqueous formulation in which the compound had adequate solubility, i.e., >0.10 mg/mL). However, no detectable plasma concentration of the compound was measured. This result underscores the limited solubility of the pyrrole[2,3-*d*]azepino analogs (**10a–h**) and their low absorption in vivo.

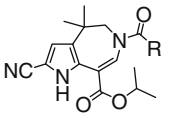
The previously disclosed crystal structure of **1** (PDB code 3FLI) bound to the FXR ligand-binding domain was used for docking studies to understand the binding mode of 2-cyanopyrrole and shed further light on residues which are important for ligand recognition within the binding site. As shown in Figure 1, the docked binding mode[13] of **10a** is similar to that of **1** in that ligand recognition is achieved through a combination of a specific hydrogen bond and large number of hydrophobic interactions observed between the ligand and FXR residues (Fig. 2).

The seven-membered azepine ring was oriented in a slightly puckered conformation, that is, in a twist-chair with the C2 group twisted out of the plane with respect to the pyrrole ring. The carbonyl oxygen of the amide group was in position to accept a hydrogen bond from the hydroxyl group of the Tyr373 residue while an intramolecular hydrogen bond was seen between the indole NH of the pyrrole and the ester carbonyl group (though it had an improper dihedral angle for N–H–O–C of ~11° out of plane). This internal hydrogen bond rigidified this template further and allowed the isopropyl ester group to orient towards the narrow hydrophobic pocket surrounded by Ile339 residue.

Previous SAR studies suggested that a longer alkyl chain branched or linear in this hydrophobic Ile339 cavity was critical for ligands potency. We hypothesized that the reduction of the vinyl amide bond of **10a** would twist the azepine ring into a non-planar conformation and result in a loss of the intramolecular hydrogen bond. This conformational change would also effect how the isopropyl group was positioned into the narrow hydrophobic cavity.

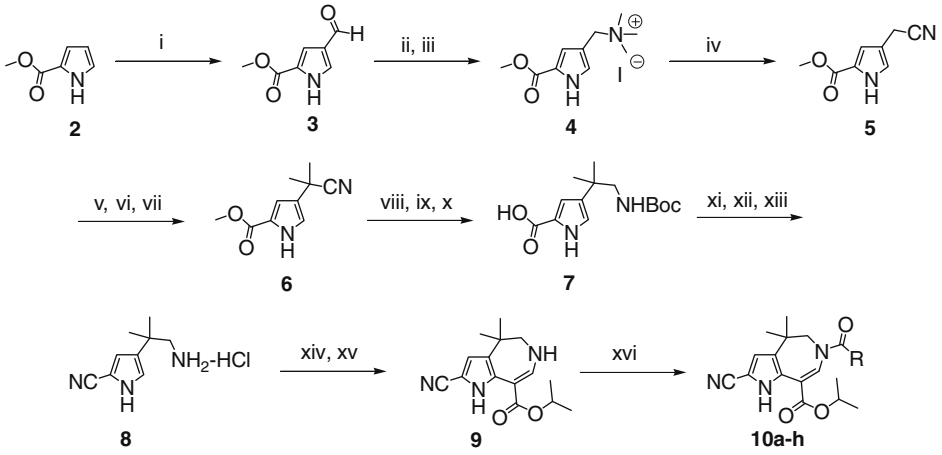
To test this hypothesis and further explore the solubility of this series of compounds, analogues were prepared that lacked the

Table 1
FXR functional activity and aqueous solubility for the pyrrole[2,3-*d*]azepino analogs



Compd	R	EC_{50}[a] (nM)	Eff.[b] (%)	*c*log *P*	Sol.[c] (μg/mL)
10a	3,4-Di-F-phenyl	6.3	121	3.89	BLD[d]
10b	3-F-Phenyl	2.5	120	3.79	BLD
10c	4-F-Phenyl	5.0	119	3.79	BLD
10d	Cyclohexyl	6.5	124	4.91	BLD
10e	4-Cyanophenyl	35	116	3.18	BLD
10f	3-Cl-Phenyl	12	122	4.36	BLD
10g	3-CF_3-Phenyl	8.5	94	4.59	BLD
10h	2-Thienyl	16	114	3.35	BLD
1	N/A (Figure 1)	16	130	5.30	BLD

[a] Induction of FXR measured in HEK293 cells stably co-transfected with an expression plasmid for an FXR-LBD-gal4 DNA binding fusion protein and a Luc12 luciferase reporter gene construct.
[b] Efficacy = [maximal fold induction of test compound/maximal fold induction of GW4064 × 100].
[c] Solubility as measured in an aqueous pH 7.4 buffer using a pION PSR4s instrument and software.
[d] BLD = Below the limit of detection.



