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Identification of an N-oxide pyridine GW4064 analog as a potent FXR agonist

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ABSTRACT

According to the docking studies and the analysis of a co-crystal structure of GW4064 with FXR, a series of 3-aryl heterocyclic isoxazole analogs were designed and synthesized. N-Oxide pyridine analog (**7b**) was identified as a promising FXR agonist with potent binding affinity and good efficacy, supporting our hypothesis that through an additional hydrogen bond interaction between the pyridine substituent of isoxazole analogs and Tyr373 and Ser336 of FXR, binding affinity and functional activity could be improved.

The Farnesoid X Receptor (FXR), a member of the nuclear receptor superfamily, has bile acids (e.g., chenodeoxycholic acid (CDCA)) as its natural ligands, and plays a key role in regulating cholesterol and bile acid homeostasis.[1–4] The key factor to this function is the ability of bile acid-activated FXR to down-regulate the expression of Cyp7a[1,5,6], which is the rate-limiting step in the liver for the conversion of free cholesterol to bile acids, and the up-regulation of the bile salt excretion pump (BSEP).[7] Treatment of *ob*/*ob* and *db*/*db* mice with the synthetic FXR agonist GW4064 (**2**) significantly improves hypercholesterolemia[8] and lowers free fatty acid[9,10] and triglyceride levels in plasma.[11] Similarly, treatment with GW4064 increases insulin sensitivity in *ob*/*ob* and *db*/*db* mice.[8,10] As such, FXR agonists may have utility in treating metabolic syndrome, a clustering of cardiovascular risk factors characterized by dyslipidemia (elevated triglyceride and low HDL levels, also add high LDL-c levels), insulin resistance, and poor glucose regulation. Several reviews have summarized the current state of FXR agonists[12,13] and the development of FXR modulators for the treatment of diabetes and metabolic syndrome[14] remains the current focus.

After deorphanization of FXR in 1999[2–4], extensive studies have been directed to the creation of FXR ligands able to modulate FXR-mediated specific gene expression, and several steroidal- and non-steroidal FXR ligands have been described (Fig. 1). Most of the reports deal with FXR agonists (**1**,[11] **2**,[15,16] **3**,[17,18] **4**[19] and **5**[20]), now compound **5** has recently entered Phase I clinic trials;[21,22] and only few FXR antagonistshave been found so far.[2,3]

GW4064 (**2**) is a well-demonstrated, non-steroid agonist towards FXR, and its biological effects on hyperlipidemia in diabetic mice have been identified and confirmed.[1–4] Since the report of GW4064, a large amount of research work has been directed towards the search replacements of the isoxazole core and the stilbene side-chain.[23–26] Many publications and patents have reported on some new modifications and SAR studies related to GW4064 analogues. So far, most of the modifications around the isoxazole 3-position were limited to modifications of the aryl substituents whereas heterocyclic substituents such as pyridines have been rarely reported.[27]

We report herein the exploration around the 3-aryl substituent of GW4064 and the discovery of an N-oxide pyridine analogue (**7b**), which exhibited highly potent binding affinity and functional activity for FXR, as well as improved physicochemical properties compared with other described GW4064 analogues.

The co-crystal structures of GW4064 and other analogues with FXR have been resolved in-house[28] and reported recently.[29] Analysis of the crystal structure of GW4064 suggested that there are good hydrogen bond interactions between the carbonyl acid group and isoxazole hetero atoms with Arg335, and His451, respectively (Fig. 2). These interactions play important roles for the binding to FXR. In addition to these interactions, some desirable substituents especially on the 3-aryl region of isoxazole could gain more favorable Hydrogen bond interaction with several amino acid residues such as Tyr373/Ser336. Therefore some modifications such as introduction of more polar heterocycles or

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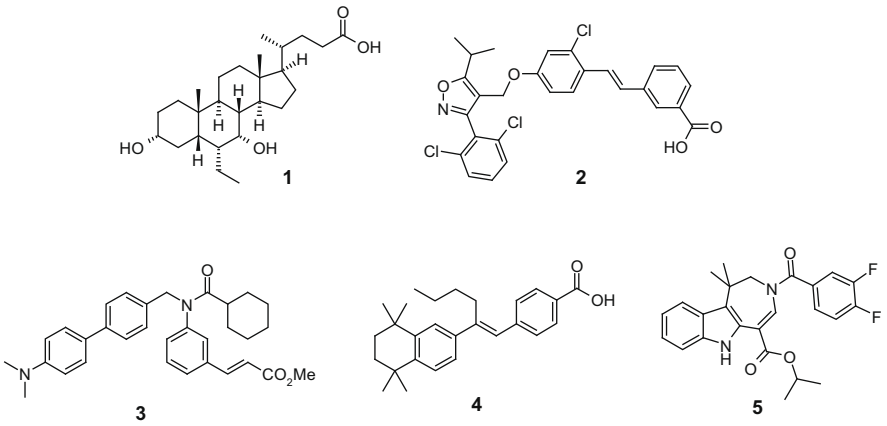

Figure 1. Structures of FXR agonists. **1** (6-ECDCA, Intercept), **2** (GW4064, GSK), **3** (Fexaramine, Amgen), **4** (AGN-31, Allergan), and **5** (Exelixis).

