N-Acylthiadiazolines, a New Class of Liver X Receptor Agonists with Selectivity for LXRβ

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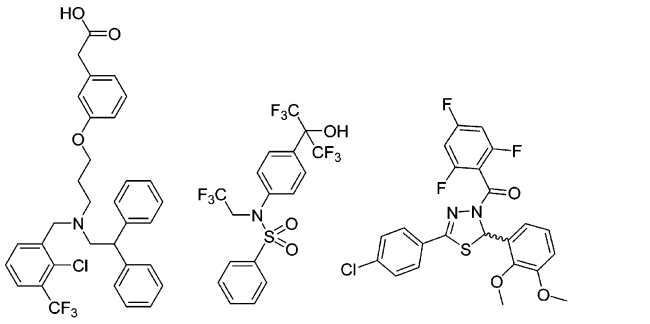
We have identified a novel liver X receptor (LXR) agonist (**2**) that activates the LXRβ subtype with selectivity over LXRα. LXRβ selectivity was confirmed using macrophages derived from LXR mutant mice. Despite its selectivity and modest potency, the compound can induce APO-AI-dependent cholesterol efflux from macrophages with full efficacy. Our results indicate that it is possible to achieve significant LXRβ selectivity in a small molecule while maintaining functional LXR activity.

Introduction

The liver X receptors (LXR)[a] α and β (NR1H3, NR1H2) are ligand-activated transcription factors of the nuclear receptor superfamily that control gene expression linked to cholesterol, lipid, and glucose homeostasis.[1,2] The endogenous ligands for LXRs are primarily oxysterols, but several synthetic ligands have been described that have facilitated the study of LXR function.[3−10] In particular, the dual LXRα/β agonists **1** (GW3965)[6] and **1a** (T0901317;[7] Chart 1) have been widely utilized as nonsteroidal chemical tools to explore the biology of the LXRs. Use of these agonists has shown the potential of the LXRs as drug targets for cardiovascular disease, diabetes, inflammation, and neurodegenerative disease.[11−16] However, development of LXR ligands as therapeutic agents has been hampered because dual LXR agonists induce a detrimental increase in hepatic triglyceride production. Because LXRα is the dominant subtype in the liver, where LXRβ is expressed at very low levels, it is thought that an LXRβ-selective agonist may retain efficacy without deleteriously increasing hepatic lipogenesis.[17−19] Hence, the identification of LXRβ-selective agonists may be of significant therapeutic value.

The crystal structures of the LXRs reveal that all the residues in the ligand binding domain (LBD) that form the ligand binding pocket (LBP) are conserved between the two subtypes, hinting that isolation of subtype-selective LXR ligands may be a challenging endeavor.[20−22] However, LXR transcriptional activity in vivo is the result of a complex interplay between recruitment of co-activators and release of co-repressors, the nature of which cannot be anticipated simply by the in vitro binding properties of a compound to the LBD. Thus, while LXR subtype selectivity measured as a separation in binding potency (EC_{50}) may be difficult to attain given the degree of similarity between the two ligand binding pockets, it is conceivable that functional selectivity measured as a separation in the degree of transcriptional activation induced (i.e., efficacy) may be achievable, even if it cannot be described at a molecular level.

Chart 1



1 **1a** **2** = racemate; **3** = R enantiomer; **4** = S enantiomer

Results and Discussion

To identify novel LXR modulators, a cell-based HTS using a chimeric GAL-hLXRβ LBD construct was run. A few N-acylthiadiaziolines with high micromolar activity that appeared selective for LXRβ over LXRα under the conditions used were identified. These molecules activated LXRβ but failed to activate LXRα in transfection assays. Through a round of optimization of the HTS hits, a more potent LXRβ-selective N-acylthiadiazoline **2** was synthesized (GAL-hLXRβ assay $EC_{50} = 1.29\ \mu$M, % efficacy = 55; no activity on GAL-hLXRα). This thiadiazolidine was inactive in cell-based assays for a panel of nuclear receptors (RXR, FXR, PXR, PPARs, GR, VDR) both in agonist and in antagonist mode (10 μM top dose, not shown). To explore the molecular basis of the apparent selectivity of this compound, we profiled it on cell-free LXR binding assays (Table 1). In scintillation proximity assays (SPA) measuring total binding, **2** bound to LXRβ with 30-fold greater potency than to LXRα, and to a more complete extent (100% vs 52% occupancy). Similarly, in FRET-based coactivator recruitment assays measuring agonist activity, **2** stimulated co-activator recruitment to the LXRβ LBD with 10-fold greater potency than to the hLXRα LBD and with greater efficacy (100% vs 53% efficacy).

