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Optimization of a novel class of benzimidazole-based farnesoid X receptor (FXR) agonists to improve physicochemical and ADME properties

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ABSTRACT

Structure-guided lead optimization of recently described benzimidazolyl acetamides addressed the key liabilities of the previous lead compound **1**. These efforts culminated in the discovery of 4-{(*S*)-2-[2-(4-chloro-phenyl)-5,6-difluoro-benzoimidazol-1-yl]-2-cyclohexyl-acetylamino}-3-fluoro-benzoic acid **7g**, a highly potent and selective FXR agonist with excellent physicochemical and ADME properties and potent lipid lowering activity after oral administration to LDL receptor deficient mice.

The farnesoid X receptor (FXR, NR1H4) is a ligand-activated transcription factor and member of the nuclear hormone receptor superfamily. Cloned in 1995[1] and de-orphanized in 1999,[2] FXR has been identified as a key sensor for bile acids, with chenodeoxycholic acid (CDCA, Fig. 1) being the most effective endogenous activator.

Consistent with its function in regulating bile acid synthesis and profile, FXR is mainly expressed in organs that are involved in the enterohepatic circulation of bile acids that is, liver and intestine as well as kidneys and adrenal glands.[3] Upon activation of FXR by bile acids it represses the expression of CYP7A1 and CYP8B1, the rate-limiting enzyme of bile acid synthesis from cholesterol and of bile acid hydrophilicity, respectively, by increasing the expression of the atypical nuclear receptor small heterodimer partner (SHP). SHP then decreases the pro-transcriptional regulation of these genes by a third nuclear receptor liver receptor homologue 1 (LRH-1). FXR also controls enterohepatic circulation of bile acids by regulating the expression of bile acid transporters and binding proteins such as BSEP, IBABP, and NTCP.[4]

The combined effects of FXR agonism on bile acid composition and pool size is a decrease in the intestinal absorption of dietary lipids resulting in a reduction of plasma cholesterol and triglycerides. Besides its central role in the maintenance of bile acid homeostasis, FXR also regulates genes affecting triglyceride metabolism (e.g., apoCII, apoCIII, SREBP1-1c)[5] leading to reduced plasma triglyceride levels. FXR is also involved in glucose homeostasis. In various mouse models[6] FXR agonists were shown to decrease plasma glucose levels through the modulation of different target genes involved in the regulation of hepatic gluconeogenesis (e.g., PEPCK or G6Pase) and glycogen synthesis (e.g., phosphorylation of GSK3β), respectively. In addition, FXR was shown to improve insulin sensitivity, although the underlying molecular mechanisms and the contribution of FXR-independent pathways still need to be determined. Consistent with these mechanisms and with positive results from pre-clinical animal studies, FXR modulators have a great therapeutic potential in the treatment of dyslipidemia, atherosclerosis and diabetes. Further studies also suggest a possible role for FXR agonists in treating cholestasis,[7] gallstone disease,[8] nonalcoholic steatohepatitis (NASH)[9] and diabetic nephropathy,[10] although the final proof in humans is still to be established.

Since the deorphanization of FXR several potent steroidal and non-steroidal FXR agonists have been reported (Fig. 1). GW4064,[11] the first high-affinity non-steroidal ligand, and later Fexaramine,[12] served as useful tool compounds to investigate the FXR pharmacology and to elucidate the topology and active conformation of the FXR binding site. However, sub-optimal in vitro and

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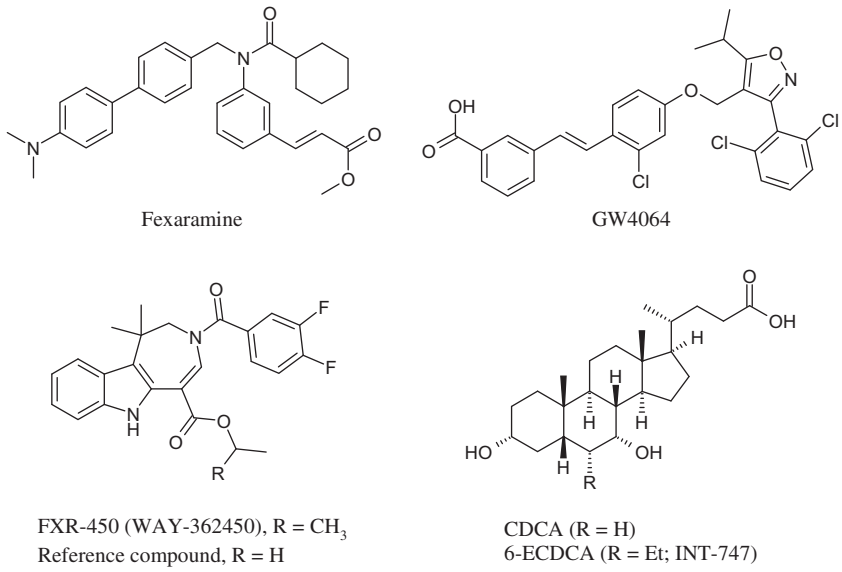