Scheme 1. Reagents and conditions: (i) dichloromethyl methyl ether, $AlCl_3$, CH_2Cl_2, 0 °C, 50%; (ii) Me_2NH, $Na(OAc)_3BH$, THF, 20 °C; (iii) MeI, THF, 20 °C; (iv) NaCN, DMF, 120 °C, 55% over 3 steps; (v) Boc_2O, Et_3N, DMAP, THF, 20 °C; (vi) NaH, MeI, DMF, 0–20 °C; (vii) TFA, DCM, 20 °C, 77% over 3 steps; (viii) H_2, Raney Ni, MeOH, NH4OH, THF, 20 °C; (ix) Boc_2O, Et_3N, DMAP, THF, 20 °C; (x) NaOH, CH_3CN, 70 °C, 72% over 3 steps; (xi) $SOCl_2$, CH_2Cl_2, 0–20 °C then NH_3, Et_2O, 20 °C; (xii) TFAA, py, 0–20 °C; (xiii) 6 N HCl, 20 °C, 49% over 3 steps; (xiv) isopropylbromopyruvate, $(CH_3)_2CHOH$, CH_3CN, 80 °C; (xv) Py, DMAP, 80 °C, 62% over 2 steps; (xvi) RC(O)Cl, Et_3N, CH_3CN, 20 °C.

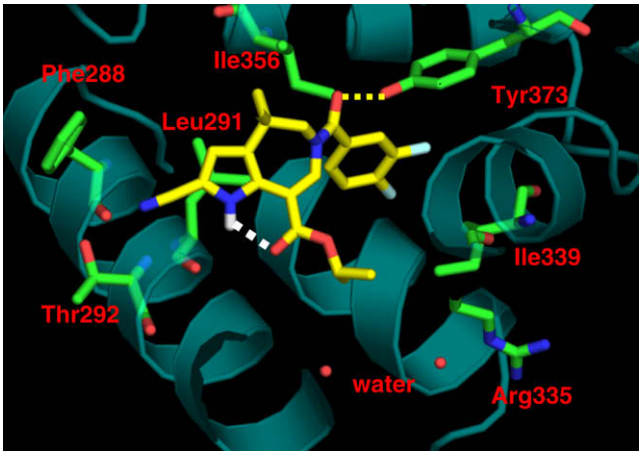


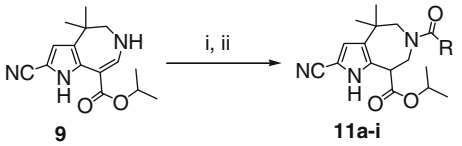
Figure 2. The docked binding mode of **10a** (in yellow) in the binding pocket of the hFXR X-ray co-crystal structure (from a complex with **1**). Only critical residues within the binding site have been shown for clarity. Hydrogen bonds are shown by dotted lines (white intramolecular, yellow intermolecular).

azpine olefin. To access these derivatives, the pyrrole[2,3-*b*]azepino core **9** was treated with sodium cyanoborohydride and subsequently acylated (Scheme 2) to yield compounds **11a–i** as racemic mixtures. The in vitro activity and aqueous solubility of these analogs is shown in Table 2.

A distinct improvement in solubility was observed among all nine of the analogues versus the unsaturated compounds. Four of the reduced compounds (**11b–d, 11i**) were soluble in aqueous media at levels greater than 100 μg/mL. Clearly, the reduction of the azepine olefin led to a dramatic improvement in solubility that may be attributed to the disruption of the planar nature of the compounds. However, a significant loss of potency was seen when the azepine olefin was removed. The 3,4-difluorophenyl analogue **11a** proved to be the most potent (EC_{50} = 280 nM) and the 4-fluorophenyl analogue **11c** (EC_{50} = 640 nM) was the only other analogue to possess an EC_{50} under 1000 nM.

