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Carboxylic acid based quinolines as liver X receptor modulators that have LXRβ receptor binding selectivity

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Abstract—A series of potent and binding selective LXRβ agonists was developed using the previously reported non-selective LXR ligand **WAY-254011** as a structural template. With the aid of molecular modeling, it was found that 2,3-diMe-Ph, 2,5-diMe-Ph, and naphthalene substituted quinoline acetic acids (such as quinoline **33**, **37**, and **38**) showed selectivity for LXRβ over LXRα in binding assays.

Liver X receptors (LXRα and LXRβ) are members of the nuclear hormone receptor superfamily and are involved in the regulation of cholesterol and lipid metabolism.[1] They are also recently reported to be glucose sensors involved in liver carbohydrate metabolism.[2] LXRs are ligand-activated transcription factors and bind to DNA as obligate heterodimers with retinoid X receptors. In macrophages, liver, and intestine, activation of LXRs induces the expression of several genes involved in lipid metabolism and reverse cholesterol transport including ATP binding cassette transporter A1 (ABCA1), ABCG1, and apolipoprotein E (ApoE). The potential to prevent or even reverse atherosclerotic process by increasing the expression of these genes makes LXR an attractive drug target in the treatment of atherosclerosis, which is one of the leading health concerns in the United States.[3] Several LXR agonists (Fig. 1), such as a natural ligand 24(*S*), 25-epoxycholesterol (**1**, EPC)[4] as well as two structurally distinct synthetic non-steroidal ligands **2** (GW3965)[5] and **3** (TO901317),[6] have been shown to increase expression of several genes involved in lipid metabolism and reverse cholesterol transport including ABCA1, ABCG1, and ApoE. These compounds reduced or even reversed atherosclerotic processes in mouse models of atherosclerosis. Currently available synthetic LXR agonists, however, also activated triglyceride (TG) synthesis in the liver by the upregulation of sterol regulatory element binding protein 1c (SREBP-1c) and fatty acid synthase (FAS) which limits the utility of these LXR synthetic agonists. Since the liver contains predominantly LXRα, LXRβ-specific agonists or tissue-specific LXR modulators may have less impact on TG synthesis, but may be effective in macrophage reverse cholesterol transport. Thus, our new efforts were focused on the identification of LXRβ selective modulators. The concept of LXR subtype selective agonists suitable for pharmacologic evaluation has been supported by a number of LXR knockout (KO) animal studies.[1] Treatment of LXRα KO mice with a potent and highly selective LXRα agonist did not have any effect on plasma TG, liver cholesterol, liver TG, whereas in wild-type mice an increase in liver TG was noted, indicating that LXRα is the subtype controlling hepatic SREBP-1c transcription.[7] In contrast, in peripheral tissues from these LXRα KO mice, LXRβ activation increased ABCA1 gene expression.[8] Therefore, selective LXRβ activation is expected to stimulate ABCA1 expression in macrophages, while having no or little effort on hepatic LXRα-dominated lipogenesis. A recent study has shown that ligand

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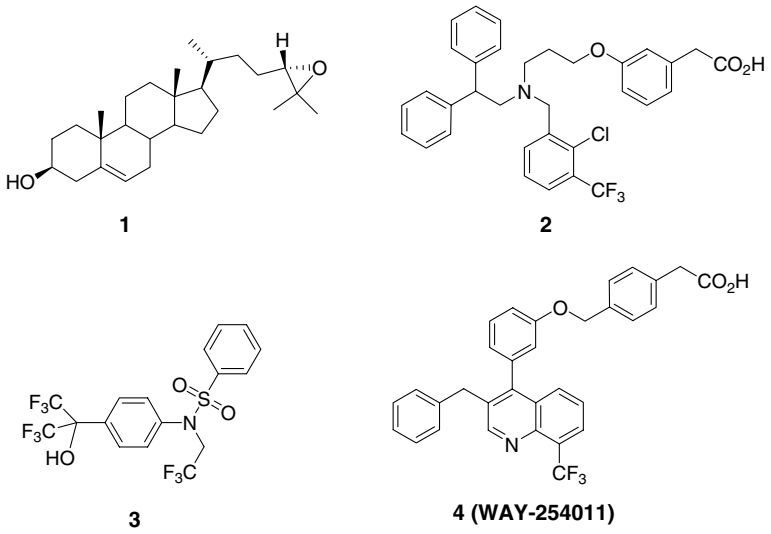


Figure 1. Reported LXR agonists.

activation of LXRβ reversed atherosclerosis and cellular cholesterol overload in mice lacking LXRα and ApoE.[9] This observation provided a strong in vivo support for LXRβ as a drug target for the treatment of atherosclerosis.

