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4-(3-Aryloxyaryl)quinoline sulfones are potent liver X receptor agonists

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ABSTRACT

A series of 4-(3-aryloxyaryl)quinolines with sulfone substituents on the terminal aryl ring (**7**) was prepared as LXR agonists. High affinity LXR ligands with excellent agonist potency and efficacy in functional assays of LXR activity were identified. In general, these sulfone agonists were equal to or superior to previously described alcohol and amide analogs in terms of affinity, functional potency, and microsomal stability. Many of the sulfones had LXRβ binding IC_{50} values <10 nM while the most potent compounds in an ABCA1 mRNA induction assay in J774 mouse cells had EC_{50} values <10 nM and were as efficacious as T0901317.

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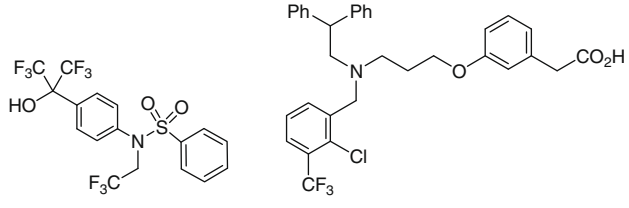
Approaches to the treatment of cardiovascular disease, a leading cause of death in developed countries, have focused on modifying the HMG-CoA reductase pathway using various statins including Lipitor® and blocking the uptake of cholesterol from the intestines using inhibitors such as Zetia®.[1] As an alternative to these therapies, we have examined interventions using LXRβ agonists to increase expression of cholesterol transporters including adenosine-binding cassette transporters (ABCs), a family of lipid transporters responsible for regulating lipid homeostasis.[2] Of particular importance is ABCA1, a key transporter in macrophages and other cells.

Adenosine-binding cassette protein transporter expression is tightly controlled by liver X receptors (LXRs), members of the nuclear hormone receptor superfamily of gene transcription factors, in conjunction with retinoid X receptors (RXRs).[3] There are two subtypes of LXR: LXRα and LXRβ. LXRα is expressed in intestine, lung, kidney, spleen, and macrophages, but especially in liver. In contrast, LXRβ is widely distributed in nearly all cell types. Both LXRα and LXRβ form heterodimers with RXRs and the resulting complex can be activated by binding of either an LXR agonist (e.g., endogenous oxysterols such as 24,25-epoxycholesterol) or an RXR agonist (e.g., 9-*cis*-retinoic acid) resulting in mRNA transcription of various genes, including ABCs.[4] In addition to their role in lipid efflux, LXRs may also have an important anti-inflammatory role which may help minimize the development of athlerosclerosis.[5]

Several pharmaceutical companies have been active in developing LXR agonists to treat dislipidemia. Among the most notable LXR agonists are Tularik's T0901317 (**1**)[6] and GlaxoSmithKline's GW3965 (**2**)[7] (Fig. 1), which are high affinity LXR ligands with potent agonism for both LXRβ and LXRα subtypes. Wyeth has identified an indazole-based LXR agonist WAY-252623 (**3**),[8] which



1 T0901317 (Tularik) **2** GW3965 (GSK)

Figure 1. LXR Agonists from Tularik and GlaxoSmithKline.

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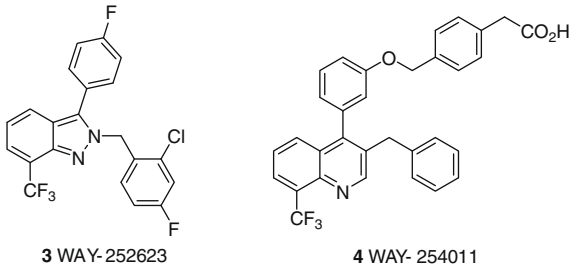
effectively treated dislipidemia in monkey but showed unacceptable CNS effects at higher doses in a phase I clinical trial (Fig. 2).[9] Another Wyeth series incorporated a quinoline as the core heterocycle leading to WAY-254011 (**4**).[10]

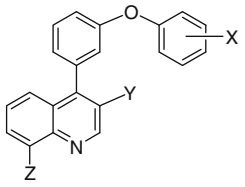
The quinoline-acids such as **4** were generally high affinity LXR ligands with potent agonist activity. Unfortunately, **4** (and related analogs) was a moderate PPAR agonist, activating all three subtypes of the receptor.[11] Modification of the benzylacetic acid group by incorporating other hydrogen bond acceptors while keeping the 4-phenyl-quinoline core led to a series of biarylether amides (**5**) which gave high affinity LXR agonists with essentially no PPAR activation (Fig. 3).[12] Aryloxybenzyl alcohols (**6**) were also potent LXR agonists.[13] To improve the stability of the hydrogen bond acceptor, we explored sulfone groups which provided a series of 4-(3-aryloxyaryl)quinoline sulfones **7** as potent LXR agonists.