To evaluate the ability of compound **2** to stimulate in vivo expression of LXR target genes, it was profiled on the natural receptors in cell-based assays. Compound **2** retained LXRβ selectivity at the level of efficacy separation (Table 1), stimulating LXRβ activity with nearly full effectiveness (82%), while

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[a] Abbreviations: LXR, liver X receptor; RXR, retinoid X receptor; FXR, farnesoid X receptor; PXR, pregnane X receptor; PPAR, peroxisome-proliferator activated receptor; GR, glucocorticoid receptor; VDR, vitamin D receptor; ABCA1, ATP-binding cassette transporter A1; FAS, fatty acid synthase; FRET, fluorescence resonance energy transfer; SPA, scintillation proximity assay.

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Table 1. Biological Properties of Racemate **2** and Its Enantiomers[a]

assay	**1**	**2** racemate	**3** R	**4** S
IC_{50} SPA-LXRα (%*E*)	0.121 (100)	9.8 (52)	5 (80)	NA
IC_{50} SPA-LXR*β* (%*E*)	0.023 (100)	0.3 (100)	0.066 (100)	NA
IC_{50} FRET-LXRα (%*E*)	0.097 (100)	2.3 (53)	1.6 (64)	NA
IC_{50} FRET-LXR*β* (%*E*)	0.027 (100)	0.25 (100)	0.067 (94)	NA
EC_{50} hLXRα (%*E*)	0.066 (100)	0.608 (19)	0.346 (41)	NA
EC_{50} hLXR*β* (%*E*)	0.004 (100)	0.098 (82)	0.063 (98)	NA
ABCA1 induction (1 *μ*M)	100%	34%	39%	0%
ABCA1 induction (10 *μ*M)	100%	59%	60%	0%

[a] NA = no activity: no significant efficacy (<15%) detected at any concentration. Reporter gene assays were performed in quadruplicate with a top dose of 10 *μ*M. EC_{50} data is reported in *μ*M. Efficacy of compounds is presented as percent efficacy (%*E*) relative to **1** and defined as the percentage ratio between maximum fold induction for the test compound and maximum fold induction for **1** in the same experiment. FRET assays were performed in duplicate with a top dose of 20 *μ*M; EC_{50} data is reported in *μ*M and efficacy of compounds is reported as percent efficacy (%*E*) relative to **1**, defined as the percentage ratio between maximum coactivator recruitment for the test compound and maximum coactivator recruitment for **1** in the same experiment. SPA assays were run in displacement mode using [^{3}H]-**1a** and a top dose of 30 *μ*M test compound; efficacy represents maximum displacement relative to **1**. ABCA1 induction in human THP-1 cells was measured using qRT-PCR.

only marginally activating LXRα (19%). As may be anticipated based on this profile, compound **2** increased expression of an endogenous LXR target gene (ABCA1) in the human macrophage cell line THP-1 that expresses similar levels of the two LXR subtypes[23] with significant efficacy relative to the dual agonist **1** (34% at 1 *μ*M and 59% at 10 *μ*M). In contrast, in liver-derived HepG2 cells that contain primarily LXRα, compound **2** induced expression of the LXR target gene FAS with considerably reduced efficacy relative to **1** (10% at 1 *μ*M and 33% at 10 *μ*M), as may be expected of an LXR*β*-selective ligand.[29] Furthermore, when profiled in human primary hepatocytes, **2** failed to induce FAS expression, while **1** and **1a** elicited robust increases in FAS message levels (Figure 1a).

Compound **2**, synthesized as per Scheme 1, was obtained as a racemic mixture.[24,25] Separation by chiral HPLC afforded the two enantiomers **3** and **4**. The *S* absolute configuration of **4** was established by X-ray crystallography. In cell-based and biochemical assays, compound **4** was found to be inactive on both LXR subtypes, while compound **3** was active (Figure 1b and Table 1). Compound **3** contributed to the functional activity of the racemic mixture, stimulating endogenous ABCA1 expression to the same degree as **2**, while **4** did not alter ABCA1 expression in THP-1 cells (Table 1). The cellular behavior of the two enantiomers correlated with their in vitro LXR-binding properties: compound **4** failed to bind either LXR, while **3** bound LXR*β* with greater potency and efficacy compared to the racemic mixture **2** (Table 1).