Figure 1. Representative FXR agonists.

in vivo characteristics, such as poor rat pharmacokinetic properties or instability under UV light and the presence of a potentially toxic stilbene moiety in the case of GW4064, precluded further development of these compounds.[13] Researchers from Wyeth and Exelixis published the synthesis of the non-steroidal agonist FXR-450 that recently entered clinical trials.[14] A follow-up paper described the approaches applied for improving the physicochemical properties of FXR-450.[15] The most advanced FXR agonist to date is the semi-synthetic bile acid derivative 6-ECDCA (a mixed FXR/GPBAR1 agonist)[16] which recently completed Phase II clinical trials in patients with primary biliary cirrhosis.[17]

We recently reported on the synthesis and structure–activity relationship (SAR) profiling of a novel class of potent and selective benzimidazole-based FXR agonists as exemplified by our lead structure **1** (Fig. 2).[18] Although **1**, a potent, partial agonist at the FXR receptor is endowed with oral activity in an in vivo model of dyslipidemia, it was found to have poor physicochemical properties such as high lipophilicity and poor aqueous solubility. Surprisingly, the compound also inhibited the hERG potassium channel in vitro with an IC_{50} of 1.6 μM.

Our lead optimization efforts, therefore, concentrated on the introduction of polarity to reduce the high lipophilicity and increase solubility and bioavailability (mouse $F = 1.7\%$) and to overcome the hERG liability of **1**.

In accordance with the hydrophobic nature of the ligand binding site of human FXR, the original SAR[18] indicated little scope for the introducing polarity into these novel agonists. However, following further analysis of structural information generated in-house, we identified a more polar, as yet unexplored region in FXR consisting of Gln267, Asn297, His298, Arg335 and three water molecules in the proximity of the amide-bound cyclohexyl group (Fig. 3). Comparison with a published co-crystal structures of rat

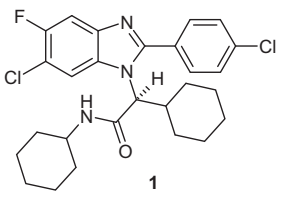

Figure 2. Lead compound from the benzimidazolyl acetamide class.

FXR with 6-ECDCA revealed that the carboxylate of 6-ECDCA is located in this region and interacts through charge-assisted hydrogen bonds with the corresponding rat Arg328.[19] We therefore initiated a structure-guided design program in which we investigated a variety of polarity-conferring substituents at different distances from and orientation to the amide group, targeting in particular an electrostatic interaction with Arg335.

Two strategies were followed for the synthesis of the novel benzimidazoles. For both, single enantiomers were obtained by chiral chromatography. The assignment of the absolute stereochemistry was based on several co-crystal structures (incl. crystallizations with racemates in which only the *S* enantiomers bound) as well as small molecule X-ray crystallography of

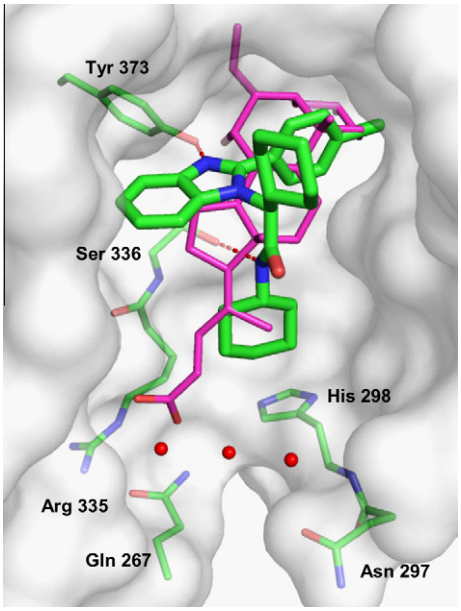

Figure 3. X-ray structure of the 5,6-di-H analogue[18] of benzimidazolyl acetamide **1** bound to human FXR (PDB: 3OKI) illustrating the targeted polar binding site region. The front of the FXR binding site is removed for clarity. Protein–ligand hydrogen bonds to Ser336 and Tyr373 are depicted as red dashed lines. The structure is overlaid with the 6-ECDCA/rat FXR complex (magenta, PDB: 1ot7, protein not shown).