Recently, we reported the identification and X-ray structures of phenyl acetic acid substituted quinolines as potent LXR pan agonists.[10] These quinolines displayed good binding affinity for LXRβ and were potent agonists in Gal4 transactivation assays. However, the compounds also increased plasma TG levels. In order to identify selective LXR modulators that would have the potential to stimulate ABCA1 gene expression without the lipogenic activity, we used a structure-based approach to improve selectivity. The design of LXR selective ligands is quite challenging since the ligand binding domains of LXRα/β share a high sequence identity (78%) and residue differences are located far away from the ligand binding pocket.[11] All residues in close contact to known ligands (i.e., 5 Å) are identical, and there is only one conservative amino acid difference within the helix-3 region (i.e., LXRβIle$_{277}$/LXRαVal$_{263}$). Examination of the X-ray structure of compound **4** (**WAY-254011**) suggested that this residue could be accessed by modification of the phenyl acetic acid head group (Fig. 2), although we were aware that this approach would have to position functional groups in the right orientation to take advantage of the small differences within these 2 amino acid residues. Using this observation we proposed the synthesis of 2,3 and 2,5-disubstituted phenyl acetic acids or α-substituted acetic acids to probe this selectivity pocket.

General synthesis of carboxylic acid based quinolines **7** is shown in Scheme 1 using methods described in the previous papers.[10] Benzylamine bond (X–Y = NHCH$_2$ or CH$_2$NH) was formed by a reductive amination reaction between aldehydes and anilines and benzyl ether bond (X–Y = OCH$_2$ or CH$_2$O) was formed by a Mitsunobu reaction. Synthesis of anilines **11** is described in Scheme 2. Treatment of 2,3 or 2,5-dimethyl substituted acetanilide **8** with acetyl chloride in the presence of aluminum chloride gave the acetamidoacetophenones **9**. The latter were oxidized to the thioamides **10** using sulfur in boiling morpholine and the amides were subsequently hydrolyzed in hydrochloric acid.[12] In Scheme 3 nitration of 1-naphthaleneacetic acid with fuming nitric acid in acetic acid gave a mixture of position isomers which were separated into 4-nitro- and 5-nitro-1-naphthaleneacetic acids (**13**[13] and **14**). The regio-chemistry of **14** was assigned by 2D NMR studies (COSY and NOESY). Compounds **13** and **14** were then reduced to anilines **15** and **16** under palladium-catalyzed hydrogenation. α-Substituted phenyl acetic acids **18** were prepared by mono or dialkylation of **17**[10a] with substituted alkyl bromides followed by basic hydrolysis of the methyl ester (Scheme 4).

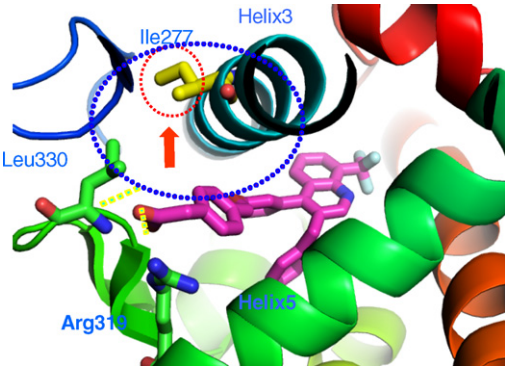
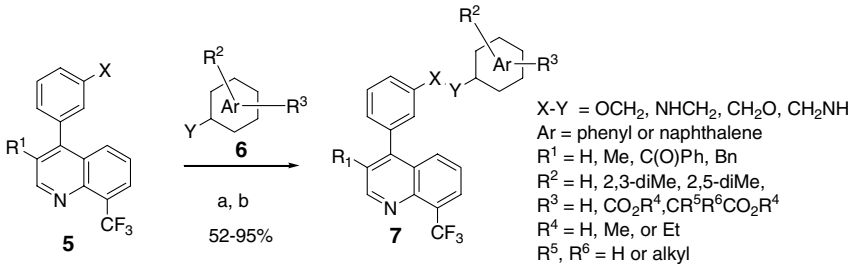
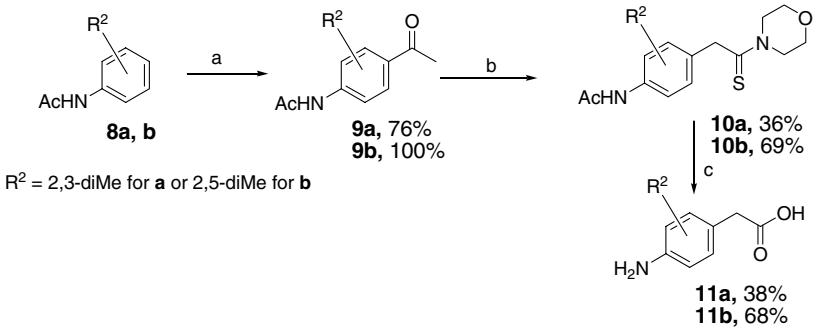


Figure 2. X-ray crystal structure of **WAY-254011** bound to hLXRβ. The red arrow draws attention to the corresponding region for which there is amino acid difference between LXRα and LXRβ. The blue dotted line indicates opportunities to improve selectivity from the phenyl group.

