Identification of a Nonsteroidal Liver X Receptor Agonist through Parallel Array Synthesis of Tertiary Amines

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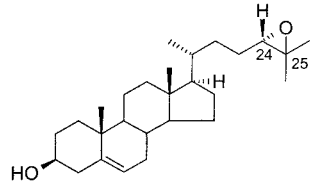
Abstract: A potent, selective, orally active LXR agonist was identified from focused libraries of tertiary amines. GW3965 (**12**) recruits the steroid receptor coactivator 1 to human LXRα in a cell-free ligand-sensing assay with an EC_{50} of 125 nM and profiles as a full agonist on hLXRα and hLXRβ in cell-based reporter gene assays with EC_{50}'s of 190 and 30 nM, respectively. After oral dosing at 10 mg/kg to C57BL/6 mice, **12** increased expression of the reverse cholesterol transporter ABCA1 in the small intestine and peripheral macrophages and increased the plasma concentrations of HDL cholesterol by 30%. **12** will be a valuable chemical tool to investigate the role of LXR in the regulation of reverse cholesterol transport and lipid metabolism.

The increased incidence of cardiovascular disease (CVD) in westernized nations has been linked to increased dietary intake of cholesterol and saturated fats and an increase in low-density lipoprotein (LDL) particles.[1] Accumulation of small, dense LDL particles in the arterial wall leads to the formation of cholesterol-laden foam cells, which are the hallmark of coronary atherosclerosis, and activation of the immune system.[2] Although cholesterol-lowering drugs, such as statins, reduce the incidence of CVD in patients with high circulating levels of LDL cholesterol (LDLc), atherosclerosis also afflicts individuals with relatively normal levels of LDLc. In contrast to LDL, the levels of high-density lipoprotein (HDL) particles are inversely related to the incidence of CVD.[3] The protective role of HDL may result from its role in mediating "reverse cholesterol transport" whereby cholesterol is transported from peripheral cells, including the macrophage-derived foam cells, back to the liver.[4] Thus, agents that promote reverse cholesterol transport by raising circulating levels of HDL could provide an alternative therapeutic option for the prevention of atherosclerotic CVD.[5]

A pivotal step in the process of reverse cholesterol transport is the efflux of free cholesterol from peripheral tissues to nascent HDL particles. Mutations in the cholesterol transporter adenosine triphosphate binding cassette (ABC) A1 were recently identified as the genetic basis of Tangier disease (TD), a condition characterized by an absence of plasma HDLc.[6−8] TD patients show a decreased capacity to effect cholesterol efflux from peripheral tissues and also develop premature atherosclerosis.[9] Notably, targeted disruption of the ABC1 gene in mice results in HDL deficiency and increased foam cell formation compared to wild-type mice.[10] Thus, drugs that upregulate ABCA1 expression could provide a method for promoting reverse cholesterol transport and preventing CVD.[4]

Chart 1. Structures of Epoxycholesterol (**1**), T0901317 (**2**), and Partial Agonist **3**



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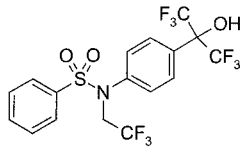

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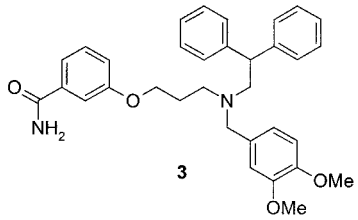

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The liver X receptors, LXRα (NR1H3) and LXRβ (NR1H2), are oxysterol-activated transcription factors that belong to the nuclear hormone receptor superfamily.[11,12] 24(*S*),25-Epoxycholesterol (EPC, **1**) may be an endogenous ligand for LXRα in the liver (Chart 1).[13,14] Upon cholesterol feeding, the hepatic levels of **1** are raised in rats to levels consistent with its putative role as a natural LXRα agonist.[15−17] The LXRs function as heterodimers with the 9-cis retinoic acid receptor RXR (NR2B) to regulate the expression of the ABCA1 gene.[18−21] A nonsteroidal LXR agonist, T0901317 (**2**), was recently reported to increase ABCA1 expression and raise HDLc levels in mice.[20,22,23] In this report we describe the identification of a novel chemical series of LXR agonists through solid-phase parallel array synthesis of tertiary amines. These compounds may provide leads for the development of drugs to increase reverse cholesterol transport.