To determine if the subtype selectivity of **2** was physiologically relevant, we measured ABCA1 expression in primary macrophages derived from LXRα- and *β*-null mice treated with **1** or **2** (Figure 2a). The dual agonist **1** induced ABCA1 in LXRα- and LXR*β*-null macrophages to a similar level. In contrast, mixture **2** induced higher levels of ABCA1 in LXRα-null than in LXR*β*-null macrophages, illustrating that the compound is functionally LXR*β*-selective at the concentrations tested. Mixture **2** also failed to induce ABCA1 in LXRα/*β* double null cells, corroborating its dependence on LXR for activity (not shown). Interestingly, when tested for its ability to promote APO-AI-dependent cholesterol efflux, mixture **2** and its active enantiomer **3** both induced cholesterol efflux in a dose-dependent manner, consistent with their ability to induce ABCA1 expression (Figure 2b). Notably, the efficacy of **3** in this assay is similar to that of **1**, despite its subtype selectivity and lower potency and efficacy.

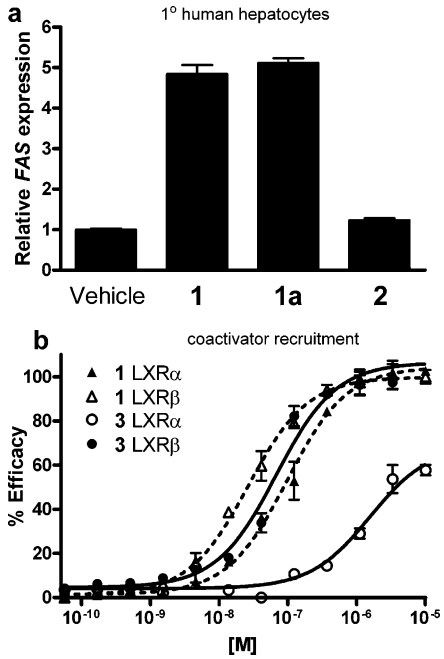
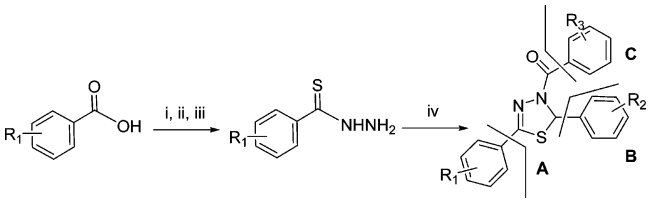


Figure 1. (a) Induction of endogenous FAS expression in primary human hepatocytes. Cells were treated with 1 *μ*M of the indicated compounds, and gene expression was analyzed by Taqman-based qRT-PCR. (b) Dose response in FRET-based coactivator recruitment assay. Note that there are several concentrations at which **3** activates LXR*β* but not LXRα.

Scheme 1. General Synthesis of Thiadiazolines[a]



[a] Reagents and conditions: (i) *t*-BuOCONHNH$_2$, EDC, DMAP, DMF/THF; (ii) Lawesson's reagent, THF, MW 80 °C; (iii) HCl (4 N), 1,4-dioxane; (iv) (1) R_2PhCHO, DIEA, CH_2Cl_2; (2) R_3PhCOCl, DIEA.

To explore the chemical basis of LXR activity and subtype selectivity, we performed SAR analysis around lead compound **2** using a FRET-based coactivator recruitment assay to guide our survey (Table 2). Substitutions around the phenyl group A (Scheme 1) appeared limited to halogens and small lipophilic substituents in the 3- and 4- position (**5**−**7**), while 4-substitutions such as MeO, CN, $SONH_2$, Et_2N, *t*-Bu, and CF_3 yielded inactive mixtures (data not shown). Substitution of ring B with only one methoxy group in the 2-position (as in **8**) yielded a 10-fold more potent mixture for LXR*β* but less selective than the parent mixture **2**. Mixture **9** with only one methoxy group in the 3-position showed complete selectivity but a 10-fold decrease in potency and a reduction in efficacy, suggesting that the 2-MeO substituent may be sufficient for activity and that the 3-MeO substituent, while not necessary for activity, may be beneficial for selectivity. Addition of a 4-fluoro substitution to the monomethoxy phenyl compounds yielded a 50-fold increase in LXR*β* potency but ablated subtype selectivity, as in **10**. 2-Trifluoromethoxy substitution, as in **11**, abolished LXR activity while substitution with 2-$OCHF_2$ still provided active mixtures (**12**). Tying the two oxygens in a cycle as in the difluoromethylenedioxy phenyl derivative **13** yielded active but

