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Discovery of a new binding mode for a series of liver X receptor agonists

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ABSTRACT

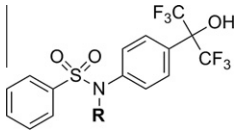
Structural modification of a series of dual LXRα/β agonists led to the identification of a new class of LXRβ partial agonists. An X-ray co-crystal structure shows that a representative member of this series, pyrrole **5**, binds to LXRβ with a reversed orientation compared to **1**.

Liver X receptors (LXRs) are nuclear receptors that function as ligand-activated transcription factors and are present in two highly similar isoforms, LXRα (NR1H3) and LXRβ (NR1H2). Heterodimerization of LXRs with retinoid X receptors (RXRs) is required to achieve DNA binding. LXRs have been implicated in cholesterol homeostasis by regulating ABCA1, a key gene involved in managing cellular HDL cholesterol transport.[1] LXRs also play a critical role in fatty acid metabolism and lipid biosynthesis by regulating sterol regulatory element-binding protein-1c (SREBP-1c) production; SREBP-1c is a transcription factor which regulates the expression of a number of key genes implicated in lipogenesis including fatty acid synthase (FAS) and stearoyl CoA desaturases (SCDs).[2]

Several reports on the viability and lipid profile of LXRα/β null mice raised our interest in developing a dual-LXRα/β antagonist for the treatment of hypertriglyceridemia, a risk factor for atherosclerotic cardiovascular disease.[3] Two observations in particular were noteworthy. First, LXRα/β null mice exhibited significantly reduced levels of VLDL plasma triglycerides relative to wild type.[3b] Second, basal expression of ABCA1 was elevated and intestinal absorption of cholesterol was unaffected in LXRα/β null mice.[1a] Taken together, these data suggest that a potent LXRα/β dual antagonist could down-regulate the SREBP-1c pathway to reduce triglyceride levels in hypertriglyceridemic patients without affecting cholesterol homeostasis. To date, several reports of synthetic LXR antagonists have been documented in the literature.[4]

A series of dual LXRαβ agonists, which include *N*-methylsulfonamide 1 and *N*-trifluoroethylsulfonamide 2 (also known as T0901317), was identified at Tularik Inc. (now Amgen) and was described previously.[5] These compounds exhibit potent affinities for both LXRα and LXRβ in a scintillation proximity ligand binding assay (SPA) using tritium-labeled **1** as the competitive binder (Table 1).[5] Compounds **1** and **2** were also shown to activate LXRα in a luciferase reporter gene assay performed in HEK293 cells.[6] An X-ray co-crystal structure of **2** bound to the LXRα has been reported and establishes the presence of a strong hydrogen bond between His421 and the acidic hydroxyl group of the 1,1,1,3,3,3-hexafluoroisopropanol moiety of **2**.[7] Further, helix 12 of the LBD of LXRα effectively seals off the exposed face of the ligand binding pocket.

Table 1
Binding affinities of early LXR agonists **1**–**2**



Compound	R	SPA binding, IC_{50} (μM)	
		LXRα	LXRβ
1	CH_3	0.04	0.02
2	CH_2CF_3	0.1	0.1

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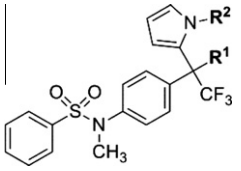
We postulated that replacement of one of the trifluoromethyl groups of **1** with a bulky aromatic moiety might force helix 12 to point away from the binding site and thus prevent the ligand-bound receptor from adopting a transcriptionally active conformation. There is precedent for this type of agonist to antagonist conversion in the nuclear receptor field. For example, the estrogen receptor (ER) agonist diethylstilbestrol was converted into the antagonist 4-hydroxytamoxifen by the attachment of a suitable bulky side chain to the agonist core structure. This structural change prevented helix 12 from adopting an agonist-bound conformation upon 4-hydroxytamoxifen binding.[8]

