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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3055–3060

SAR studies: Designing potent and selective LXR agonists

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Received 1 February 2006; revised 16 February 2006; accepted 17 February 2006
Available online 10 March 2006

Abstract—Counterscreening compounds from a Merck PPAR program discovered lead **1**, as a nanomolar LXR/PPAR dual agonist. SAR optimization developed a series of heterocyclic LXR agonists having excellent selectivity over all PPAR isoforms and possessing high LXR affinity and strong in vivo potency.

A major cause of morbidity and mortality in modern societies is atherosclerotic cardiovascular disease (CVD),[1] which is characterized by accumulation of low density lipoprotein (LDL) particles in the arterial wall leading to formation of cholesterol-laden foam cells and immune system activation.[2] Important therapeutics (e.g., statins) have been developed which lower circulating levels of LDL cholesterol and successfully reduce the incidence of CVD.[3] Despite these therapies, incidences of myocardial infarction and ischemic stroke are only reduced by one-third, and many individuals with 'normal' LDL levels suffer from atherosclerosis. Promisingly, high density lipoprotein (HDL) plasma levels are inversely correlated with the incidence of CVD.[4] This protective effect may be linked to the formation of the HDL particle via reverse cholesterol transport (RCT), whereby cholesterol is transported by plasma lipoproteins from peripheral cells (including arterial foam cells) to the liver for excretion in bile.[5] Agents that promote RCT and/or raise the level of circulating HDL hold the promise of an alternative and complimentary treatment to existing LDL-based therapies.

The liver X receptor (LXR) belongs to the nuclear hormone superfamily and is comprised of two subtypes: LXRα (NR1H3) and LXRβ (NR1H2).[6] They act as intracellular oxysterol sensors and regulate expression of genes involved in lipid metabolism.[7] The LXR target genes include many of those known to be important in the reverse cholesterol transport pathway, specifically those regulating cellular cholesterol efflux, HDL metabolism, and biliary cholesterol excretion.[7] The most prominent, ABCA1, which encodes for a lipid pump, mediates cholesterol efflux from peripheral cells to the lipid-poor apolipoprotein acceptors (e.g., apoA-1).[8] Surprisingly, only a few examples of synthetic LXR agonists have been reported.[9–11] LXR agonists offer the exciting possibility of combining the beneficial effects of HDL raising and cholesterol efflux by acting upon a single drugable target, unfortunately the accompanying agonist driven steatosis limits the utility of these agents.[12] In this report, we describe the SAR and optimization for a novel series of potent LXR agonists and their effect upon circulating HDL and triglyceride (TG) levels in vivo.

A novel LXR agonist (**1**) was identified by counterscreening ligands from our PPAR program. Although **1** possessed high LXR affinity in the binding assay,[13] it displayed poor efficacy in the functional assay (monitoring βLac transactivation, TA)[14] as well as high affinity for off-target PPAR receptors (e.g., PPARδ IC_{50} = 4 nM, Table 1). Elimination of all PPAR activity was of particular concern,[15] since evaluation of the effect of a mixed LXR/PPAR agonist in vivo would be compromised by PPAR-mediated lipid changes. Thus, a key question for these agonists was whether the LXR and PPAR activities could be separated.

Keywords: LXR; Small molecule agonist; SAR study.
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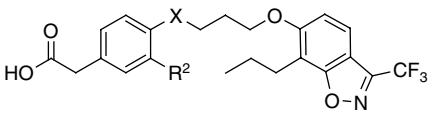
doi:10.1016/j.bmcl.2006.02.050

Table 1. Aniline substitutions and modifications[a,b]