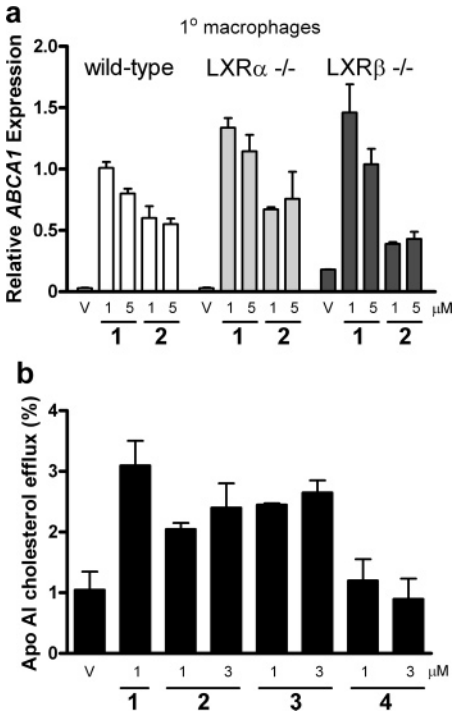


Figure 2. (a) Induction of ABCA1 expression in primary mouse macrophages. Genotype of cells is shown above bars. Two concentrations of **1** and **2** were used. Note the increased basal expression in LXRβ null cells. (b) Apo AI-dependent cholesterol efflux from primary mouse macrophages.

nonselective mixtures. Compounds where the 2-methoxy phenyl ring was exchanged with a 2-methoxy pyridine ring were found to retain activity with improved efficacy but showed less subtype selectivity, as exemplified by compound **14**.

Replacement of the hydrogen atom on the stereogenic center of the thiadiazoline ring (C_5) with an alkyl group caused almost complete loss of activity, as in compound **15**. Phenyl ring C tolerated a variety of substitutions. In general, substitution of the phenyl ring with at least one halogen atom in different positions and in combination with other groups yielded active mixtures with varying degrees of potency and efficacy that were invariably LXRβ-selective in terms of efficacy separation, as seen in compounds **17**−**27**. Other phenyl substitutions such as 3-MeO, 3-F, 4-MeO, 4-OCF_3, 4-NO_2, and 4-NH_2 were detrimental to activity (not shown).

Compounds were also tested in a qRT-PCR assay measuring upregulation of endogenous ABCA1 expression in THP-1 cells to verify the cellular properties of newly synthesized molecules (Table 2). As expected, the compounds that showed the highest level of efficacy were the less LXRβ-selective mixtures **10** and **14**. Among the more selective mixtures, **5** elicited expression of ABCA1 comparable to mixture **2** despite being slightly more potent in the FRET assay. In contrast, mixture **25** showed a comparable level of activity in the FRET assay, but demonstrated a higher degree of efficacy in terms of ABCA1 expression when compared to mixture **2**.

Unfortunately, despite adequate in vivo PK,[30] when dosed in mice, both mixtures **2** and **5** failed to elicit induction of ABCA1 and SREBP-1c in liver or other tissues. One likely reason may be that mixtures **2** and **5**, as well as most of the

Table 2. SAR Analysis[a]