Building on previously reported syntheses of **5** and **6**, the key step in the preparation of targets **7** was formation of a bond between a 4-(3-hydroxyphenyl)quinoline core and a halogenated phenyl ring. The halogenated phenyl sulfones **8** were generally prepared by three different approaches (Scheme 1). The first relied on the partial reduction of a sulfonyl chloride to the sodium sulfinate salt which was reacted in the same pot with an alkylating agent, typically an alkyl bromide or iodide.[14] The desired sulfone was often accompanied by some sulfinate ester, though except with more hindered alkylating agents, this was a very minor byproduct. An alternative approach for methylsulfones utilized Ma's copper iodide mediated coupling procedure[15] to prepare arylsulfones directly from aryl iodides and aryl bromides. A third approach to sulfones was the alkylation of thiophenols followed by Oxone® treatment in one pot. The wealth of starting materials for these three approaches allowed the incorporation of a variety of substituents and substitution patterns.



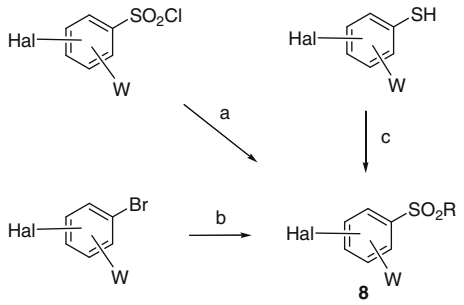
3 WAY-252623 **4** WAY-254011

Figure 2. LXR Agonists from Wyeth.



5 X = C(O)NR^{1}R^2
6 X = CR^{1}R^{2}OH
7 X = SO$_2$R

Figure 3. Modifications of 4-[3-(ArO)-Ph]quinolines.



Scheme 1. Reagents and conditions: (a) Na_2SO_3, $NaHCO_3$, H_2O, 95–100 °C, 1 h, then tetrabutylammonium bromide, R-Br or R-I; (b) $NaSO_2Me$, CuI, L-proline, NaOH, DMSO, 95 °C, 18 h; (c) R-Br or R-I, K_2CO_3, acetone, then Oxone®, aq $NaHCO_3$.

The synthesis of several of the quinoline cores (**10c–f**) has been described previously[10,12,13] and generally utilized a Friedlander reaction[16] with a 2-aminophenone and an aldehyde or acetal. Syntheses of the 3-unsubstituted 4-(3-hydroxyphenyl)quinolines involved the conversion of commercial 4-chloro-quinolines **9** by Suzuki reaction with 3-hydroxyphenylboronic acid into **10a–b** (Scheme 2). Compound **10a** was also prepared from 4-bromo-8-trifluoromethylquinoline.

Two complementary approaches were used to complete the targets from phenols **10** and halogenated arylsulfones **8**. Copper-mediated coupling of **10** to an aryl bromide or aryl iodide, typically employing another Ma procedure, afforded biarylethers **7**.[17] Alternatively, biarylether formation could be effected for ortho, meta or para substitution patterns by heating **10** with a fluoro or chlorophenylsulfone **8** in the presence of base. Not surprisingly, fluorophenylsulfones tended to react at lower temperatures compared to chlorophenylsulfones and *meta*-substitution required longer reaction times and higher temperatures.