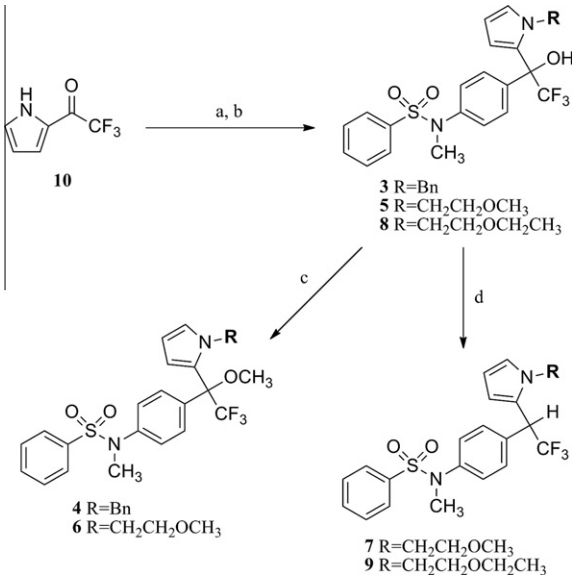
In our case, the replacement of one of the trifluoromethyl groups of compound **1** with a variety of aryl and heterocyclic moieties failed to generate compounds displaying appreciable levels of LXR antagonism.[9] During the course of this study, however, a surprising SAR trend for a series of racemic *N*-substituted pyrrole derivatives was identified (Table 2). As expected, removal of the hydrogen bond donating capability of *N*-benzylpyrrole **3** significantly reduced both LXRα and LXRβ binding affinities for methyl ether analog **4** relative to **3**. On the other hand, *N*-methoxyethylpyrrole **5** unexpectedly becomes a more potent binder upon *O*-alkylation (compound **6**) or deoxygenation (compound **7**). A similar trend is observed for *N*-ethoxyethylpyrrole **8** and its corresponding deoxygenated analog **9**.

Pyrroles **3**–**9** were synthesized from commercially available 2-(trifluoroacetyl)pyrrole (**10**) (Scheme 1). *N*-alkylation of 10 with an appropriate bromide followed by a *tert*-butyllithium-mediated condensation with *N*-(4-bromophenyl)-*N*-methylbenzenesulfona-

Table 2
Binding affinities of pyrrole derivatives **3**–**9**



Compound	R^1	R^2	SPA binding, IC_{50} (μM)	
			LXRα	LXRβ
3	OH	Bn	0.8	0.8
4	OCH_3	Bn	>10	>10
5	OH	$CH_2CH_2OCH_3$	1.5	1.8
6	OCH_3	$CH_2CH_2OCH_3$	0.4	0.9
7	H	$CH_2CH_2OCH_3$	0.2	0.8
8	OH	$CH_2CH_2OCH_2CH_3$	0.5	1.5
9	H	$CH_2CH_2OCH_2CH_3$	0.2	0.7



Scheme 1. Reagents and conditions: (a) NaH, RBr, DMF, rt (for R = Bn) or 60 °C, 75–80%; (b) (i) *t*-BuLi, *N*-(4-bromophenyl)-*N*-methylbenzene-sulfonamide, Et_2O, −100 °C, 10 min; (ii) add ketone, Et_2O, −100 °C to rt, 65–70%; (c) NaH, MeI, DMF, 45–60%; (d) $BF_3{\cdot}OEt_2$, Et_3SiH, CH_2Cl_2, 33–34%.