cmpd	R_1	R_2	R_3	FRET LXRα EC_{50} (%*E*)	FRET LXRβ EC_{50} (%*E*)	ABCA1 induction (%) 1 μM	ABCA1 induction (%) 10 μM
1				0.097 (100)	0.027 (100)	100	100
2	4-Cl	2,3-di-MeO	2,4,6-tri-F	2.3 (42)	0.3 (99)	34	59
3	4-Cl[b]	2,3-di-MeO	2,4,6-tri-F	1.6 (64)	0.067 (94)	39	60
4	4-Cl[c]	2,3-di-MeO	2,4,6-tri-F	no fit	no fit	0	0
5	4-F	2,3-di-MeO	2,4,6-tri-F	1.4 (62)	0.076 (98)	42	53
6	4-Me	2,3-di-MeO	2,4,6-tri-F	1.4 (52)	0.2 (98)	24	34
7	3,4-di-Cl	2,3-di-MeO	2,4,6-tri-F	no fit	>10 (51)	18	46
8	4-Cl	2-MeO	2,4,6-tri-F	0.18 (80)	0.035 (110)	57	54
9	4-Cl	3-MeO	2,4,6-tri-F	no fit	3.7 (60)	ND[d]	ND
10	4-F	2-MeO-4-F	2,4,6-tri-F	0.07 (92)	0.006 (100)	99	59[e]
11	4-Cl	2-OCF_3	2,4,6-tri-F	no fit	3.4 (31)	ND	23
12	4-F	2-$OCHF_2$	2,4,6-tri-F	1.9 (76)	0.15 (90)	26	ND
13	4-Cl	2,3-difluoromethylenedioxy	2,4,6-tri-F	3.9 (71)	0.8 (74)	35	61
14	4-F	[f]	2,4,6-tri-F	0.4 (71)	0.21 (93)	100	79
15[g]	4-F	2-MeO	2,4,6-tri-F	no fit	4 (50)	ND	ND
16	4-Cl	2,3-di-MeO	H	no fit	No Fit	ND	ND
17	4-Cl	2,3-di-MeO	2-F	no fit	7.7(48)	ND	ND
18	4-Cl	2,3-di-MeO	2,4-di-F	6.7 (50)	0.98 (80)	23	40
19	4-F	2,3-di-MeO	2,3,6-tri-F	no fit	0.97 (74)	35	42
20	4-F	2,3-di-MeO	2,4,5-tri-F	no fit	0.6 (40)	30	48
21	3,4-di-F	2,3-di-MeO	3-F	1.5 (30)	0.56 (83)	27	47
22	4-F	2,3-di-MeO	3,5-di-F	2.1 (40)	0.47 (95)	21	37
23	4-F	2,3-di-MeO	3-Cl	no fit	0.25 (82)	13	36
24	4-F	2,3-di-MeO	2-Me, 5-F	2.4 (46)	0.14 (90)	21	32
25	4-F	2,3-di-MeO	2,6-di-F, 4-MeO	0.9 (47)	0.17 (100)	81	42[e]
26	4-F	2,3-di-MeO	2,6-di-F, 4-Br	0.9 (43)	0.16 (92)	ND	42
27	4-F	2,3-di-MeO	2,4,5-tri-F, 3-OMe	0.7 (64)	0.081 (100)	30	59

[a] No fit = no significant efficacy (<15%) detected at any concentration. For FRET assays, data is reported in μM, assay performed in duplicate, variance < 15%. Efficacy of compounds is reported as percent efficacy (%*E*) relative to **1** and defined as the percentage ratio between maximum coactivator recruitment for the test compound and maximum coactivator recruitment for **1** in the same experiment. ABCA1 induction in human THP-1 cells was measured using qRT-PCR. [b] *R*-enantiomer. [c] *S*-enantiomer. [d] ND = not determined. [e] Drop-off in activity is due to toxicity. [f] 2-Methoxy-3-pyridyl. [g] Methyl-substituted compound on thiadiazoline ring C_5.

compounds shown, were found to be highly protein-bound (>99.9%). This was reflected by a dramatic decrease in their ability to induce ABCA1 in THP-1 cells when the assay was run using a higher concentration of serum (20% FBS, not shown). This liability limits the utility of these molecules in animal models of disease; further optimization of this class of thiadiazolines is required to increase plasma-free fraction and establish their therapeutic potential in vivo.

Conclusions

In summary, we have described the first examples of LXRβ agonists with separation of efficacy from LXRα. Functional selectivity was confirmed using macrophages derived from LXR-null mice: compounds showed significantly higher expression of ABCA1 in LXRα-null macrophages than in LXRβ-null macrophages. In spite of their selectivity and modest potency, these compounds can induce APO-AI-dependent cholesterol efflux with similar efficacy to that of the potent dual LXR agonist **1**. Our results indicate that it is possible to achieve significant LXRβ selectivity in a small molecule while maintaining functional LXR activity in a relevant setting.