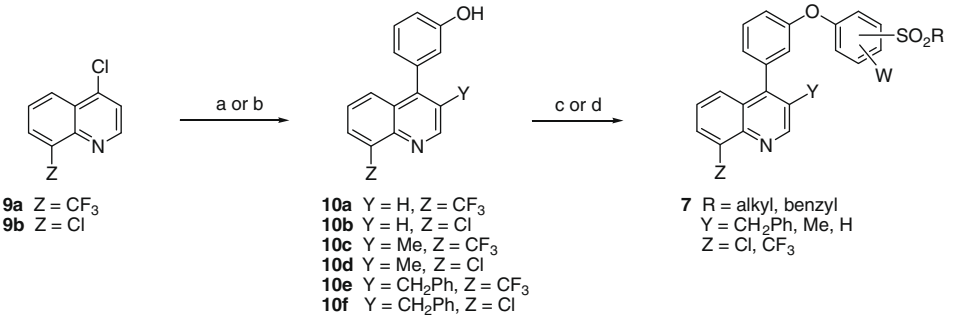
Final targets **7** were tested to determine binding affinity for the two LXR subtypes (Table 1).[10] The binding assays used the recombinant human ligand binding domains (LBDs) of the respective LXRα and LXRβ subtypes measuring displacement of [^{3}H]T0901317 from the LBD.[18] We first examined the effects of regioisomers **7a–c**. While *para*-methylsulfone **7a** had good LXRβ affinity (IC_{50} = 42 nM), meta isomer **7b** was over an order of magnitude *higher* affinity (IC_{50} = 1.0 nM) while ortho isomer **7c** had more than 500-fold *weaker* affinity for the receptor compared to **7b**. This result led us to focus on *meta*-sulfones. The effect of the Y-substituent was investigated using 3-benzyl-, 3-methyl-, and 3-H-quinolines. Comparing **7d**, **7b**, and **7e**, there was little change in affinity despite moving from a large, lipophilic benzyl group to methyl to hydrogen. The similar affinity, regardless of the quinoline 3-substituent, was also seen in the related series of 8-chloroquinolines **7f–h**, though here the 3-hydrogen analog dropped to an IC_{50} value of 7.2 nM. Comparing 8-CF_3 quinolines to 8-chloroquinolines (e.g., **7d** to **7f**; **7b** to **7g**; **7e** to **7h**) also showed comparable affinity. Variations in the sulfone from methyl to hydroxypropyl (**7i** to **7q**) showed little change in LXR affinity, though larger groups tended to reduce affinity. Finally, introduction of an additional substituent on the sulfone-bearing ring also had little effect on the high affinity of the *meta*-sulfone series (compare **7b** to **7r–t**). Binding selectivity for LXRβ over LXRα subtypes was only moderate, though **7e**, **7h**, and **7p** showed binding selectivity (LXRα IC_{50}/LXRβ IC_{50}) of >10-fold. However, the in vivo functional implications of this level of binding selectivity are unclear.

The compounds were also tested in a Gal4β functional assay for LXR activity.[10] These LXR transactivation assays used Huh7 cells transfected with human LXR ligand binding domains fused to Gal4 DNA binding domains. The preference for *meta*-sulfone substitution over para in the functional assay was apparent comparing **7a** to **7b**, with a 500-fold greater potency favoring meta over para isomers. Another SAR point addressed was the importance of the 3-substituent on the quinoline. Benzyl and methyl groups typically gave comparable activity but hydrogen at this position was significantly weaker (compare **7b** to **7d** to **7e**, and **7f** to **7g** to **7h**). We focused on the methyl group to minimize molecular weight and

lipophilicity while retaining potency. Similarly, larger R groups on the sulfone generally did not improve potency in the assay. As in the binding assay, 8-chloro and 8-trifluoromethyl quinolines were comparable in the Gal4β assay. Additional substituents on the phenylsulfone ring reduced potency (cf. **7b** to **7r**–**t**). Essentially none of the sulfones had PPAR agonism when tested in PPAR functional assays reported earlier.[12]

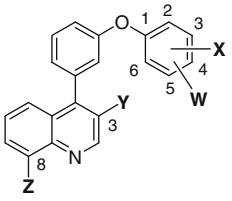
Most compounds were further tested for upregulation of ABCA1 mRNA in a J774 mouse macrophage cell line (Table 1).[18] Efficacy was measured relative to **1** with the maximum increase in mRNA using **1** taken as 100% efficacy. Overall, the same SAR trends in the Gal4β assay were seen here. For example, 8-chloro and 8-trifluoromethyl were similar to each other, while both benzyl and methyl at the 3-position of the heterocycle had comparable activity but were significantly more potent compared to hydrogen at that position. Larger R groups on the sulfone and additional phenylsulfone ring substituents did not help activity and often gave weaker compounds. Based on increased mRNA levels, sulfones **7b**, **7d**, **7i**, and **7k** were the most potent compounds and essentially as efficacious as **1**.