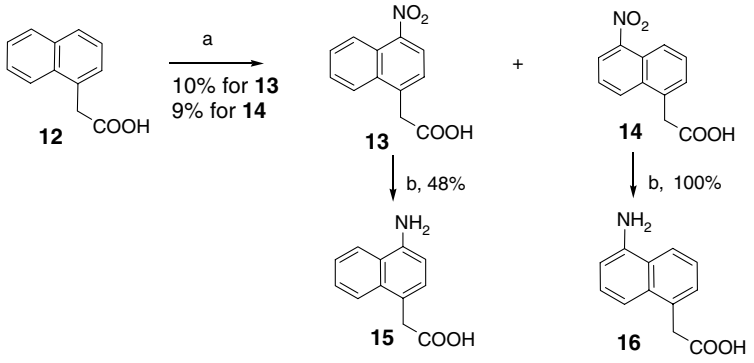
The LXR binding activity of the newly synthesized compounds was evaluated in binding assays as reported.[10] As a reference, TO-901317 (compound **3**) was tested in



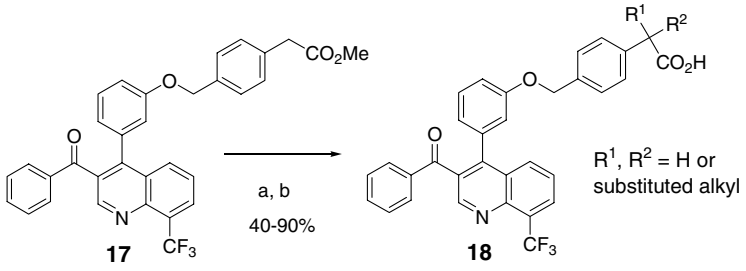
Scheme 1. Reagents: (a) benzyl bromides, K_2CO_3 or benzyl amines, $NaBH(OAc)_3$, DMF, or PPh_3/DEAD; (b) LiOH, THF/MeOH/H_2O.



Scheme 2. Reagents: (a) AcCl, $AlCl_3$; (b) S/morpholine; (c) 6 N HCl.



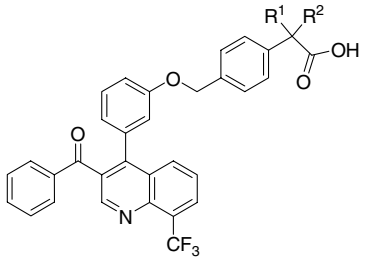
Scheme 3. Reagents: (a) HNO_3, CH_3COOH; (b) H_2, Pd/C, EtOH.



Scheme 4. Reagents: (a) substituted alkyl bromides, Cs_2CO_3; (b) LiOH, THF/MeOH/H_2O.

our binding assays and was found to be a potent LXR pan agonist (Table 1). Initial docking studies suggested that R^1/R^2 group of α-substituted acid analogs **18** would perturb the LXRβIle$_{277}$/LXRαVal$_{263}$ pocket to achieve the desired binding selectivity. Compared to the unsubstituted compound **19**[10a] the binding activity for a small mono-substitution α to the carboxylic acid functionality (**23**–**25**) was tolerated while a large substitution (**20**–**22**) decreased LXR binding affinity. No major binding selectivity for LXRβ over LXRα was observed. For example, 2-allyl substituted compound **23** had the same binding affinity as **19**. Increasing the size of the substituent from benzyl (**20**) to substituted benzyls (**21** and **22**) reduced LXRβ binding activity with no improvement on the

Table 1. α-Substitution[a]