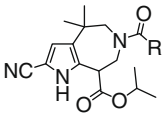
Further docking studies carried out on the saturated analog **11a** revealed that although one of the enantiomers (i.e., *S*) was able to fit and orient the isopropyl group in a similar position as seen in the X-ray structure of **1**, the azepine ring was in an energetically unfavorable conformation, that is, twist-boat (Fig. 3). The ester carbonyl group was further twisted out of plane and resulted in loss of the intramolecular hydrogen bond (as it had an improper dihedral angle for N–H–O–C of ~38° out of plane). A conformational search on the unbound ligand **11a** revealed that none of the low energy conformers within a ~3 kcal energy window, favored a ligand-bound-like conformation of **10a**, that is, azepine ring in a twist-chair conformation and the presence of an intramolecular hydrogen bond. We believe that this high conformational energy cost for **11a** was responsible for the reduction in ligand potency. However, we cannot rule out that other factors such as ligand desolvation energy and electronic effects could also play a role.

In conclusion, we have discovered a novel class of pyrrole[2,3-*d*]azepines with low nanomolar in vitro potency as FXR agonists.



Scheme 2. Reagants and conditions: (i) $NaCNBH_4$, AcOH, 20 °C, 61%; (ii) RC(O)Cl, Et_3N, CH_3CN, 20 °C, 80%.

Table 2
FXR functional activity and aqueous solubility for the reduced pyrrole[2,3-*d*]azepino analogs



Compd	Structure	EC_{50}[a] (nM)	Eff.[b] (%)	*c*Log *P*	Sol.[c] (μg/mL)
11a	3,4-Di-F-phenyl	280	115	3.67	31
11b	3-F-Phenyl	1300	84	3.57	>100
11c	4-F-Phenyl	640	85	3.57	>100
11d	Cyclohexyl	1900	127	3.74	>100
11e	4-Cyanophenyl	5700	91	2.96	21
11f	3-Cl-Phenyl	1300	103	4.14	17
11g	3-CF_3-Phenyl	2700	114	4.36	10
11h	4-Cl-Phenyl	4100	115	4.14	5
11i	4-Tetrahydropyran	3300	112	1.34	>100

[a] Induction of FXR measured in HEK293 cells stably co-transfected with an expression plasmid for an FXR-LBD-gal4 DNA binding fusion protein and a Luc12 luciferase reporter gene construct.
[b] Efficacy = [maximal fold induction of test compound/maximal fold induction of GW4064 × 100].
[c] Solubility as measured in an aqueous pH 7.4 buffer using a pION PSR4s instrument and software.

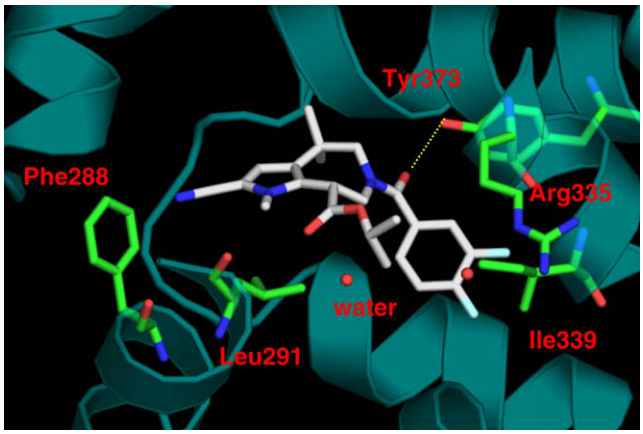


Figure 3. The docked binding mode of **11a** (in white) in the binding pocket of the hFXR X-ray co-crystal structure (from a complex with **1**). Only critical residues within the binding site have been shown for clarity. Hydrogen bonds are shown by dotted yellow lines. The ester carbonyl group is now twisted out of plane and not in position to form hydrogen bond with NH of pyrrole.

Furthermore, by disrupting the planarity of the core azepine ring system, we have identified the first aqueous soluble analogues of the series, albeit with an associated loss of potency.

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