In an assay testing for stability in rat and human microsomes, many of the compounds had greater than 50% remaining after



Scheme 2. Reagents and conditions: (a) 3-(HO)PhB(OH)$_2$ (2 equiv), Pd(PPh$_3$)$_4$ (0.3 equiv), 2M aq Na$_2$CO$_3$ (4 equiv), toluene, EtOH, reflux, 18 h (**9a**); (b) 3-(HO)PhB(OH)$_2$ (1 equiv), Pd(PPh$_3$)$_4$ (0.07 equiv), K$_3$PO$_4$, 1,4-dioxane, reflux, 16 h (**9b**); (c) Hal-ArSO$_2$R (Hal = Br or I, 1.5 equiv), CuI (0.2 equiv), Me$_2$NCH$_2$CO$_2$H hydrochloride (0.3 equiv), Cs$_2$CO$_3$ (3 equiv), 1,4-dioxane, 100-110 °C, 16-24 h (d) Hal-ArSO$_2$R (Hal = F or Cl, 1.1–1.5 equiv), K$_2$CO$_3$ (2 equiv), DMF or DMA, 120-150 °C, 18–24 h.

Table 1
Biarylether sulfone quinolines **7**[a]



Compd	W	X	Y	Z	LXRβ IC$_{50}$ (nM)	LXRα IC$_{50}$ (nM)	Gal4β EC$_{50}$ (nM) (% agonism)	ABCA1 EC$_{50}$ (nM) (% agonism)	Microsomal Stability $t_{1/2}$ (min)
1		—	—	—	9	13	170 (100%)	35 (100%)	>30 (r, h)
5a	H	3-C(O)NHMe	Me	Cl	9	125	800 (66%)	276 (80%)	4 (r), 21 (h)
6a	H	3-CH$_2$OH	Me	CF$_3$	3	12	1,510 (73%)	555 (108%)	>30 (h)
7a	H	4-SO$_2$Me	Me	CF$_3$	42	153	1,550 (30%)	453 (76%)	>30 (r, h)
7b	H	3-SO$_2$Me	Me	CF$_3$	1.0	2.4	31 (78%)	6 (91%)	>30 (r, h)
7c	H	2-SO$_2$Me	Me	CF$_3$	560	>1000	Not tested	Not tested	8 (r)
7d	H	3-SO$_2$Me	CH$_2$Ph	CF$_3$	0.92	2.0	11 (78%)	5 (108%)	16 (r), 12 (h)
7e	H	3-SO$_2$Me	H	CF$_3$	2.8	40	214 (44%)	70 (87%)	>30 (r, h)
7f	H	3-SO$_2$Me	CH$_2$Ph	Cl	1.2	3.3	30 (85%)	Not tested	3 (r), 11 (h)
7g	H	3-SO$_2$Me	Me	Cl	1.1	3.4	44 (103%)	Not tested	8 (r)
7h	H	3-SO$_2$Me	H	Cl	7.2	129	382 (45%)	81 (70%)	>30 (r, h)
7i	H	3-SO$_2$Et	Me	CF$_3$	1.6	6.1	55 (73%)	3 (100%)	>30 (r)
7j	H	3-SO$_2$-1-Pr	Me	CF$_3$	2.6	23	251 (50%)	10 (99%)	6 (r)
7k	H	3-SO$_2$-2-Pr	Me	CF$_3$	1.8	7.9	56 (49%)	3 (88%)	23 (r)
7l	H	3-SO$_2$-2-Pr	H	CF$_3$	2.4	4.0	299 (44%)	22 (77%)	12 (r), 18 (h)
7m	H	3-SO$_2$CH$_2$CHMe$_2$	Me	CF$_3$	8.1	61	592 (55%)	55 (107%)	10 (r)
7n	H	3-SO$_2$CH$_2$CH$_2$CHMe$_2$	Me	CF$_3$	13	118	1030 (56%)	154 (135%)	8 (r)
7o	H	3-SO$_2$CH$_2$CH$_2$CH$_2$OH	Me	CF$_3$	1.4	4.7	70 (68%)	24 (112%)	>30 (r)
7p	H	3-SO$_2$CH$_2$CH$_2$CMe$_2$OH	Me	CF$_3$	8.2	121	962 (62%)	636 (96%)	>30 (r)
7q	H	3-SO$_2$CH$_2$CH$_2$CH$_2$OMe	Me	CF$_3$	3.2	19	274 (48%)	Not tested	1 (r)
7r	4-OMe	3-SO$_2$Me	Me	CF$_3$	8.2	56	688 (43%)	256 (112%)	11 (r), >30 (h)
7s	5-F	3-SO$_2$Me	Me	CF$_3$	1.9	6.6	90 (79%)	14 (114%)	>30 (r, h)
7t	5-Cl	3-SO$_2$Me	Me	CF$_3$	3.0	28	445 (89%)	162 (135%)	>30 (r, h)

[a] Results are given as the mean of two independent experiments. The standard deviations for the binding assays were typically ±30% of the mean or less. The standard deviations for the Gal4β assay were typically ±30% of the mean or less. % of efficacy is relative to **1**. r = rat liver microsomes, h = human liver microsomes.