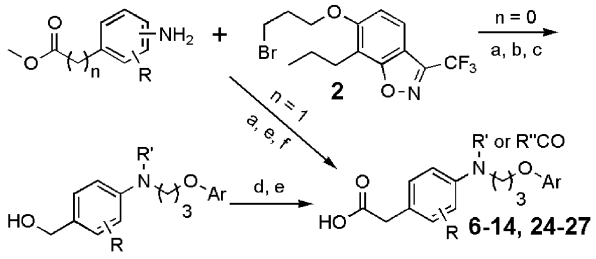


Compound	X	R^2	LXR IC_{50} (nM)		LXR TA EC_{50} (nM)		PPAR IC_{50} (nM)		
			α	β	α	β	α	δ	γ
1	S	Cl	32	28	5900	7300	280	4	195
3	O	Cl	39	65	wa	9700	230	4	280
4	O	H	730	100	wa	wa	19	8	270
5	CH_2	H	530	970	na	na	na	na	na
6	NMe	Cl	630	170	wa	wa	wa	49	wa
7	NMe	H	18	25	450	360	310	310	2900
8	NH	H	68	89	8000	7700	16	88	102
9	NEt	H	110	140	wa	wa	2600	8600	wa
10	*N*-Formyl	H	6400	wa	na	na	na	na	na
11	NAc	H	6400	wa	na	na	na	na	na

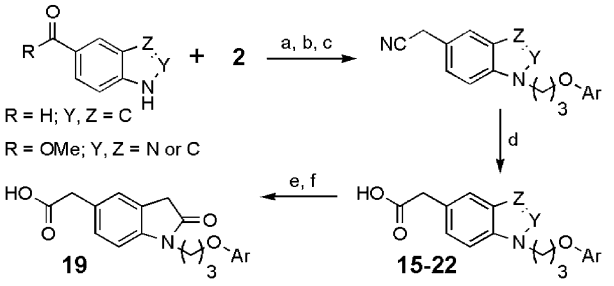
[a] Values are means of at least two independent titrations.
[b] na, not active, <10% activation at 15 μM; wa, weak active, <50% activation at 10 μM.

The synthesis of the lead agonist **1**[16] and the oxy-analogs **3** and **4**[17] has been previously described. The *N*-methyl aniline agonists were synthesized by heating the corresponding benzoate amines and the bromide **2**[18] in toluene (Scheme 1, $n = 0$). The secondary amines were alkylated, and the esters reduced with diisobutylaluminum hydride (DIBAL). The resulting alcohols were converted to nitriles via a two-step, one-pot mesylation/cyanide displacement sequence. Saponification then afforded the desired analogs **6**–**14** and **24**–**27**. Alternatively, aminophenyl acetic acid was alkylated with **2**, followed by hydrolysis to afford analog **8** (Scheme 1, $n = 1$). This material was then directly acylated to afford analogs **10** and **11**.

The indole and polar heterocyclic analogs were prepared analogously by alkylation of the appropriate heteroaromatic with the bromide **2** (Scheme 2). In the case of indole analogs, the starting aldehyde was reacted with sodium borohydride to generate a secondary alcohol. For other heteroaromatics, DIBAL reduction of the starting esters furnished the alcohols. Subsequent mesylation/cyanide displacement yielded the nitrile precursors, which were saponified to afford analogs **15**–**22**. Treatment of the indole **15** with *N*-chlorosuccinimide



Scheme 1. Synthesis of various aniline agonists. Reagents and conditions: (a) Bu_4NI, toluene, reflux; (b) NaH, R′–X, THF; (c) DIBAL, THF; (d) i—MsCl, TEA, THF; ii—Bu_4NCN; (e) NaOH (50% aq), 2:1 MeOH/THF; (f) $(R''CO)_2O$, DMF, TEA.

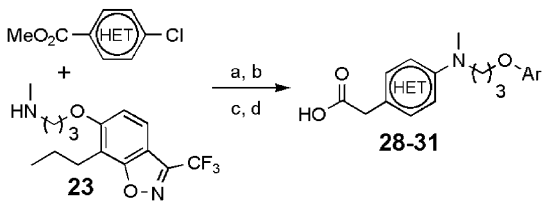


Scheme 2. Synthesis of various heterocyclic agonists. Reagents: (a) Cs_2CO_3; DMF; (b) $NaBH_4$, MeOH; or DIBAL, THF; (c) i—MsCl, TEA, THF; ii—Bu_4NCN; (d) NaOH (50% aq), 2:1 MeOH/THF; (e) NCS, DCM; (f) H_3PO_4, acetic acid, 100 °C.

followed by hydrolysis with phosphoric acid afforded the oxyindole **19**.

