Bioorganic & Medicinal Chemistry Letters 21 (2011) 191–194

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel and orally active FXR agonists for the potential treatment of dyslipidemia & diabetes

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ARTICLE INFO

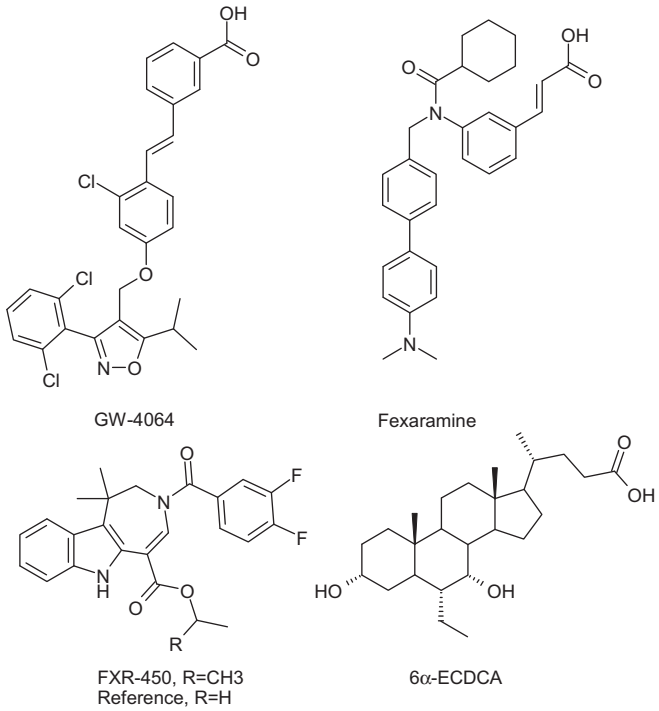
Article history:
Received 14 October 2010
Revised 4 November 2010
Accepted 4 November 2010
Available online 12 November 2010

Keywords:
Farnesoid X receptor
Nuclear hormone receptors
Ugi-reaction
Benzimidazoles

ABSTRACT

Herein we describe the synthesis and structure activity relationship of a new class of FXR agonists identified from a high-throughput screening campaign. Further optimization of the original hits led to molecules that were highly active in an LDL-receptor KO model for dyslipidemia. The most promising candidate is discussed in more detail.

The farnesoid X receptor (FXR) belongs to the superfamily of nuclear hormone receptors which are gene regulating transcription factors involved in several diverse physiological functions.[1] FXR is mainly expressed in the liver, intestine, adrenal gland and kidney, where, upon activation by certain bile acids (BA), it regulates, among others, the expression of various genes involved in cholesterol and BA synthesis, metabolism and enterohepatic circulation. Hence, BA present in the enterohepatic circulation maintains the size and composition of the BA pool partially by feedback regulation via FXR. Activation of FXR by non-BA agonists leads to a reduced BA pool size and a change in its composition which again results in reduced cholesterol absorption from the small intestine and reduced plasma cholesterol levels. FXR agonists also regulate genes involved in triglyceride synthesis and metabolism, leading to a reduction in plasma triglyceride.[2] More recently it has been shown that activation of FXR in the liver plays a key role in gluconeogenesis, glycogen synthesis and insulin sensitivity making this protein a very promising target for treating metabolic and vascular diseases. Further pharmacological effects mediated through FXR activation have been reported such as improved liver regeneration and protection against hepatocarcino-genesis.[3] Hence, FXR agonists are of great interest and, indeed, several small molecule agonists have previously been described in the literature. FXR agonists have been proposed for the treatment of dyslipidemia,



Scheme 1. Representative FXR agonists with reported in vivo activity.

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doi:10.1016/j.bmcl.2010.11.039

cholestasis, non-alcoholic steatohepatitis (NASH) as well as type-2 diabetes. The five best characterized molecules so far are shown in Scheme 1.[4–7]

To identify valuable starting points for a chemistry program we initiated a high-throughput screening campaign of our corporate compound library. Several interesting hit clusters were identified with the benzimidazolyl acetamides **1** showing the most promising overall profile (Scheme 2).