Tertiary amine **3** was identified from a high-throughput screen of the GlaxoSmithKline compound file using a cell-free ligand-sensing assay (LiSA) for human LXRα. The LXRα LiSA measures the ligand-dependent recruitment of a 24 amino acid fragment of the steroid receptor coactivator 1 (SRC1) to the ligand-binding domain of the receptor.[24] Tertiary amine **3** showed an EC_{50} of 260 nM

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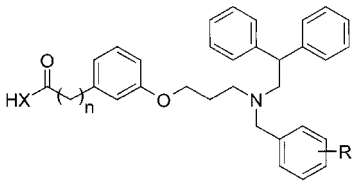
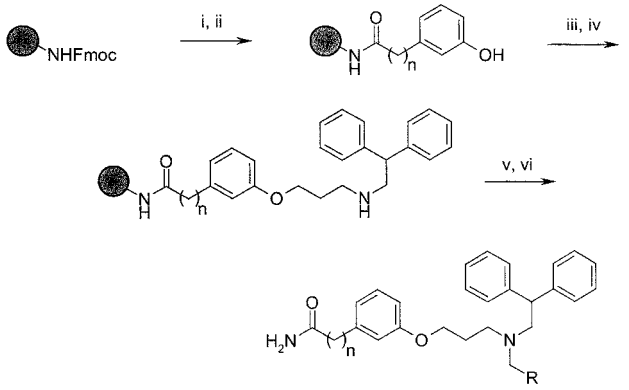
Table 1. LXR Activity of Tertiary Amines[a]



compd	*n*	X	R	LXRα/SRC1 LiSA EC$_{50}$ (nM)	LXRα/SRC1 LiSA RE	LXRα-GAL4 EC$_{50}$ (nM)	LXRα-GAL4 RE
1			24(*S*),25-epoxy-cholesterol	95 ± 4	1.0	3000 ± 750	1.0
2			T0901317	60 ± 4	1.0	85 ± 10	2.5
3	0	NH	3,4-dimethoxy	260 ± 60	0.2	nt	nt
4	1	NH	4-methoxy	260 ± 10	0.8	ia	
5	1	O	4-methoxy	860 ± 50	0.8	8000 ± 3200	0.9
6	1	NH	2,4-dimethoxy	660 ± 10	0.7	4000 ± 1300	0.6
7	1	NH	3-fluoro-4-methoxy	250 ± 40	0.7	910 ± 20	0.5
8	1	NH	2-fluoro-4-methoxy	190 ± 50	0.9	700 ± 220	0.4
9	1	NH	3-trifluoromethyl	85 ± 20	0.7	650 ± 300	0.3
10	1	NH	4-fluoro-3-trifluoro-methyl	85 ± 5	0.9	945 ± 365	0.4
11	1	NH	2-chloro-3-trifluoro-methyl	45 ± 10	1.1	425 ± 115	1.5
12	1	O	2-chloro-3-trifluoro-methyl	125 ± 20	1.0	190 ± 30	1.7

[a] EC$_{50}$ = concentration of compound that leads to half-maximal activity ± standard error, $n = 3$. RE = relative efficacy compared to 24(*S*),25-epoxycholesterol (**1**). nt = not tested. ia = inactive at 10 μM.

Scheme 1[a]



[a] Reagents: (i) 20% piperidine, DMF; (ii) four phenolic acids, HATU, 2,6-lutidine, NMP; (iii) 3-bromopropanol, DIAD, Ph_3P, toluene, 0 → 25 °C; (iv) 2.0 M diphenethylamine, DMSO; (v) RCHO, $NaHB(OAc)_3$, 8% AcOH/DMF; (vi) 10% TFA, DCM.