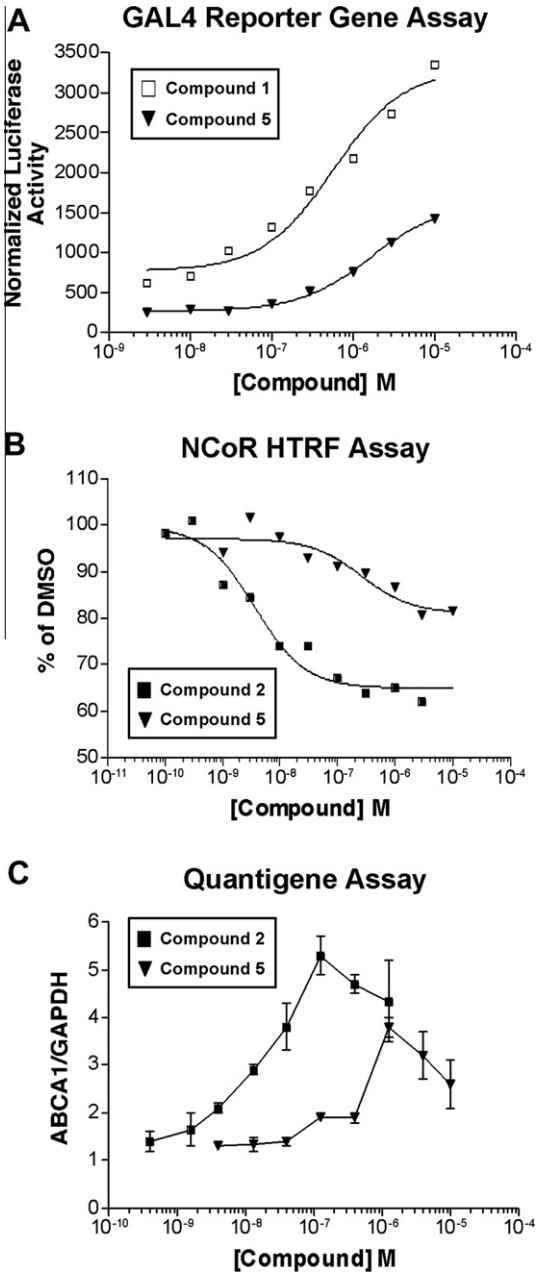
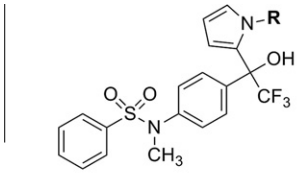
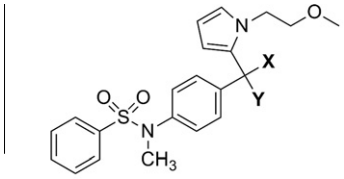


Figure 1. (A) Compounds **1** and **5** increase transcriptional activation by LXRβ in a cell-based reporter gene assay. HEK-293 cells were transfected with a plasmid containing a gene encoding a fusion of the GAL4 DNA-binding domain and the LXRβ ligand-binding domain, a luciferase reporter gene, and a β-galactosidase gene to control for transfection efficiency. (B) Compounds **2** and **5** reduce the basal interaction of LXRβ and the nuclear receptor co-repressor NCoR in a homogeneous time-resolved fluorescence (HTRF) assay. The emission intensity ratio has been expressed as a percentage relative to the control (DMSO) emission ratio. (C) Compounds **2** and **5** increase endogenous ABCA1 gene expression in Caco-2 cells. Expression level is normalized by expression of the housekeeping gene GAPDH.



11 R=H
12 R=CH_3
13 R=CH_2CH_3
14 R=$CH_2CH_2CH_3$
15 R=CH_2CH_2OH
16 R=$CH(CH_3)CH_2CH_3$
17 R=$CH_2CH_2CH_2CH_3$
18 R=$CH_2CH_2CH_2OH$
19 R=$CH_2(CH_2)_4CH_3$

Figure 2. Pyrrole *N*-alkyl and *N*-hydroxyalkyl derivatives.



20 X=H, Y=H
21 X,Y=O
22 X=H, Y=OH
23 X=H, Y=OCH_3
24 X=H, Y=OCH_2CH_3
25 X=OH, Y=CH_3
26 X=H, Y=CH_3
27 X=OH, Y=CH_2CH_3
28 X=H, Y=CH_2CH_3
29 X=OH, Y=$CH_2CH_2CH_3$
30 X=H, Y=$CH_2CH_2CH_3$
31 X=H, Y=CH_2OH

Figure 3. Pyrrole derivatives with modified central linkers.

mide[10] generated alcohols **3**, **5**, and **8**. From these alcohol intermediates, methyl ether formation was accomplished with methyl iodide to afford **4** and **6**. Alternatively, deoxygenation with triethylsilane in the presence of boron trifluoride etherate led to **7** and **9**.

We were initially surprised that despite the presence of a bulky pyrrole side chain, several compounds in this series behave as partial agonists. For example, compound **5** exhibits approximately 33% of the maximal response of **1** in an LXRβ cell-based reporter gene assay (Fig. 1).[6] Compounds **6** and **8** also act as partial agonists, while **3** is a very weak antagonist (data not shown). Moreover, **5** displays partial agonism (as compared to **2**) in a biochemical assay measuring LXRβ co-repressor recruitment, and in a cell-based assay measuring endogenous expression of ABCA1 (Fig. 1).

