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Discovery of Highly Potent Liver X Receptor β Agonists

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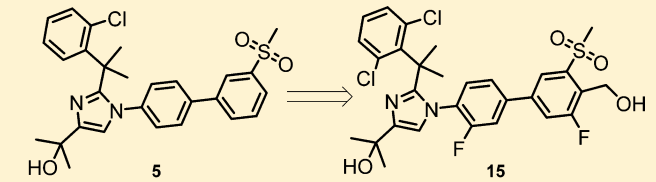
Supporting Information

ABSTRACT: Introducing a uniquely substituted phenyl sulfone into a series of biphenyl imidazole liver X receptor (LXR) agonists afforded a dramatic potency improvement for induction of ATP binding cassette transporters, ABCA1 and ABCG1, in human whole blood. The agonist series demonstrated robust LXRβ activity (>70%) with low partial LXRα agonist activity (<25%) in cell assays, providing a window between desired blood cell ABCG1 gene induction in cynomolgus monkeys and modest elevation of plasma triglycerides for agonist **15**. The addition of polarity to the phenyl sulfone also reduced binding to the plasma protein, human α-1-acid glycoprotein. Agonist **15** was selected for clinical development based on the favorable combination of *in vitro* properties, excellent pharmacokinetic parameters, and a favorable lipid profile.



KEYWORDS: *Liver X receptor, LXRα, LXRβ, ABCA1, ABCG1, α-1-acid glycoprotein*

Identification of drugs to treat coronary heart disease (CHD) patients continues to be an important research area. Despite significant advances in treatment including statin therapy, CHD caused by atherosclerosis remains a major cause of morbidity and mortality in the United States.[1] Owing to the powerful effect of liver X receptor (LXR) agonists on reverse cholesterol transport (RCT)[2] and immune system modulation,[3,4] which culminate in reduced atherosclerosis lesions in animals,[5−7] there have been many LXR agonist medicinal chemistry campaigns to treat atherosclerosis driven cardiovascular disease.[8] LXRα and LXRβ agonists increase RCT by induction of ATP binding cassette transporters ABCA1 and ABCG1,[9,10] which efflux cholesterol from cells to HDL particles, and transporters ABCG5 and ABCG8, which traffic cholesterol from liver to the feces and promote its excretion. The immune system effects of LXR receptors continue to be elucidated, and LXRs are involved in innate and acquired immunity processes.[3] LXRs regulate many additional pathways in lipid homeostasis and energy utilization, and as such LXR agonists have been suggested as therapeutic treatments for other diseases including several types of cancers,[11] skin conditions,[12] and heart failure.[13]

While many LXR agonists have been reported including the well-studied TO-091317 (**1**)[14] and GW3965 (**2**),[15] a challenge in the field has been to develop agonists that maintain the positive effects described above while not causing increases in low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG). The TG increases are primarily caused by LXR induction of the transcription factor sterol regulatory element binding protein 1c (SREBP1c) and the enzyme fatty acid synthase (FAS) in liver, leading to increased very low density lipoprotein (VLDL) production and secretion from liver,[14] as well as upregulation of hepatic angiopoietin-like protein 3 and down-regulation of apoA-V expression resulting in decreased lipolysis of circulating TG-rich lipoproteins.[16,17] Increased LDL-C has been reported after repeat dose treatment in hamsters and cynomolgus monkeys with LXR agonists.[18] The LDL-C increases may be caused by a combination of multiple mechanisms, including increased VLDL production, induction of cholesteryl ester transfer protein (CETP),[19] and induction of inducible degrader of LDL receptor (IDOL).[20] The lipid effects have been proposed to be driven by hepatic LXRα based on knockout mice,[21,22] influencing the field to optimize for LXRβ selectivity.[8]

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Several reports of LXR agonists with improved therapeutic windows compared to full pan-agonists have been described (Figure 1). For instance, the agonist LXR-623 (**3**) has been