on LXRα but was only partially effective at recruiting the SRC1 protein compared to EPC (**1**) (RE = 17%, Table 1). To improve the efficacy of **3**, a solid-phase synthesis of tertiary amines was developed that would allow exploration of the benzamide and benzylamine functionalities (Scheme 1). Among the solid-phase linkers that were evaluated were the Sasrin linker, which yields a carboxylic acid upon cleavage of the resin, and the Rink linker, which yields the corresponding amide. The Rink amide linker was chosen for initial array synthesis, since it provided the highest overall yield and purity of final products in preliminary studies (data not shown). Four commercially available phenolic acids were loaded onto solid support and subsequently reacted with 3-bromopropanol under Mitsunobu conditions. The resin-bound bromides were treated with a solution of diphenylethylamine in DMSO, and the resulting secondary amines were subjected to reductive amination with 30 commercially available benzaldehydes. Cleavage from the solid support provided an array of 120 tertiary amines in approximately 83% average purity and 50% overall yield as determined by HPLC and chemiluminescent nitrogen detection (CLND). The array of tertiary amines was screened at a concentration of 1 μM using the LXRα/SRC1 LiSA. Phenylacetamide **4** was identified as a potent LXRα ligand with increased efficacy for recruitment of the SRC1 peptide compared to **3** (Table 1). Unfortunately, **4** was inactive at doses up to 10 μM in cell-based LXRα reporter gene assays[13] (Table 1). In an attempt to change the physical properties of amide **4**, the corresponding carboxylic acid **5** was synthesized using Sasrin resin in place of Rink amide resin. Although **5** was less potent in the LXRα/SRC1 LiSA, it showed improved activity with an EC$_{50}$ of ~8 μM in the cell-based reporter gene assay (Table 1).

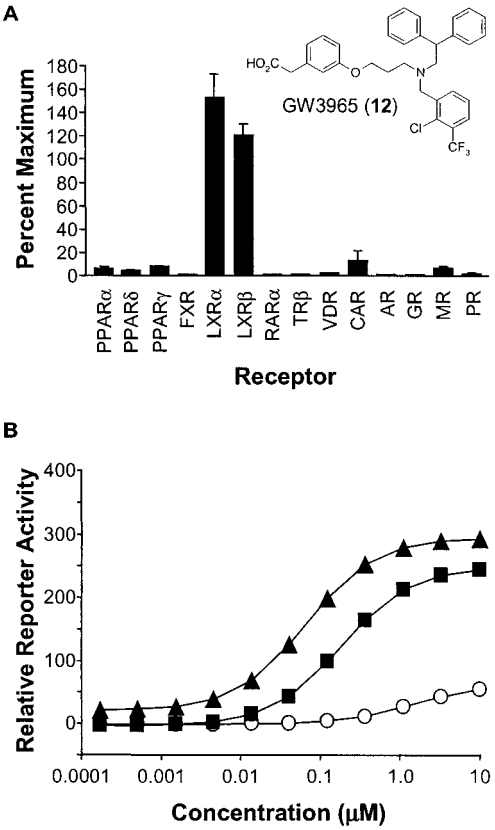


Figure 1. (A) Activity of **12** (1 μM) on hPPARα-GAL4, hPPARδ-GAL4, hPPARγ-GAL4, hFXR-GAL4, hLXRα-GAL4, hLXRβ-GAL4, hRARα-GAL4, hTRβ-GAL4, hVDR-GAL4, full-length hCAR, full-length hAR, full-length hGR, full-length hMR, and full-length hPR. Percent maximum is the activity relative to a positive control for each receptor. Data are representative of two or more independent experiments. (B) Dose−response analysis of **12** on hLXRα-GAL4 (■), hLXRβ-GAL4 (▲), and hPXR-GAL4 (○). Reporter activity is relative to EPC (**1**) for LXR and rifampicin for PXR. Data are representative of four independent experiments.