Further structural modification of LXR agonist **5** was examined in an effort to identify pyrrole-based agonists with enhanced binding affinities and cellular potencies. To this end, the pyrrole nitrogen was functionalized with a number of flexible, non-bulky alkyl and hydroxyalkyl chains (compounds **11**–**19**, Fig. 2).[11] Also, a series of analogs **20**–**31** with modified aryl-heteroaryl linkers was made (Fig. 3).[12] Unfortunately, no significant improvements in binding were observed for either set of derivatives.

Co-crystal structures of compounds **1** and **5** bound to LXRβ have been determined and provide a rational explanation for these unanticipated results (Fig. 4). For compound **1**, the usual agonist binding conformation is observed, including a strong hydrogen bond between the hydroxyl group of **1** and His435 (analogous to His421 in LXRα). On the other hand, compound **5** adopts a novel reversed agonist binding mode in the ligand binding site of LXRβ, presumably due to the large steric size of the functionalized pyrrole. In this structure, the sulfonamide region of the molecule points toward helix 12, and no hydrogen bond interaction is present between **5** and the protein. The lack of a hydrogen bond between compound **5** and the ligand binding site in this reversed agonist binding mode could account for the reduced LXRβ binding affinity of **5** relative to **1**. This weakened interaction with helix 12 might also explain why compound **5** behaves as a partial agonist in comparison to **1**. Note that while compound **5** is racemic, only the (*S*)-epimer of **5** is observed in the co-crystal structure.

In conclusion, an attempt to transform dual-LXRα/β agonists **1** and **2** into an antagonists via structural modification of the 1,1,1,3,3,3-hexafluoroisopropanol moiety led to the unexpected discovery of a new series of LXR agonists. An X-ray co-crystal structure of **5** bound to LXRβ displayed a novel, reversed agonist binding mode characterized by the lack of a hydrogen bond anchoring the substrate into the binding site. This new binding mode accommodates for the bulky substituted pyrrole side chain of **5** without significant reorientation of helix 12 relative to its position when bound to **1**.

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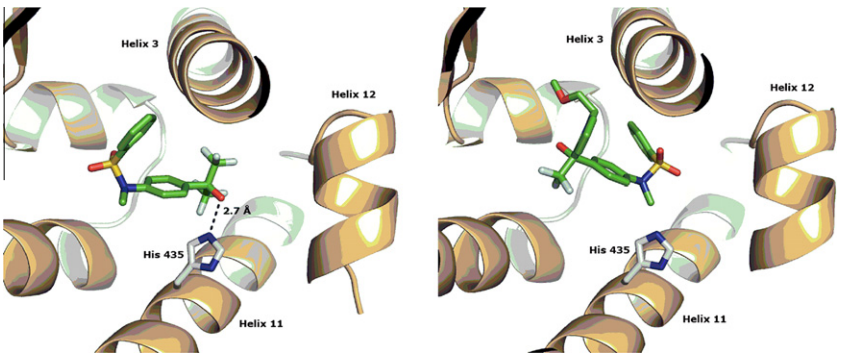

Figure 4. X-ray co-crystal structure of full agonist **1** bound to LXRβ (left picture, resolution = 2.45 Å, pdb code 4DK7) shows that a key hydrogen bond interaction exists between **1** with His435. The X-ray co-crystal structure of partial agonist **5** bound to LXRβ (right picture, resolution = 2.75 Å, pdb code 4DK8) shows that **5** binds in a 'flipped' orientation with no hydrogen bond interactions. The electron density map indicates that only the (*S*)-epimer of **5** is present. In both cases, Helix 12 adopts a closed conformation that seals off the ligand binding site.

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11. Compounds **11**–**19** were synthesized from 2-(trifluoroacetyl)pyrrole (10) by a similar route to that described in Scheme 1.
12. Compounds **20**–**24** and **27**–**31** were synthesized from pyrrole-2-carboxaldehyde, while compounds **25**–**26** were made from 2-acetylpyrrole.