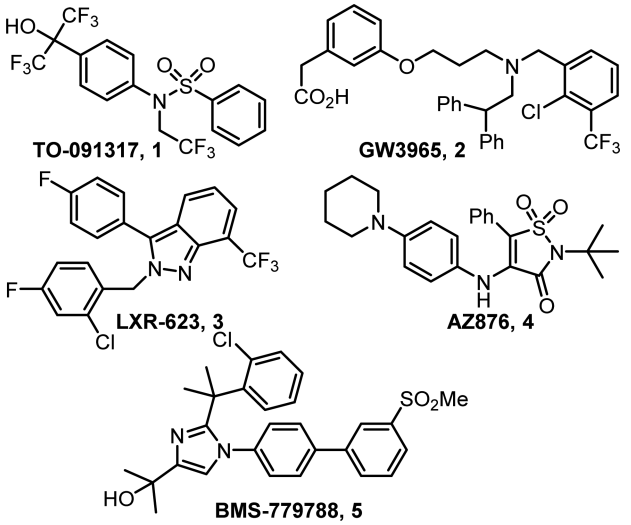


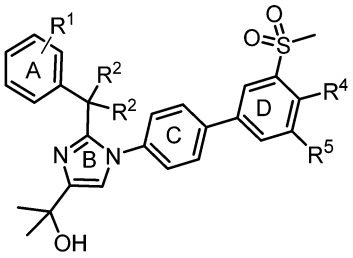
Figure 1. Examples of LXR agonists reported in the literature.

reported to decrease LDL-C in cynomolgus monkeys, while inducing transporters such as ABCA1; however, the mechanism for the LDL-C decrease has not been reported.[23,24] Unfortunately neurological effects were observed in humans after 1 day of dosing **3**.[25] Further supporting that efficacy can be achieved without lipid effects, AZ876 (**4**) was reported to reduce atherosclerosis plaques and improve heart failure outcomes in mice at doses that did not cause increased TGs.[26] We have previously reported that an improved therapeutic window can be achieved with agonist BMS-779788 (**5**).[27,28]

Agonist **5** shows a preference for LXRβ in binding and functional assays and induces LXR target genes ABCA1 and ABCG1 in human whole blood with an EC_{50} value of 1.2 μM and 55% efficacy (Table 1).[27] An improved lipid profile was observed in mouse and cynomolgus monkeys with **5** compared to TO-091317 (**1**), and agonist **5** had a good pharmacokinetic and safety profile so it was taken into human trials.[28] Key goals for optimization were to identify a molecule with lower LXRα agonist activity to minimize the TG effects, while improving the potency for on-target ABCA1 and ABCG1 induction in human whole blood.

The imidazole agonists were prepared as reported previously[27] and as described in Supporting Information. Agonists were profiled in a suite of five LXR assays. The binding affinity was determined with full-length LXRα–RXRα and LXRβ–RXRα heterodimers.[29] Functional isoform activity was assessed using LXRα and LXRβ transactivation assays in CV-1 cells,[30] and in HeLa cells with endogenous LXRα and LXRβ receptors and an ABCA1 LXREx3 reporter. Compounds were tested for ABCA1 and ABCG1 induction in a human whole blood assay (hWBA). The hWBA potency was a key driver because we anticipated it would predict clinical efficacy. The hWBA EC_{50} value for ABCA1 gene induction is reported because we used that data to evaluate SAR; in general, ABCG1 EC_{50} values were within 2-fold and are reported for **5** and **15** in the Supporting Information.

Table 1. SAR Optimization of Lead 5



Example #	R^1	R^2	R^4	R^5
5	2-Cl	Me	H	H
6	2,6-diCl	Me	H	H
7	2-Cl	H	CH_2OH	H
8	2-Cl	Me	CH_2OH	H
9	2-Cl	Me	CH(Me)OH	H
10	2-Cl	Me	CH_2OH	F

#	LXRβ/LXRα Binding K_i, nM	LXRβ EC_{50} nM (% eff)	LXRα EC_{50} nM (% eff)	ABCA1 HeLa EC_{50} nM (% eff)	hWBA EC_{50} nM (% eff)
5	14/68	250 (72%)	220 (38%)	33 (50%)	1200 (55%)
6	11/16	170 (41%)	99 (20%)	18 (42%)	380 (32%)
7	5/31	60 (50%)	100 (39%)	9 (54%)	300 (72%)
8	13/74	170 (74%)	110 (46%)	12 (56%)	300 (46%)
9	40/180	220 (93%)	120 (47%)	21 (68%)	870 (47%)
10	10/53	72 (83%)	76 (29%)	8 (43%)	57 (47%)

[a]Standard deviations are reported in the Supporting Information when $n > 2$.