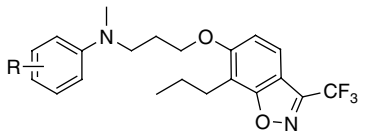
Lastly, synthesis of the *N*-methyl amino heteroaromatics began by SnAr displacement of the corresponding chloro aryl ester with amine **23**[18] (Scheme 3). These esters were then reduced, mesylated, displaced with cyanide, and subsequently saponified to afford the desired acetic acids **28**–**31**.

An initial SAR investigation revealed that the benzisoxazole fragment of **1** is well optimized for LXR binding. All modifications were poorly tolerated, including



Scheme 3. Synthesis of various *N*-methyl amino heteroaromatics. Reagents: (a) TEA, CH_3CN; (b) DIBAL, THF; (c) i—MsCl, TEA, THF; ii—Bu_4NCN; (d) NaOH (50% aq), 2:1 MeOH/THF.

Table 2. Acid modifications[a]



Compound	R	LXR IC_{50} (nM)		LXR TA EC_{50} (nM)		PPAR IC_{50} (nM)		
		α	β	α	β	α	δ	γ
12	*m*-CH_2CO_2H	102	98	2600	3500	1500	93	3000
13	H	372	428	na	na	na	na	na
14	*p*-CO_2H	79	190	6000	5100	wa	na	na

[a] na, not active, <10% activation at 15 μM; wa, weak active, <50% activation at 10 μM.

replacement of the ether oxygen with carbon, any change of the propyl residue, and alteration of the benzisoxazole ring. Only the trifluoromethyl position allowed some small variation (i.e., phenyl, ethyl, and *tert*-butyl methyl), yielding only compounds of inferior potency.

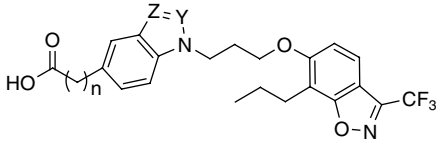
The benzisoxazole moiety was then held constant, allowing the SAR investigation to focus on optimization of the acetic acid fragment (Table 1). Arbitrarily, initial studies of **1** began with replacement of the sulfur heteroatom. Oxy-analog **3** had comparable affinity, but functional potency was reduced. Removal of the chlorine substituent (**4**) significantly decreased affinity and potency. Carbon substitution (**5**) for sulfur was poorly tolerated and resulted in inactive analogs. Introduction of a *N*-methyl group gave a weak agonist of modest affinity (**6**), but intriguingly, replacement of the chloride with hydrogen (**7**) in this case dramatically improved affinity (~10-fold), increased functional potency (~10-fold), and modestly improved selectivity.

To follow up the aniline modification, a simple set of nitrogen substituents were surveyed. Replacement of the *N*-methyl with a smaller (i.e., hydrogen, **8**) or a larger group (ethyl, **9**) greatly decreased functional potency (Table 1). Acylation of the aniline to form the *N*-formyl **10** or *N*-acetyl analog **11** resulted in inactive compounds.

The optimized *N*-methyl group of **7** was held constant, while the contribution of the acetic acid to affinity and selectivity was investigated (Table 2). The *meta*-acetic acid analog **12** retains moderate affinity, however functional potency is notably reduced. The complete loss of functional activity upon deletion of the acetic acid (**13**) indicates that the acid is essential for efficacy. Truncation of the acid to the benzoate **14** improves the selectivity, but reduces both affinity and functional potency. Additionally, the pharmacokinetic profiles of the benzoates are greatly inferior to related acetic acid analogs, providing inadequate drug levels for a convincing in vivo test.[19] This combination of superior potency and pharmacokinetics leads to retention of the aryl acetic acid pharmacophore and pursuit of alternative approaches to improve selectivity.