Experimental Section

General Procedure for the Preparation of Benzothiohydrazides Hydrochloride Salt. To a solution of a benzoic acid (35.7 mmol) in 72 mL of a mixture of DMF and THF (1:1) were added *tert*-butyl carbazate (37.5 mmol), EDC (39.3 mmol), and *N*,*N*-dimethylaminopyridine (0.54 mmol). After 10 min, the mixture became homogeneous and stirring was continued for 3 h until the reaction was complete by TLC and LC/MS. The reaction mixture was poured into ice. Upon addition of diethylether, the organic layer was separated. The organic layer was washed with sodium bisulfite, satd sodium bicarbonate, and satd sodium chloride solution, dried over magnesium sulfate, and concentrated to yield the benzoylhydrazinecarboxylic acid *tert*-butyl ester. To a mixture of the benzoylhydrazinecarboxylic acid *tert*-butyl ester (11.1 mmol) in 10 mL of dry THF, Lawesson's reagent (11.6 mmol) was added, and the mixture was heated in the microwave oven at 80 °C for 20 min. The reaction mixture was concentrated and purified by automated column chromatography using hexanes/EtOAc. The thiobenzoyl hydrazinecarboxylic acid *tert*-butyl ester obtained (18.5 mmol) was added to HCl (4 N) in 1,4-dioxane (185 mmol). The mixture was stirred at room temperature for 1 h. Hexanes were added to further precipitate the product, which was filtered off, yielding the benzothiohydrazide.

General Procedure for the Preparation of N-Acyl Thiadiazolines. To a heterogeneous mixture of a benzothiohydrazide hydrochloride salt (0.107 mmol) in CH_2Cl_2 (1 mL) an aldehyde (0.128 mmol) and DIEA (0.128 mmol) were added. After 10 min, the mixture became homogeneous and the reaction was complete by TLC and LCMS to give the 2,5-substituted-2,3-dihydro-[1,3,4]-thiadiazole, which was used in the next step without evaporation of the solvent. To the solution was added DIEA (0.16 mmol) and 2-fluorobenzoyl chloride (0.16 mmol), and the reaction mixture was stirred for 12 h at room temperature. After evaporation of the solvent, the residue was purified by automated chromatography (hexane/EtOAc) or preparative LC/MS (20 to 100% MeCN/H_2O).

Biological Assays. Transcriptional Assays: Expression constructs for chimeric GAL-hLXR LBDs were generated by fusing the hLXRα and β LBDs to the DBD of GAL4 and inserting them into pcDNA3 (Invitrogen) as previously described.[5] Assays were validated using known ligands. The pTK-2xLXRE-luciferase reporter and pcDNA3 expression plasmids for full-length human and mouse LXRα/β have been described.[5,26] Cells were cultured in DMEM with 10% FBS. For transcriptional assays, cells were plated in 384-well plates in DMEM with 10% fetal bovine lipoprotein-deficient serum (FBLDS, Intracel) in the presence of 7.5 μM lovastatin and 100 μM mevalonic acid. HepG2 cells were transfected using FuGENE 6 (Roche). After 24 h, ligands were added as 11-point dose−response curves starting at 10 μM. After an additional 16 h, luciferase activity was read.

Fluorescence-Resonance Energy Transfer Assay: FRET assay was performed in 384-well plates in a 20 μL final volume. A mix of 20 nM His-tagged hLXR, 300 nM biotinylated SRC-1 peptide (Biotin-CPSSHSSLTERHKILHRLLQEGSPSC-OH), Europium-labeled anti-His antibody (Perkin-Elmer), and allophycocyanin-labeled streptavidin (Prozyme) was prepared. Ligands were tested in 12-point dose−response curves starting at 20 μM. Plates were incubated for 1 h at 37 °C and FRET was read on an AnalystGT (Molecular Devices).

Scintillation Proximity Assay: SPA was performed as in Janowski et al.[27] Scintillant-filled beads (Amersham) were diluted in SPA buffer (10 mM K_2HPO_4, 10 mM KH_2PO_4, 2 mM EDTA, 50 mM NaCl, 1 mM DTT, 2 mM CHAPS, and 10% glycerol) to a final concentration of 5 mg/mL. The assay was performed in 384-well plates (Packard) in 20 μL containing beads (0.2 mg per well) and His-tagged hLXR LBD (200 ng per well). The amount of protein did not deplete ligand concentration. Displacement assays used 30 nM of [^{3}H]-**1a** (Amersham). Ligands were tested in 12-point dose−response curves starting at 20 μM. Plates were shaken for 3 h at room temperature and read in a Packard Topcount at 1 min per well. Wells devoid of competitor represented 100% binding. Nonspecific binding was measured by leaving LXR protein out of the SPA reaction.