In pursuit of improving properties, several areas were explored simultaneously, including substitutions on the A and D rings (Table 1). While many compounds were prepared with different R^1 substitution patterns that had similar activities to **5** (structures and data not shown), the 2,6-dichloro substitution (**6**) achieved a 3-fold boost in hWBA potency with lower LXRα efficacy (20%) compared to **5** (38% LXRα efficacy). Substituted phenyl sulfones were prepared based on the LXRβ crystal structure obtained with agonist **5** that had a water channel where R^4 is positioned.[27] Investigations to exploit this position led to the synthesis of the hydroxymethyl sulfones **7** and **8**. Small gains in potency were observed in LXRα and LXRβ agonist assays, which translated to 300 nM EC_{50} values for hWBA ABCA1 induction. Since **7** was prepared first it was dosed to mice at 10 mg/kg, and good plasma exposure was observed (Supporting Information); however, when dosed to cynomolgus monkeys, the observed clearance was higher than hepatic blood flow at 61 mL/min/kg (Table 2). Cynomolgus monkeys were critical to compound progression because they were a key model to study the lipid effects. High clearance was also observed with other analogues containing this D-ring substitution (data not shown), so compounds were prepared to address the high clearance.

Anticipating that a secondary alcohol would have reduced clearance, **9** was prepared and found to show partial 47% activity at LXRα with an hWBA EC_{50} value of 870 nM.

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Table 2. PK in Cynomolgus Monkeys after i.v. Dosing

example	5	5	7	9	10	15
dose (mg/kg)	1.0	0.2	0.25	0.2	0.2	3.0
Cl (mL/min/kg)	1.9	2.9	61	5.6	8.4	8.0
$t_{1/2}$ (h)	7.4	8.9	0.8	5.5	5.6	12

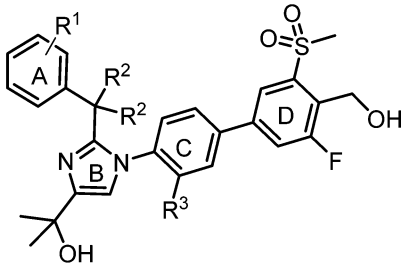
[a] n = 2 for 0.2 and 0.25 mg/kg doses, and n = 3 for 1 mg/kg doses. Standard deviations for **5** and **15** are in the Supporting Information.

Analogue **10** was prepared with an adjacent electron withdrawing fluorine at R^5 to try to slow metabolism of the hydroxymethyl R^4 substituent. Compound **10** had LXRβ agonist potency of 72 nM with a dramatic improvement in hWBA potency to 57 nM (47% efficacy), which was 20-fold better than **5**. While limited selectivity was observed in binding assays, **10** showed differential agonist activity with 29% LXRα efficacy and robust LXRβ 83% efficacy. Both **9** and **10** had improved clearance rates of 5.6 and 8.4 mL/min/kg in cynomolgus monkeys (Table 2). Due to the significant potency improvement with **10** we progressed this series and did not test the closest comparator **8** in cynomolgus monkey PK to confirm the improved clearance was entirely due to the fluorine.