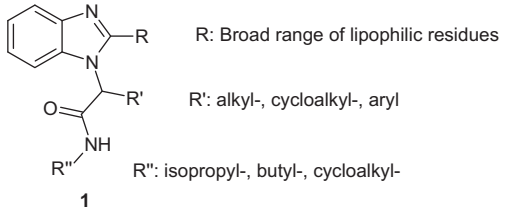
The compounds originate from an earlier in-house combinatorial chemistry effort where library design was based on availability of the building blocks rather than on drug-likeness of the final products. While R varied extensively, the diversity of R′ and R″ was very limited. No modifications were made at the benzene part (Ar) of the benzimidazole core. This compound class caught our attention because benzimidazoles are a well known substructural element in nature and thus appear quite frequently in pharmaceutical products, for example, astemizole, omeprazole and candesartan. Although compounds with the same core structure are well described in the literature, this particular substitution pattern led to rather novel chemical structures allowing us to investigate the structure activity relationship of the compound class more broadly. A set of approximately one hundred analogs was retested for hit confirmation in a scintillation proximity assay (SPA) using human FXR (hFXR) ligand binding domain with some compounds even displaying nanomolar binding affinities. The general synthesis route, based on the four component Ugi reaction, is depicted in Scheme 3.

Compounds of type **1** can be generated easily by treating BOC-protected phenylenediamine with carboxylic acids, aldehydes and isonitriles. The resulting intermediates **6** are further treated with neat TFA after evaporation of the organic solvent.[8] We slightly modified the procedure described by Hulmes[9] using equimolar amounts of components **2**–**5** to avoid the use of polymer supported reagents. In some cases the cyclization to the imidazole was not completed even after 12 h. Here, the TFA was evaporated and the residue taken up in acetic acid and maintained at 80 °C overnight which usually led to complete conversion of intermediate **6** to product **1**. All final compounds were purified via preparative HPLC. Using the synthesis protocol described above we were able to quickly generate a preliminary SAR and investigate the physicochemical properties of the products. All compounds described in Tables 1–4 were tested as racemic mixtures. As a control we used GW4064 which showed an IC_{50} = 0.11 μM in our assay.

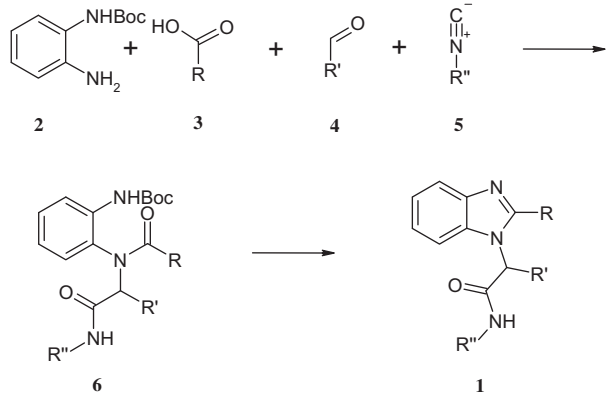
From the compound set generated it became evident that the ligand binding site tolerates a variety of modifications in position R as long as the residues are lipophilic. A wide range of residues were investigated with 4-chlorophenyl being the most potent both in terms of binding affinity as well as functional activity. Polar groups were not tolerated at this position as exemplified in Table 1 where four analogs are presented with R′ and R″ both being cyclohexyl.

With 4-chlorophenyl as a very potent residue for R we further investigated the SAR at position R′. Using the synthesis protocol described above we generated a follow-up library varying the aldehyde part (R′). Four representatives are shown in Table 2 (R″ = cyclohexyl).

As already observed for R the introduction of polarity at R' was quite challenging. Lipophilic groups such as alkyl-, cycloalkyl- and aryl- were tolerated (data not shown) but even minor changes such as **1g** led to a loss of binding affinity by ~two log units (compared to **1a**). Interestingly, by shifting the oxygen in **1g** from the 4 to the 2 position of the tetrahydropyran ring, low nanomolar binding



Scheme 2. Representative hit cluster identified from HTS.



Scheme 3. General synthesis route for benzoimidazolyl acetamides **1**. Reagents and conditions: (i) equimolar amounts of reagents in MeOH, rt, 1 h; (ii) neat TFA, rt, 16 h.

Table 1
SPA-binding affinities of **1a–d** with both R′ and R″ being cyclohexyl. Binding affinity was assessed in a radioligand displacement assay as described.[10] Values are means of at least two experiments

Compound	R	*c* log *P*	IC_{50} (μM)
1a	4-Cl-Ph	7.78	0.07
1b	4-CN-Ph	6.50	1.25
1c	4-$MeSO_2$Ph	5.52	12.44
1d	4-Pyridyl	5.65	30.44

Table 2
SPA-binding affinities of **1** with R being 4-chlorophenyl and R″ being cyclohexyl. Values are means of at least two experiments