Next, a series of *N*-methyl anilide ring-fused analogs were prepared (Table 3). While the *N*-methyl aniline was intolerant of simple modification (Table 1), fusion of the anilide nitrogen into a five-membered indole ring was remarkably well tolerated (**15**); although selectivity remained unchanged. In contrast, the other possible

Table 3. Effect of indole modifications and heteroatom replacements[a]



Compound	Y	Z	*n*	LXR IC_{50} (nM)		LXR TA EC_{50} (nM)		PPAR IC_{50} (nM)		
				α	β	α	β	α	δ	γ
15	C	C	1	16	16	590	450	400	57	1900
16	C	N	1	180	410	wa	8600	na	1800	na
17	N	C	1	73	87	480	580	780	24	na
18	N	N	1	410	350	2300	1700	4400	79	na
19	CO	CH_2	1	19	13	180	140	na	na	na
20	CO	CH_2	0	97	700	3000	4400	na	na	na
21	C	C	2	18	6	820	700	450	70	1200
22	C	C	0	72	199	1900	2800	8300	na	na

[a] na, not active, <10% activation at 15 μM; wa, weak active, <50% activation at 10 μM.

nitrogen ring-fusion analog, a C2-linked *N*-methyl indole, dramatically decreased LXR affinity (>100-fold) and was not pursued.

The carbons of the five-membered indole ring were systematically replaced with heteroatoms to investigate if strategic placement of a polar atom could effectively achieve discrimination between LXR and the undesired PPAR receptors (Table 3). Sequential substitution of C2 and C3 of **15** with nitrogen yielded the benzimidazole **16**, indazole **17**, and benzotriazole **18** analogs. In general, polarity was tolerated at C2 with affinity and potency well maintained, while poorly tolerated at C3. Disappointingly, this series of heterocyclic replacements only moderately improved selectivity, but indicated that it was possible to increase agonist polarity while maintaining high affinity.

As a second generation analog, an exocyclic oxygen was introduced at C2 forming an oxyindole (**19**, Table 3). The oxyindole **19** retained high LXR affinity and full functional potency while not binding to any of the PPAR subtypes. Remarkably, this represents a >3100-fold increase in designed selectivity and achieves the discrimination desired for in vivo evaluation (PPAR's $IC_{50} > 50\ \mu M$). Oxyindole **19** was further profiled against a representative set of problematic channels and receptors (i.e., IKr, PXR, and RXR) and found to be inactive (IC_{50}'s >10,000 nM). As previously observed, truncation of the acetic acid to the benzoate **20** provides favorable selectivity, while again yielding an unacceptable pharmacokinetic profile.

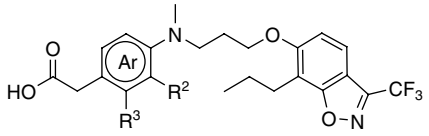
An attempt to gain receptor selectivity by α-methyl substitution of the carboxylate moiety was also explored. The two chiral α-methyl and the α,α-dimethyl indoles were prepared, and while affinity was well maintained no meaningful enhancement in selectivity was observed.[19] Additionally, homologation of the acid by one carbon (**21**) left both affinity and selectivity unchanged. Deletion of the α-methylene to give benzoate **22** improved selectivity but reduced LXR potency.

Since heteroatom substitution dramatically improved selectivity for the indole series, a small set of substituted anilines were investigated. *ortho*-Methyl agonist **24** was tested and, like the prior *ortho*-chloro analog **6**, *ortho*-substitution decreased affinity and produced essentially functionally inactive compounds (Table 4). In contrast, *meta*-substitution was tolerated (**25**–**27**), though these simple substituents had no effect upon selectivity.

Next, the aniline carbons were substituted with heteroatoms, thus formally replacing the phenyl with pyridine (**28**), thiazole (**29**), pyrazine (**30**), and pyrimidine (**31**). The amino heterocyclic analogs were generally well tolerated, possessing good affinities and excellent selectivity profiles (Table 4). The pyrazine **30** is representative of the series having >550-fold LXR selectivity over PPAR. The only exception was the basic pyridine analog **28**, which had good affinity but poor functional potency.