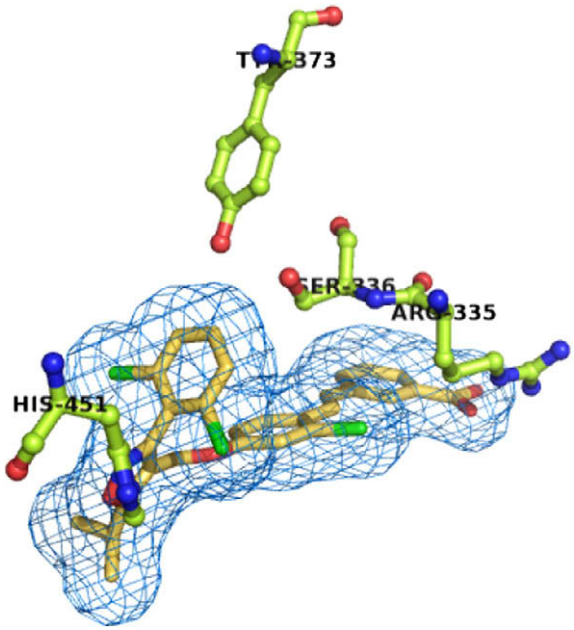

Figure 2. Co-crystal structure of GW4064 bound to FXR ligand binding domain (LBD). FXR species: human; resolution: 2.3 Å. Residues in H-bonds are Arg335 and Tyr373, both of which are conserved in FXR (according to PFAM alignment).

substituents toward this position could potentially improve the binding affinity and interaction with the transactivation helix-12. On the other hand, from the point of drug-like properties, the envisaged modifications should help to improve the physicochemical properties while maintaining a high degree of binding affinity at least.

According to the previous hypothesis, more polar moieties such as methoxy and methylsulfonyl substituents were introduced to the *ortho*- and *para*-positions at the phenyl moiety of isoxazole (Fig. 3, **6b**, **6c**), respectively. Dichloro-pyridine also was utilized to replace the dichloro-phenyl moiety affording compound **6d**. The synthesis of **6b**–**d** is quite similar to the reported method.[11,30] Dozens of heterocyclic GW4064 analogues with pyridine, N-oxide pyridine, and pyridone as 3-aryl substituent replacements have been designed and synthesized (**7a–h**). Since the phenyl indole side-chain of isoxazole has been revealed recently by Eli Lilly' patent as a good replacement of stilbene[23,25], the phenyl indole carboxylic acid was selected to combine into our design.

The typical synthetic strategy for the preparation of compound (**7b**) is outlined in Scheme 1. The key intermediate **8** was prepared mainly according to the reported synthesis procedure of GW4064 (**6a**) analogues.[11] Pyridine ester **8** was firstly oxidized with *m*-CPBA in refluxing DCE, and then followed by the treatment with trifluoro acetic anhydride to furnish another important intermediate, pyridone ester **9**. The following *o*-methylation of **9** was realized via refluxing with silver carbonate and methyl iodide in chloroform to afford methyl ester **10**. Reduction of the ester moiety in **10** did not work very well in a number of different solvents, however DCM was found to be effective, providing the desired alcohol **11** in 60% yield. Chlorination of alcohol **11** with thionyl chloride in chloroform, followed by oxidation with *m*-CPBA produced N-oxidated pyridine **13**. Deprotonation of phenol **16** with mild base in DMF and subsequent reaction with 4-chloromethyl isoxazole **13** provided coupled intermediate indole aldehyde **14**. Finally, oxidation of the corresponding aldehyde **14** with sodium hypochlorite in *t*-butene/dioxane solution mediated by monobasic sodium phosphate gave rise to the target compound **7b**. Compounds **7a** and **7c–h** were synthesized following similar procedures.

The FXR binding and functional activity data of **6a–d** and **7a–h** are listed in Table 1. Replacement of the 2,6-dichloro substituent with either *ortho*-methoxy (**6b**) or *para*-methylsulfonyl (**6c**) groups resulted in a 3- to 4-fold decrease in binding affinity concomitant with a significant loss in functional activity. Most likely the large methylsulfonyl substituent interferes with the hydrogen bond interaction with FXR, and weakens its activity. 2,6-Dichloro pyridine analogue **6d** showed reduced binding affinity (0.69 μM), however maintained the weak transactivation activity, of which permeability was improved slightly compared with GW4064. Combination of the pyridine head with an optimized stilbene replacement, *N*-methyl indole ring afforded a promising compound **7a**, which exhibited good activity (0.094 μM) in the human SPA binding assay. The predicted membrane permeability was improved, too. Screening of additional analogs led to the discovery of the most potent compound (**7b**) in this series, with a binding affinity of 45 nM. The compound has proved to be a partial agonist (54% and 89% max efficacy relative to GW4064) with an EC_{50} at 0.1 μM, 0.22 μM, **7b** also revealed the best PAMPA data among these series. All other analogues (**7c–h**) exhibited moderate to weak activity either in binding or functional assay, with the exception of **7c** which showed good transactivation activity. The reduced activity might result from the minor changes of the head group orientation, leading to less favorable interactions between the ligands and the binding pocket of FXR.