Compound	R′	*c* log *P*	IC_{50} (μM)
1f	2-Methylbutyl	7.11	0.91
1g	4-Tetrahydropyranyl	5.38	3.8
1h	4-Acetylpiperidinyl	4.40	14.78
1i	4-Pyridyl	5.47	25.12

Table 3
SPA-binding affinities of **1** with R being 4-chlorophenyl and R′ being cyclohexyl. Values are means of at least two experiments

Compound	R″	*c* log *P*	IC_{50} (μM)
1k	Ph	7.60	0.15
1l	4,4-Difluorocyclohexyl	7.16	2.72
1m	Cyclopropyl	6.10	3.61
1n	4-Tetrahydropranyl	5.38	3.71

Table 4
SPA-binding affinities of **1** with modification at position 6 of the benzimidazole core structure. R being 4-chlorophenyl and R′ & R″ being cyclohexyl. Values are means of at least two experiments

Compound	Ar	*c* log *P*	IC_{50} (μM)
1p	6-Methoxy–	8.02	0.45
1q	6-Dimethylamino–	8.34	5.17
1r	6-Carboxy–	7.79	5.72
1s	6-Hydroxymethyl–	6.74	6.19

affinity was regained. Obviously, burying the electronegative oxygen within the molecule reduces the polar character of the ligand. Unfortunately, this derivative did not show any improvement in terms of physicochemical properties or microsomal stability but rather increased the complexity of the molecule due to the introduction of a second chiral center. In analogy to the above, R″ was investigated keeping R (4-chlorophenyl) and R' (cyclohexyl) constant. Again four representatives are depicted in Table 3.

Exploration of substitutions in this position showed that only a phenyl group could be introduced as a substitute for the cyclohexyl ring at position R″. All other, even minor changes led to a substantial loss of binding affinity. We also investigated the aromatic part of the benzimidazole core structure (Ar). Using proprietary *N*-Boc protected phenylenediamines, originating from an earlier lead optimization program. A library of over 100 analogs was generated using state-of-the-art parallel synthesis and purification equipment. From that series it became evident, that significant modifications were only tolerated at position 6 of the benzimidazole core structure. Position 4, 5 and 7 were shown to be fairly conservative. Four representatives with 6-modifications are shown in Table 4 with R being 4-chlorophenyl and R′ and R″ being cyclohexyl.

Here also, the functionalization of the aromatic moiety with polar groups was very limited whereas the introduction of further lipophilic residues (e.g., F or Cl) was well tolerated and even beneficial in terms of binding and functional activity.

The crystal structure of compound **1a** bound to the hFXR receptor could be solved with a resolution of 2.3 Å (Fig. 1). The structure nicely shows that the benzimidazole scaffold interacts through a strong hydrogen bond (d = 2.6 Å) with Tyr373 and is well suited to orient its substituents R, R′ and R″ into three binding pockets in an almost perpendicular fashion. As observed in previous FXR X-ray structures, the binding site is highly lipophilic, hence, providing a rational for the observed loss of affinity when introducing polar groups. A second, weak polar interaction is observed for the ligand amide NH which is engaged in a rather long hydrogen bond with Ser336 (d = 3.6 Å). Fig. 1 shows the more active enantiomer of the racemic mixture of **1a**, which turned out to be S configured. Various analogs of **1a** bound to hFXR were successfully crystallized and characterized showing that the hydrogen bonding of Tyr373 to the N3 of the benzimidazole core is highly conserved making this a key interaction for efficient ligand binding. This hypothesis was also confirmed by generating the corresponding indole derivative (N3 substituted by a C) which showed only very weak binding affinity in the high micromolar range and completely lacking functional activity (data not shown).

Using preparative chiral HPLC, we were able to separate most of the racemic material into their pure enantiomers. The R enantiomers were usually at least 2–4 log units less active than their corresponding S analogs. For example, compound **7** (depicted in Scheme 4) shows very high binding affinity and strong functional potency in the cell based transactivation (TA) assay in contrast to the corresponding R enantiomer which was inactive both in binding and function (data not shown). All compounds of this series were partial agonists. Further investigations concerning the physicochemical properties and the in vitro DMPK profile of this compound were undertaken and are discussed in more detail below. Not surprisingly the log *D* of **7** is very high resulting in negligible aqueous solubility. Nevertheless, in simulated fasted and fed state intestinal fluid (Fassif and Fessif, respectively) the solubility was medium to high. The permeation data from the parallel artificial membrane permeability assay (PAMPA) were low, but the activity observed in the functional assay showed that this compound and analogs thereof could easily penetrate cells. Concerning metabolic stability of **7** the in vitro results were, unfortunately, not consistent. Clearance by both, human and mouse microsomes were very high whereas hepatic clearance was moderate to low. Cytochrome P450 interactions for isoforms 3A4, 2D6 and 2C9 were investigated as well showing only weak affinities for all three enzymes. The compound selectivity of **7** for FXR was evaluated using a reporter-gene transcriptional assay versus a panel of other nuclear receptors including PPAR-α, -β, -δ, LXR-α, -β and RXR-α. No cross-reactivity was observed at a concentration of 10 μM (data not shown).