Dose−response and competition curves were generated by nonlinear regression analysis using GraphPad Prism, and the apparent equilibrium association/dissociation constants (K_d and K_i values) were determined using the method described by DeBlasi and colleagues.[28]

Cholesterol Efflux: Primary peritoneal macrophages were plated at 50% confluence in 24-well plates. On day 2, the cells were washed and incubated for 24 h in medium A supplemented with an ACAT inhibitor (58−035; 2 μg/mL) and [^{3}H]cholesterol (1.0 μCi/mL). Compounds were added to the cells as indicated. On day 3, the cells were washed twice with PBS and then incubated for 2−4 h in fresh medium, devoid of radiolabeled cholesterol, but containing the indicated compounds. The cells were rewashed before addition of 1.0 mL medium B (DMEM containing 0.2% BSA) in the absence or presence of apoAI (15 μg/mL). After 4 h incubation, the medium was removed and centrifuged at 14 000 × *g* for 10 min, and the radioactivity was determined by liquid scintillation counting. The cells were dissolved in isopropanol, and an aliquot was used to determine total cell-associated radioactivity. The apoAI-dependent efflux of radioactive cholesterol from the cells into the medium was determined as a percentage of the total radioactivity in the cells and medium for each condition. Each efflux assay was performed in triplicate.

Gene Expression Analysis: Human THP-1 cells were grown in propagation media (10% defined FBS, 2 mM L-glutamine, 10 mM HEPES, 1.0 mM sodium pyruvate, 4.5 g /L glucose, 1.5 g/L bicarbonate, and 0.05 mM 2-mercaptoethanol in RPMI 1640). On day 1, 125 000 cells/well were plated in propagation media plus 40 ng/mL PMA on a 48-well dish. On day 2, media was replaced with fresh assay media (same as propagation media but with 2% FBLDS as the serum supplement) and compounds were added 6 h later (1 or 10 μM in DMSO). Cells were then incubated for 24 h before being harvested, and RNA was isolated using the RNeasy kit (Qiagen) with DNaseI option. RNA was analyzed by TaqMan qRT-PCR using the one-step Superscript III platinum reagent (Invitrogen). Samples were run in triplicate as multiplexed reactions with an internal control (36B4). Probe and primer sequences for ABCA1 and FAS are available on request. Fold induction induced by compound was calculated in reference to DMSO. Primary peritoneal macrophages were elicited from LXR mutant mice by IP injection of 4% thioglycollate (Difco) and subsequent lavage.[12] Cells were washed extensively and then plated for 24 h. Adherent cells were treated in all subsequent steps in identical fashion to the THP-1 cells described above, except no PMA was used to prompt

differentiation. Primary human hepatocytes were cultured and analyzed as described in reference 14.

Animal Experiments: The 10-week-old male C57BL/6 mice were maintained on a standard chow diet. Mice were gavaged daily with vehicle, **1** (50 mpk), or test compound (20−200 mpk) for 3 days before sacrifice. Mice were fasted overnight prior to the last dose of compound and sacrificed 3 h later. RNA was isolated from tissues and gene expression analyzed using TaqMan-based qRT-PCR. All experiments were approved by the Institutional Animal Care and Use Committee of The Genomics Institute of the Novartis Research Foundation.

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Supporting Information Available: Spectral and purity data for compounds **2**−**27**. This material is available free of charge via the Internet at http://pubs.acs.org

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(29) Compound **2** was tested for stability in human liver microsomes (1 μM). After 30 minutes, 70% of the compound was still present.
(30) Pharmacokinetic studies IV and PO were performed for mixtures **2** and **5** in mice (2.5 mg/mL in PEG300/Tween 80 [4:1]). Intravenously, mixture **2** dosed at 5 mpk showed AUC = 428.24 min·ug/mL, C_{max} = 20.4 μM, Cl = 12.23 (mL/min/kg), V_{ss} = 1.65 (L/kg). Mixture **5** dosed at 5 mpk showed AUC = 2143.69 min·ug/mL, C_{max} = 70.9 μM, Cl = 2.26 (mL/min/kg), V_{ss} = 0.44 (L/kg). Orally, mixture **2** dosed at 20 mpk demonstrated an AUC = 475 min·ug/mL and C_{max} of 4.9 μM; bioavailability was 28% and $t_{1/2}$ was 6.7 hours. Mixture **5** dosed at 20 mpk showed an AUC = 1005 min·ug/mL, C_{max} of 6.9 μM but poorer bioavailability (11%); $t_{1/2}$ was 4.5 hours.

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