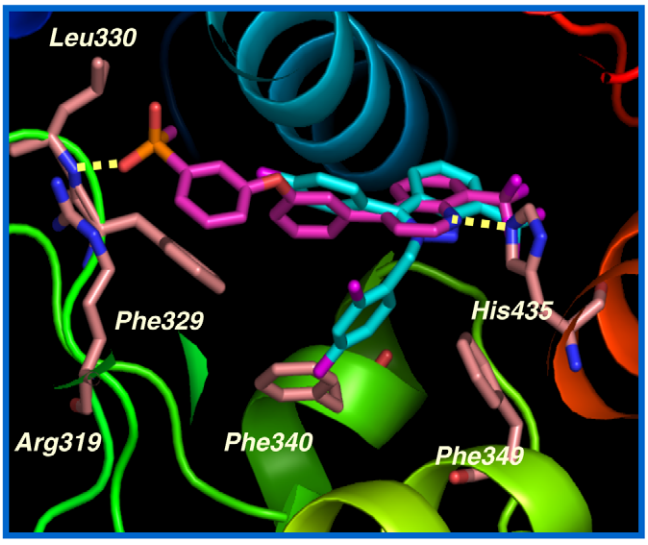


Figure 4. Overlay of X-ray structures of hLXRβ/**3** complex (blue) and hLXRβ/**7e** complex (magenta). Only key residues and helices are shown. Hydrogen bonds to key Leu330 and His435 residues are shown by dotted yellow lines.

30 min. However, longer alkyl groups on the sulfone tended to shorten the half-life in a rat microsomal preparation, especially when the linear chain length exceeded two carbons. Typically, benzyl substitution at Y gave less stable compounds. Methoxy substituents, either as part of R (**7q**) or on the phenylsulfone ring (**7r**), also reduced stability. 8-Chloro compounds were somewhat less stable compared to 8-trifluoromethyl.

Compound **7e** was cocrystallized with LXRβ to examine the ligand binding mode and understand which residues are important for affinity (Fig. 4).[19] An overlay of the previously disclosed[8] X-ray structure of the ligand binding domain of hLXRβ/**3** with hLXRβ/**7e** is also shown. Ligand recognition was achieved by hydrogen bond interaction between the His435 residue in the active site and the quinoline nitrogen and the N-1 indazole nitrogen in **7e** and **3**, respectively. The trifluoromethyl groups in both structures were in close proximity to His435 residue (d(N–F) = 3.0 Å) and may have a favorable electrostatic interaction with the histidine. The N2-benzyl group in **3**, positioned in a hydrophobic pocket surrounded by three Phe residues (329, 340 and 349), is replaced by a hydrogen. In **7e** the loss of these hydrophobic interactions was apparently compensated for by a strong hydrogen bond interaction between a sulfone oxygen and the backbone NH group of Leu330. However, the loss in potency in both the Gal4β and ABCA1 assays suggests at least a methyl group is needed for good functional activity.

Replacement of alcohols and amides with sulfones led to a series of high affinity LXR ligands **7**. Efficient synthetic approaches allowed variation at key positions on the molecules furthering the SAR. Some of the compounds showed modest binding selectivity for LXRβ over LXRα. Many of the compounds had potent LXRβ agonist activity in a Gal4β functional assay. A second functional assay using mouse J774 cells demonstrated increases in mRNA for ABCA1, a key protein for lipid transport. Several of the compounds had potency and efficacy comparable to **1**, with a few compounds having greater potency. Structure–activity relationships for the two functional assays were generally consistent. Stability in rat and human microsomes was high for many analogs, especially those with smaller alkyl groups. 4-(3-Aryloxyaryl)quinolines with a *meta*-sulfone group are high affinity LXR ligands, often with excellent agonist potency for upregulating ABCA1 mRNA and no PPAR activity.

Acknowledgments

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