Based on the promising data obtained so far for this compound class we investigated the in vivo effects of **7** on lipid lowering in LDL-receptor knockout mice. The animals were fed a high fat diet for 15 days prior to compound administration for an additional five days. The reduction of total cholesterol (TC), low density lipoprotein (LDL) and triglycerides (TG) was measured. The ethyl ester version of FXR-450 (see Scheme 1) was used as a reference. Both compounds were orally dosed at 30 mg/kg. The efficacy data obtained are shown in Table 5. PK/PD monitoring revealed a plasma exposure for **7** of 0.47 μg/ml and 4.3-fold increased expression of hepatic SHP (FXR regulated gene) 2 h after administration.

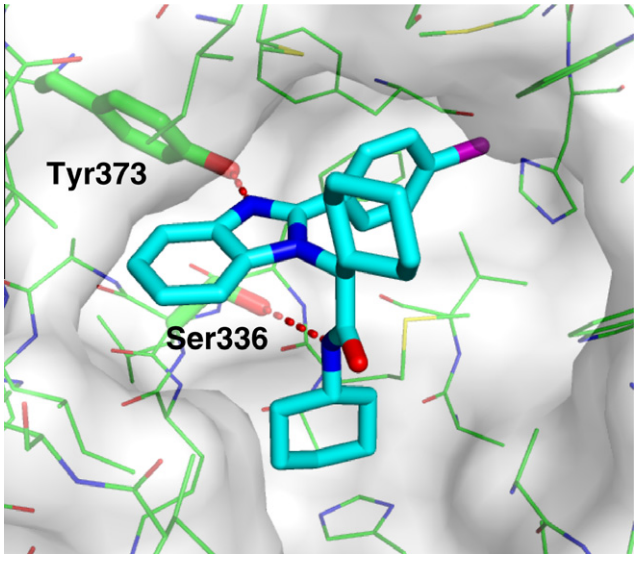
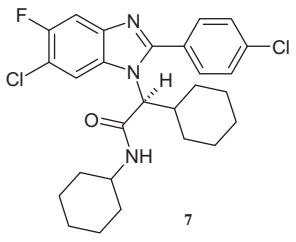


Figure 1. X-ray crystal structure of **1a** bound to hFXR (PDB id: 3OKI). Hydrogen bonds between the ligand and the side chains of Tyr373 and Ser336, respectively, are shown as red, dashed lines.



IC_{50} (SPA): 0.013 μM
EC_{50} (TA): 0.141 μM (43%)
logD: >4
Solubility: <1 μg/ml
Fassif/Fessif: 36/113 μg/ml
PAMPA (Pe): <0.2 x 10^{-6}cm/s
hMic(CL cat.): 17% (high)
mMic(CL cat): 4% (high)
hFH(CL cat): 100% (low)
mFH(CL cat): 66% (medium)
CYP3A4/2D6/2C9: 9.8/14.0/8.3 μM

Scheme 4. In vitro profile of lead compound **7**. TA: transactivation activity assay. TA activity was measured by a luciferase transcriptional reporter gene assay as described.[11] Efficacies are relative to GW4064 (EC_{50} = 0.91 μM set at 100%), the value is a mean. CL cat: clearance category.

Table 5
In vivo lipid lowering effects of **7** versus reference (30 mg/kg, p.o.) as determined in high-fat diet fed $LDL^{-/-}$ mice. (six animals per group, ANOVA followed by Dunnett's post-hoc tests: $^{*}p$ <0.05, $^{**}p$ <0.01)

Compound	TC	LDL	TG
Reference	−37 ± 5%**	−35 ± 5%*	−54 ± 7%
7	−45 ± 3%**	−48 ± 3%**	−52 ± 9%*

Further analysis of the available structural information revealed a more polar, yet unexplored region in the direction of the R″ vector. Specifically targeting this area of the FXR binding site, we subsequently refined our lead molecules to generate further analogs with improved physicochemical properties, in vitro efficacy and in vivo pharmacological characteristics. More detailed information will be reported in due course.

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