With optimized D-ring substituents, we focused SAR exploration on the A and C aryl rings (Table 3). Our goal was to identify the most potent analogues with LXRα efficacy ≤25% and robust LXRβ efficacy. The 2-fluoro substitution (**11**) at R^1 had a similar profile to **10**, although the % efficacy was modestly reduced across all agonist assays. Mirroring the observation with **6**, the 2,6-dichloro analogue **12** had a 3−4-fold improvement in hWBA potency to 15 nM with limited 25% LXRα efficacy. Interestingly, the 2-Cl,3-F analogue **13** had very limited LXRα activity at 12% while maintaining a hWBA EC_{50} value of 76 nM. The R^3 fluorine substitution (**14**) maintained similar activity assays compared to **10**. When the fluorine R^3 substitution was combined with the 2,6-diCl A-ring (**15**) very potent hWBA activity was observed with an EC_{50} value of 9 nM (26% efficacy). Although **15** has similar LXRα and LXRβ binding K_i values (19 and 12 nM, respectively), in agonist assays the compound achieved 88% efficacy toward LXRβ and only 20% efficacy toward LXRα compared to a full pan-agonist. When tested in antagonist mode, **15** was a potent LXRα antagonist with an IC_{50} value of 69 nM (83% inhibition); whereas no antagonism was observed in LXRβ assays up to 10,000 nM (Supporting Information). Further SAR investigation with a 2-Cl,6-F R^1 substitution pattern provided **16** with a hWBA potency of 41 nM (33%). Introduction of an R^3 chlorine atom in **17** caused a decrease in efficacy in all four agonist assays with a potent hWBA EC_{50} value of 5 nM just above the limit of assay detection (16%). Analogue **18** with hydrogen at R^2 had *in vitro* activity consistent with the gem-dimethyl analogue **12**. The monomethyl analogue **19** was identified as a metabolite of **15**, and upon synthesis the profile showed it to be a potent, partial LXR agonist as well.

During the characterization of our lead molecules the clinical single ascending dose PK results were available for **5**. The human plasma $t_{1/2}$ was 100−200 h, which was 5−10-fold longer than predicted by preclinical studies. Studies with clinical plasma samples showed high binding (i.e., >99.9%) of **5** to plasma proteins. The volume of distribution in humans was at least 10× smaller than the projected values based on preclinical species, suggesting limited distribution of this molecule outside of systemic circulation. One hypothesis brought forward to explain the unexpected long half-life, limited volume of distribution and slow clearance in humans was tight binding to α-1-acid glycoprotein (α1 AGP), which was different between human and preclinical species (manuscript in preparation). A similar explanation has been proposed for the human PK of UCN-01 (7-hydroxystaurosporine).[31] Equilibrium dialysis with **5** demonstrated 99.9% binding to human α1 AGP. In contrast **5** has moderate binding of 97, 92, and 98% to human serum albumin (HSA) and rat and dog α1 AGP, respectively. Binding to human α1 AGP was measured for several agonists (Table 4). Whereas the biphenyl sulfones **5** and **6** had >99% binding to α1 AGP, introduction of the polar R^4 hydroxymethyl group in **8**, **10**, **13**, and **15** reduced α1 AGP binding, consistent with the hWBA potency improvement. Binding to HSA, a major plasma protein, did not differentiate the analogues with 94−98% bound.

Table 3. Optimization of R^1, R^2, and R^3 with (2-Fluoro-6-(methylsulfonyl)phenyl)methanol D-Ring



Example #	R^1	R^2	R^3
11	2-F	Me	H
12	2,6-diCl	Me	H
13	2-Cl, 3-F	Me	H
14	2-Cl	Me	F
15	2,6-diCl	Me	F
16	2-Cl, 6-F	Me	F
17	2,6-diCl	Me	Cl
18	2,6-diCl	H	H
19	2,6-diCl	Me, H	F

#	LXRβ/LXRα/Binding K_i, nM	LXRβ EC_{50} nM (%Eff)	LXRα EC_{50} nM (%Eff)	ABCA1 HeLa EC_{50} nM (% Eff)	hWBA EC_{50} nM (%Eff)
11	14/81	160 (68%)	130 (13%)	12 (15%)	43 (28%)
12	6/38	42 (72%)	30 (25%)	3 (23%)	15 (43%)
13	14/53	72 (68%)	72 (12%)	8 (16%)	76 (34%)
14	18/9	50 (79%)	57 (25%)	2 (29%)	46 (35%)
15	12/19	24 (88%)	8 (20%)	0.6 (29%)	9 (26%)
16	14/70	20 (86%)	11 (15%)	1 (30%)	41 (33%)
17	48/50	27 (51%)	8 (6%)	2 (12%)	5 (16%)
18	11/75	140 (54%)	69 (17%)	5 (43%)	42 (51%)
19	13/17	25 (67%)	12 (18%)	2 (9%)	23 (17%)

[a] Standard deviations are reported in the Supporting Information when n > 2.