Agonists that possessed superior potency and selectivity profiles were evaluated for their mouse pharmacokinetic properties (Table 5). In general, these analogs exhibited

Table 4. Phenyl substitution and heteroatom replacements[a]



Compound	Ar	R^2	R^3	LXR IC_{50} (nM)		LXR TA EC_{50} (nM)		PPAR IC_{50} (nM)		
				α	β	α	β	α	δ	γ
24	Ph	Me	H	580	320	na	na	600	39	460
25	Ph	H	Me	117	26	1300	303	870	150	4600
26	Ph	H	Cl	12	10	170	124	na	1400	na
27	Ph	H	CF_3	60	18	3600	1300	140	540	wa
28	2,5-Pyridyl			105	630	5000	wa	na	na	wa
29	2,5-Thiazolyl			67	100	1100	650	na	wa	na
30	2,5-Pyrazinyl			90	40	540	150	na	na	na
31	2,5-Pyrimidyl			26	55	620	470	na	na	na

[a] na, not active, <10% activation at 15 μM; wa, weak active, <50% activation at 10 μM.

Table 5. Mouse pharmacokinetic parameters for selected LXR agonists[a]

Compound	Cl (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	AUC_n (μM h kg/mg)
15	18	2.7	3.9	2.7
19	46	2.9	1.0	1.0
29	13	1.2	1.2	3.1
30	17	2.8	2.1	7.5
GW3965	8	1.2	3.4	2.0

[a] 0.5/20 mpk iv/po.

Table 6. In vivo HDL raising with LXR agonists[b,c]

Compound	Day	Dose[a]	HDL (%)	Tot. Chol. (%)	TG (%)	Liver TG (%)
GW3965	8	1-qd	23†	21†	62‡	10‡
	8	10-qd	46	48	50	44
	8	30-qd	40	69	110	69
15	8	30	64	97	115	405
19	9	10	40	55	1‡	—
	9	30	32	62	33‡	—
	14	10	64	91	47‡	145
	14	30	61	120	4‡	174
29	8	30	23	48	143	178
30	10	30	48	101	130	256

[a] All doses (mpk) are b.i.d. unless otherwise noted.
[b] In a typical experiment, normal mice were po dosed b.i.d. for 8–14 days and HDL, total cholesterol, and triglycerides were monitored.
[c] All P values are $\leqslant 0.01$ except as noted. P values of $\leqslant 0.05$ and >0.05 indicated by † and ‡, respectively.

moderate clearance and good half-lives. Oral dosing was performed at the anticipated use dose (20 mg/kg), and adequate po plasma exposures were achieved with b.i.d. dosing.

The most promising analogs were tested in C57BL/6 mice (Table 6). These mice were responsive to known agonists[9] (e.g., GW3965) for both the desired effect of raised HDL and the undesired TG elevation in plasma and liver.

Dosing of the unselective indole **15** resulted in excellent LXR efficacy (HDL increased 65% relative to control), yet as previously observed with other small molecule agonists, triglycerides were increased to an unacceptable level (116%/388% plasma/liver, respectively). A structurally unrelated LXR agonist, GW3965, increased HDL 39% and elevated plasma TG (32%) in this assay. The selective oxyindole **19** was tested at two different dose levels (10 and 30 mpk b.i.d.) and the lipid levels monitored at days 9 and 14. While excellent in vivo efficacy was observed for both doses (10 and 30 mpk; HDL = 60% and 64%, respectively), triglyceride elevation was also dose dependent. Moreover, the lower dose of **19** (10 mpk) slows the onset of, but does not prevent, steatosis.

Two of the selective amino heteroaryls (**29** and **30**) were also tested as two structurally diverse agonists. Both were effective LXR agonists in vivo, raising HDL 24% (**29**) or 48% (**30**), respectively (Table 6). Again, these agonists also elevated plasma triglycerides and histology revealed the development of fatty liver.

From these data, pyrazine **30** was selected for advanced in vivo testing to elucidate the effects of selective activation of LXRα and LXRβ on lipid metabolism.[20] In this study, mice genetically deficient in either LXRα or LXRβ were dosed with **30**, a LXR dual agonist, and the lipid effects on HDL cholesterol and plasma TG were monitored, and these results are reported in a separate monograph.[20]

The SAR for a series of acidic LXR agonists has been described. Atomic substitution of either an indole or aniline template gave highly potent heterocyclic agonists with excellent LXR selectivity versus PPAR. These selective agonists were evaluated in vivo and determined to be highly efficacious in raising HDL and promoting RCT, while unavoidably increasing plasma TG.

Acknowledgments

We thank Charlotte Burton for measurement of liver TG content and James Pivnichny for pharmacokinetic analysis.

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