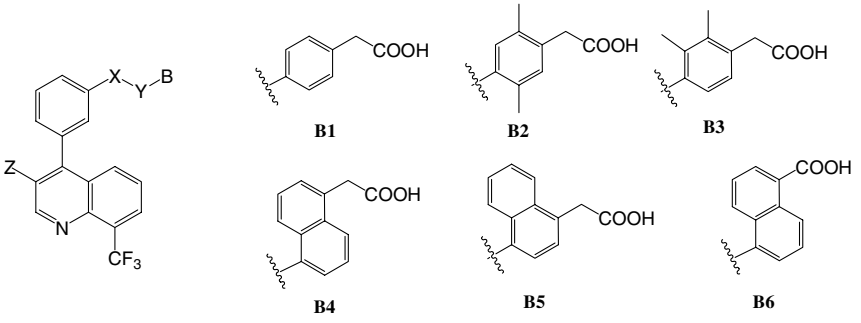
Compound	R^1	R^2	hLXRβ IC_{50} (nM)	hLXRα IC_{50} (nM)	Ratio α/β (β/α)
3			9	13	1.4
19	H	H	5	17	3.4
20	H	Bn	67	112	1.7
21	H	4-Bu^{t}Bn	81	65	0.8
22	H	4-Ph–Bn	473	528	1.1
23	H	Allyl	5	16	3.2
24	H	2-Butynyl	2	6	3.0
25	H	2-Pentynyl	10	21	2.1
26	2-Pentynyl	2-Pentynyl	219	147	0.67 (1.5)
27	Propargyl	Propargyl	26	2	0.075 (13)

[a] Results are given as means of at least two independent experiments. The standard deviations for these assays were typically ±30% of mean or less.

binding selectivity. Apparently mono-alkyl was not oriented through the narrow cavity toward the LXRβIle$_{277}$/LXRαVal$_{263}$ pocket. Interestingly, a bispentynyl group α to the carboxylic acid functionality (**26**) gave minor LXRα binding selectivity (1.5-fold) although the binding affinity on LXRα was weak (IC_{50} 147 nM). A moderate selectivity for LXRα over LXRβ (13-fold) was observed for the bispropargyl substituted compound **27** and the compound also had excellent binding affinity (IC_{50} 2 nM for LXRα). However, LXRα selective agonists might enhance the lipogenic activity.[1] Therefore, no further analogs were pursued in this line.

The contribution of the 2,3 or 2,5-disubstituted phenyl acetic acid to the LXRβ binding selectivity was then investigated (Table 2) as an alternative approach to orient groups toward the LXRβIle$_{277}$/LXRαVal$_{263}$ selectivity pocket. Similar to unsubstituted compound **4**[10a] the 2,5-dimethyl analog **28** showed no difference on binding affinity for both LXRα and LXRβ. Modifications on the

Table 2. C3, linker, and phenyl modifications[a]



Compound	X–Y	Z	B	hLXRβ IC_{50} (nM)	hLXRα IC_{50} (nM)	Ratio α/β
4	OCH_2	Bn	B1	2	10	5.0
28	OCH_2	Bn	B2	3	11	3.7
29	CH_2O	Bn	B2	4	31	7.8
30	CH_2NH	Bn	B2	2	21	11
31	CH_2NH	Bn	B1	7	47	6.7
32	CH_2NH	Bn	B3	4	58	15
33	CH_2NH	Bn	B4	5	123	25
34	CH_2O	Bn	B4	11	161	15
35	CH_2NH	Bn	B5	2	21	11
36	CH_2NH	Bn	B6	35	157	4.5
37	CH_2NH	Me	B4	10	211	21
38	CH_2NH	H	B4	15	745	50

[a] Results are given as means of at least two independent experiments. The standard deviations for these assays were typically ±30% of mean or less.

linker region were undertaken and it was found that the analog with a CH_2NH linker (**30**, α/β 11-fold) was slightly more LXRβ selective than the corresponding analogs with a CH_2O (**29**, α/β 7.8-fold) or OCH_2 linker (**28**, α/β 3.7-fold). The corresponding 2,3-dimethyl analog with the CH_2NH linker, namely **32**, showed a similar selectivity profile (α/β 15-fold). A slightly better selectivity was observed for the 2-(naphthalen-1-yl)acetic acid analog **33**, which had the binding selectivity α/β of 25-fold, and binding affinity on LXRβ of 5 nM. As seen earlier (**29** vs **30**), the benzyl amine **33** (α/β 25-fold) was slightly more selective than the corresponding benzyl ether analog **34** (α/β 15-fold). Compared to **33** the positional isomer **35** showed similar binding affinity (IC_{50} for LXRβ = 2 nM) but reduced selectivity (α/β 11-fold). The benzoic acid analog **36** also showed reduced selectivity (α/β 4.5-fold).

Replacements for the C-3 benzyl substituent were also evaluated. Compared to C-3 benzyl analog **33**, the C-3 methyl analog **37** offered no advantage in terms of binding affinity or binding selectivity. Interestingly, the most selective analog in the series was found with the C-3 H analog **38**, which was 50-fold selective for LXRβ against LXRα, and with LXRβ binding IC_{50} of 15 nM.