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Table 4. Equilibrium Dialysis with Human α1 AGP and HSA

example	human α1 AGP (% bound)	HSA (% bound)
5	99.9 ± 0.0	97.2 ± 0.3
6	99.6 ± 0.2	97.9 ± 0.5
8	90.2 ± 4.2	96.1 ± 0.7
10	86.6 ± 0.9	95.5 ± 0.2
13	87.6 ± 1.3	94.4 ± 1.1
15	97.3 ± 0.1	96.6 ± 0.1

[a]The average is reported with standard deviation (n = 3).

The crystal structure of **15** complexed with the ligand binding domain of LXRβ has been determined to 2.4 Å resolution (Figure 2). The complex crystallized in space group

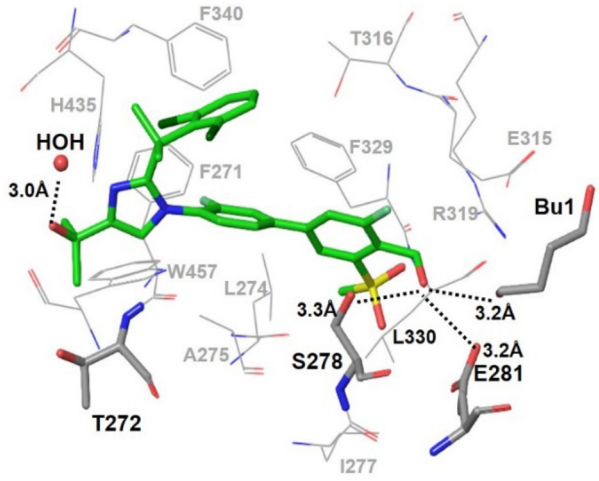

Figure 2. LXRβ complexed with **15** to 2.4 Å resolution (PDB code: 5JY3).

C2 with four independent subunits in the asymmetric unit, with subunits A and B forming a canonical dimer, as did C and D. Helix 12 from subunit A was bound in the coactivator binding pocket of subunit B. The binding mode of **15** is very similar to that of **5**, and there do not appear to be any major changes to side chain positions. The sulfone interacts with the backbone Leu330 as was observed with **5**. The hydroxymethyl interacts with Ser-278, Glu281, and a bound butane diol molecule from the crystallization solvent. The fluorine gives an improved shape complementarity to the pocket near the "D" ring of **15** compared to **5**, likely providing some of the improved potency. The benzylic phenyl (A-ring) forms a pi-stacking interaction with Phe340. The second chlorine substituent causes the benzylic methyl groups to rotate compared to **5**, improving the molecular shape complementarity to the LXR pocket. Whereas many LXR agonists have a direct interaction with His435 stabilizing helix 12[8] that is important for LXR agonist activity, the carbinol hydroxyl group of **17** appears to interact with a water in the active site that looks to be positioned to H-bond with His435. While we are not able to give a definitive structural reason for the low LXRα agonist activity from the LXRβ structure, it is possible that the indirect interaction through a water molecule with His 435 provides some of the observed differences between LXRα and LXRβ agonist activity.

Agonists **13** and **15** were nominated for further study because both compounds had robust LXRβ efficacy with low LXRα agonist efficacy (<20%), which was anticipated to improve the separation of desired efficacy from TG and LDL-C effects. In addition, **15** was very potent in the hWBA. Analogues **13** and **15** were not active in 16 nuclear hormone receptor agonist assays (>10 μM), except PXR with EC_{50} values of 3 μM (85% of full agonism) and 1 μM (108% of full agonism), respectively. When dosed in mice at 10 mg/kg, the C_{max} coverage was high compared to the hWBA potency (Supporting Information). Given that LXR agonists could have deleterious effects in brain, as observed with LXR-623, the brain levels were measured and found to be low with **15** having a brain to plasma ratio of <0.05. In cynomolgus monkeys, **13** and **15** displayed good bioavailability, moderate clearance rates, and 10−12 h plasma half-lives (Supporting Information).