In an effort to further increase the LXRα activity of the tertiary amines, an array of 1280 carboxamides were synthesized using Rink amide linker and screened for activity in the LXRα/SRC1 LiSA at 1 μM. Six carboxamides (**6**−**11**) were identified from the array with activity less than 1 μM in the LXRα/SRC1 LiSA (Table 1). Several of these analogues contained a *m*-trifluoromethyl functionality in the benzylamine substituent (**9**−**11**), with the 2-chloro-3-trifluoromethylbenzylamine **11** identified as the most potent analogue with an EC$_{50}$

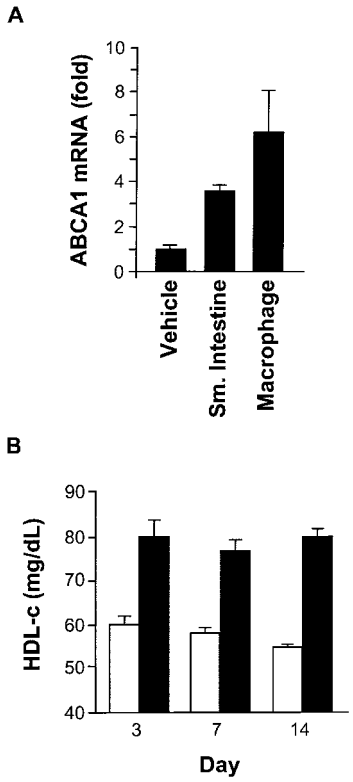


Figure 2. Effects of dosing **12** at 10 mg/kg bid to C57BL/6 mice ($n = 3$) on ABC1 expression levels in small intestine and peripheral macrophages (A) and plasma levels of HDLc (B).

of 45 nM in the LXRα/SRC1 LiSA. As was seen in the earlier series of carboxamides, **11** showed a reduction in potency when tested in the cell-based LXRα-GAL4 reporter gene assay. Since cellular potency had been improved through conversion of amide **4** to carboxylic acid **5**, the corresponding carboxylic acid **12** was synthesized using the Sasrin linker. Carboxylic acid **12** showed an EC_{50} of 125 nM in the LXRα/SRC1 LiSA with comparable efficacy to EPC (**1**) for recruitment of the SRC1 peptide. To our delight, **12** maintained its potency in the LXRα cell-based reporter gene assay with an EC_{50} of 190 nM (Table 1, Figure 1B). When screened against a panel of nuclear receptors, **12** showed cross reactivity with only LXRβ (Figure 1A) and the pregnane X receptor (PXR) (data not shown). Full dose−response analysis on LXRβ- and PXR-GAL4 chimeras showed **12** was >10-fold selective for activation of LXR compared to PXR (Figure 1B). Thus, carboxylic acid **12** is a potent LXR agonist with good cellular activity and excellent selectivity over other nuclear receptors.

In mice, **12** showed 70% oral bioavailability with $C_{max} = 12.7$ μg/mL and $t_{1/2} = 2$ h after dosing at 10 mg/kg. Analysis of the pharmacokinetic data indicated that the serum levels of **12** were 5-fold above its EC_{50} in cells for up to 7 h after dosing. The pharmacological activity of **12** was evaluated in C57BL/6 mice by dosing at 10 mg/kg bid for 14 days. By day 3, ABCA1 expression was increased 8-fold in the small intestine and 7-fold in peripheral macrophages (Figure 2A), while plasma levels of HDLc increased 30% at day 3 and was maintained until day 14 (Figure 2B). Thus, **12** is an orally active LXR agonist that upregulates ABCA1 expression and raises circulating levels of HDL in C57BL/6 mice.

In summary, carboxylic acid **12** has been identified through parallel array synthesis of tertiary amines as a potent LXR agonist with selectivity over other nuclear receptors including PXR. Carboxylic acid **12** is active on LXRα and LXRβ in cell-based reporter gene assays and shows oral bioavailability in mice with plasma concentrations reaching 5-fold above the EC_{50} for LXR activation. Moreover, **12** raises plasma levels of HDLc in C57BL/6 mice and increases ABCA1 expression in multiple tissues that are known to be involved in the process of reverse cholesterol transport. GW3965 (**12**) is structurally distinct from the oxysterol[24] and sulfonamide[20,22,23] classes of LXR agonists and represents a promising lead for the development of drugs to prevent atherosclerotic CVD.

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Supporting Information Available: Experimental procedures for the research described in this letter. This material is available free of charge via the Internet at http://pubs.acs.org.

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