A few binding selective LXR agonists were tested in Gal4 functional transactivation assays (Table 3, for assay condition see Ref. 10a). Although compound **33**, **37**, and **38** showed good binding affinity ($IC_{50} \leqslant 15$ nM) for LXRβ and good binding selectivity for LXRβ over LXRα (α/β > 20-fold), the compounds showed much less selectivity (~2-fold in potency against LXRα and some differences in efficacy) in the Gal4 transactivation assays. Nevertheless, compared to the reference compound **3**, these three compounds showed reduced potency with partial agonism on LXRα (13 ~ 57% efficacy) in Gal4, suggesting potentially less lipogenic impact on the TG synthesis. In fact, relative to **3** and **4** compounds **33**, **37**, and **38** showed reduced potency and efficacy in SREBP-1c gene expression and TG accumulation in liver cells (HegG2 cells).[14] However, these LXRβ binding selective compounds also had reduced potency and efficacy relative to **3** and **4** on the LXRβ-Gal4 activity. The lower LXR-Gal4 activity translated into reduced potency and efficacy in ABCA1 activation in the human macrophage cell line (THP-1).[15] To better assess the cellular activity for LXRβ these compounds were also evaluated in murine J774 macrophages which express LXRβ predominantly with little or no LXRα expression[8,16] in contrast to differentiated THP-1 cells[17] which have roughly equivalent LXRα and LXRβ expression. In the J774 macrophages, binding selective compounds **33**, **37**, and **38** showed enhanced potency and efficacy for stimulating ABCA1 gene expression over THP-1 cells whereas a pan agonist such as **3** did not show any difference in these two cell lines. Unfortunately, compounds **33**, **37**, and **38** also had unwanted peroxisome proliferator-activated receptor (PPAR) activity (data for PPARα and PPARγ not shown), especially PPARγ activity[18] ($EC_{50} < 100$ nM, efficacy > 50%) which precluded further development of the series.

Table 3. Gene expression activity and cross reactivity in Gal4 human transactivation assays

Compound	hLXRβ IC_{50} (nM)	Ratio α/β	Gal4 LXRβ EC_{50} (nM) (Ag%)[a,b]	Gal4 LXRα EC_{50} (nM) (Ag%)[a,b]	Human macrophage ABCA1 EC_{50} (nM) (Ag%)[a,b]	Murine macrophage ABCA1 EC_{50} (nM) (Ag%)[a,b]	Hepatocyte SREBP1c EC_{50} (nM) (Ag%)[a,b]	Hepatocyte TG accumu. EC_{50} (nM) (Ag%)[a,b]	PPARγ EC_{50} (nM) (Ag%)[c]
3	9	1.4	178 (100%)	135 (100%)	44 (100%)	35 (100%)	67 (100%)	137 (99%)	NT[d]
4[10a]	2	5.0	90 (63%)	240 (90%)	84 (155%)	41 (115%)	168 (103%)	224 (85%)	680 (47%)
33	5	25	355 (48%)	685 (57%)	280 (75%)	39 (91%)	263 (60%)	1451 (83%)	84 (54%)
37	10	21	4167 (51%)	7866 (47%)	1910 (64%)	475 (98%)	4050 (51%)	2748 (39%)	40 (83%)
38	15	50	622 (32%)	1189 (13%)	2740 (47%)	669 (75%)	4040 (38%)	2952 (22%)	69 (49%)

[a] Results are given as means of two to three independent experiments. The standard deviations for these assays were typically ±50% of mean or less. The % of efficacy is relative to reference compound **3**.
[b] LXR transactivation assay used Huh7 cells transfected with human LXR LBD fused to Gal4 DBD; ABCA1 gene regulation by LXR ligands was measured in THP1 (human) cells or J774 (murine) cells; SREBP1c gene regulation by LXR ligands was measured in HepG2 cells; LXR-mediated TG accumulation was measured in HepG2 cells.
[c] Recombinant human PPAR LBD. % of efficacy is relative to reference Rosiglitazone.
[d] NT, not tested.

In summary, modifications on previously reported **WAY-254011** via molecular modeling guided SAR study produced a series of novel carboxylic acid based quinolines that showed some selectivity for LXRβ over LXRα in binding assays and reduced efficacy in the TG accumulation assay, however, compounds in this series displayed more modest selectivity in the Gal4 functional assays. Unwanted PPAR agonist activity was also observed in this quinoline carboxylic acid series.

Acknowledgments

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