While **15** was considered the lead compound due to exceptional hWBA potency coupled with low LXRα efficacy (Table 3), both **13** and **15** were studied in cynomolgus monkeys for 14 days to investigate the ABCG1 dose response to the lipid effects compared to those of **1**. The agonists showed robust induction of the RCT target gene ABCG1 in plasma at drug concentrations that were predicted by the cynomolgus monkey WBA potency (**1** cynoWBA EC_{50} = 310 nM (100%); **13** cynoWBA EC_{50} = 52 nM (29%); **15** cynoWBA EC_{50} = 5 nM (32%)). ABCA1 had shown variable vehicle effects in multiple cynomolgus monkey studies, precluding its use as a pharmacodynamic biomarker. Both **13** and **15** had improved TG profiles compared to **1**. Fourteen days of dosing **1** at 10 mg/kg (200 nM plasma concentration at 5 h) caused a 6-fold ABCG1 induction in blood cells with TGs elevated 140% over baseline values (p < 0.05, ANOVA). After 14 days, the 1 and 3 mg/kg doses of **13** afforded 4- and 10-fold ABCG1 induction in blood cells with 85 and 310 nM plasma exposures, respectively. These doses yielded TGs of 2% and 58% above baseline (not significant). Comparatively the 0.1, 0.3, and 1 mg/kg doses of **15** provided 5 h plasma exposures of 7.5, 22, and 57 nM with 4.7-, 15-, and 11-fold ABCG1 induction on day 14. The TGs were elevated nonsignificantly 20, 8, and 10% over baseline, respectively. As anticipated, **15** provided robust ABCG1 induction at very low plasma drug concentrations, with little effect on plasma TGs. A full data set from this cynomolgus monkey study **15** is reported elsewhere.[32]

In summary, we have identified a potent biphenyl imidazole series of LXR partial agonists containing a (2-fluoro-6-(methylsulfonyl)phenyl)methanol substituent. Importantly, agonist **15** induces ABCA1 and ABCG1 RCT targets in human whole blood at nanomolar drug exposures with robust LXRβ agonism and limited LXRα agonist activity (Table 3 and Supporting Information). In cynomolgus monkeys this profile gave robust blood cell activity with limited elevations of TGs. Based on coupling the *in vitro* properties with an excellent pharmacokinetic profile and favorable lipid profile in cynomolgus monkeys, **15** (**BMS-852927**) advanced into clinical studies. In human trials the PK was well predicted by preclinical data; however, TGs and LDL-C were observed to be elevated after multiple days of dosing with a limited therapeutic window, indicating that the low LXRα efficacy was not sufficient to protect from deleterious lipid elevations.[32]

■ ASSOCIATED CONTENT

Supporting Information

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LXR assay data with standard deviations, mouse and cyno PK data for **5**, **7**, **13**, and **15**, antagonist and WBA data for **5** and **15**, synthesis methods, compound characterization, biology assays, crystallization protocol, and *in vivo* study protocols (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

α1 AGP, α-1-acid glycoprotein; ABCA1, ABCG1, ABCG5, or ABCG8, ATP binding cassette transporter; apoA, apolipoprotein A; CHD, coronary heart disease; cyno, cynomolgus monkey; HDL, high density lipoprotein; HSA, human serum albumin; hWBA, human whole blood assay; LDL-C, low-density lipoprotein cholesterol; LXR, liver X receptor; PAMPA, parallel artificial membrane permeability assay; PK, pharmacokinetics; PXR, pregnane X receptor; RCT, reverse cholesterol transport; RXR, retinoid X receptor; SAR, structure−activity relationship; TG, triglygerides; VLDL, very low density lipoprotein

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