Combination of Virtual Screening and High Throughput Gene Profiling for Identification of Novel Liver X Receptor Modulators

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We conducted virtual docking studies using GLIDE with modified LXRβ ligand-binding domain (LBD) on internal compound collection followed by the gene profiling with ArrayPlate mRNA assay. A total of 69 compounds were found to upregulate LXRα and certain LXR regulated genes from 1308 compounds selected by virtual screen (hit rate: 5.3%). Compound **4** was shown to significantly induce the expression of LXR target genes such as ABCA1, ABCG1, APOE, SCD-1, and SREBP-1c in THP-1 differentiated macrophages. In vitro binding assay confirmed that **4** binds to both LXRα and LXRβ directly and recruits coactivator peptide SRC-1. It functions as a full LXR agonist in stimulating cholesterol efflux in THP-1 differentiated macrophages and induces lipogenesis in HepG2 cells. This study demonstrates that the combination of virtual screen and high throughput gene profiling is an efficient approach for rapid identification of novel LXR modulators.

Liver X receptor-α (LXRα,[a] NR1H3) and LXRβ (NR1H2) are oxysterol-activated nuclear receptors composed of a ligand-binding domain (LBD) and a DNA-binding domain (DBD).[1] Upon the ligand binding to the LBD, LXR forms a heterodimer with the retinoid X receptor (RXR; NR2B1). The LXR/RXR heterodimer binds to LXR response elements in promoter regions of specific genes, resulting in adaptation of gene transcription by recruiting coactivators or corepressors. LXRs are activated by natural ligands, such as oxysterols (e.g., **1**)[2] and glucose,[3] and control the expression of various genes involved in cholesterol, glucose, and fatty acid metabolisms. Thus, synthetic LXR agonists are of potential utilities as antiatherosclerotic, anti-inflammatory, and antidiabetic agents.[1] For instance, treatment with agonists such as **2** (GW3965)[4] or **3** (T0901317)[5] attenuated development of atherosclerosis in apolipoprotein E-deficient (ApoE$^{-/-}$) and low-density lipoprotein (LDL) receptor-deficient (Ldlr$^{-/-}$) mice.[6,7] ABCA1 in β-cells is shown to be critical for insulin secretion through modulating cellular cholesterol homeostasis, suggesting that LXR modulators may also affect β-cell function in type 2 diabetes.[8] However, synthetic LXR agonists may cause undesirable lipogenesis-related side effects, primarily through the upregulation of genes related to fatty acid and lipid synthesis (SREBP-1c, FAS etc). Up to date identification of new LXR modulators has mainly relied on conventional approaches such as receptor binding, cofactor recruitment, or reporter assays.[9] These approaches require screening of large compound libraries and lack the capability of differentiating compounds with preferable selectivity profiles in gene regulation.[10] In this report, we describe the rapid and successful identification of novel LXR modulators using the combination of structure-based virtual screening and high-throughput gene profiling technologies.

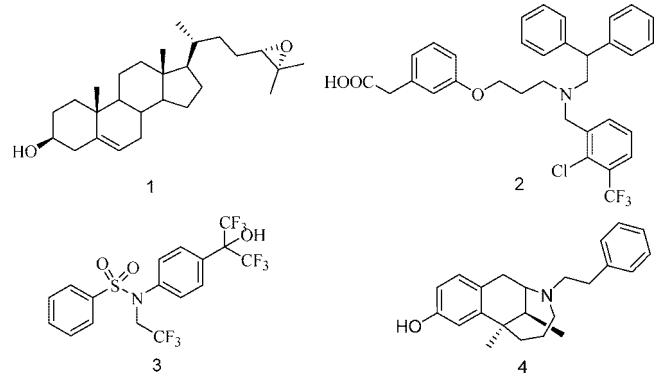

Figure 1. LXR ligands.

The crystal structures of the ligand-binding domain (LBD) of LXR in complex with native or synthetic ligands have been reported, which contribute to the current understanding of the activation mechanism of LXR.[9,11] Like most if not all nuclear hormone receptors, binding of a LXR agonist stabilizes a conformation of the receptor, where C-terminal H12 helix folds like a lid onto the ligand, thereby generating a hydrophobic surface on the outside of the receptor that serves as platform for the recruitment of coactivators. It is suggested that LXR agonists maintain an active conformation through interaction with His453 in H12 to position its imidazole ring against Trp457 indole ring.[11a]

To establish a valid virtual docking method, we selected 127 representative LXR agonists in six different chemical classes from the literature, including patent applications (Table 1, see Supporting Information),[12] and docked those compounds to a panel of LXR crystal structures. Most of those compounds are reported to be LXR agonists with binding data. The 2D agonist structures were converted to 3D structures using CORINA program[13] prior to docking with GLIDE.[14] Among the seven

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[a] Abbreviations: ABCA1, ATP binding cassette transport A1; ACTB, β-actin; Apo, apolipoprotein; B2M β-2-microglobulin; DBD, DNA-binding domain; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LXR, liver X receptor; LXRE, LXR response elements; RXR, retinoid X receptor; SCD-1, stearoyl-coA desaturase-1; SREBP-1c, sterol regulatory element binding protein 1c; SRC-1, steroid receptor coactivator-1; TBP, TATA box-binding protein; THP-1, human acute monocytic leukemia cell line.

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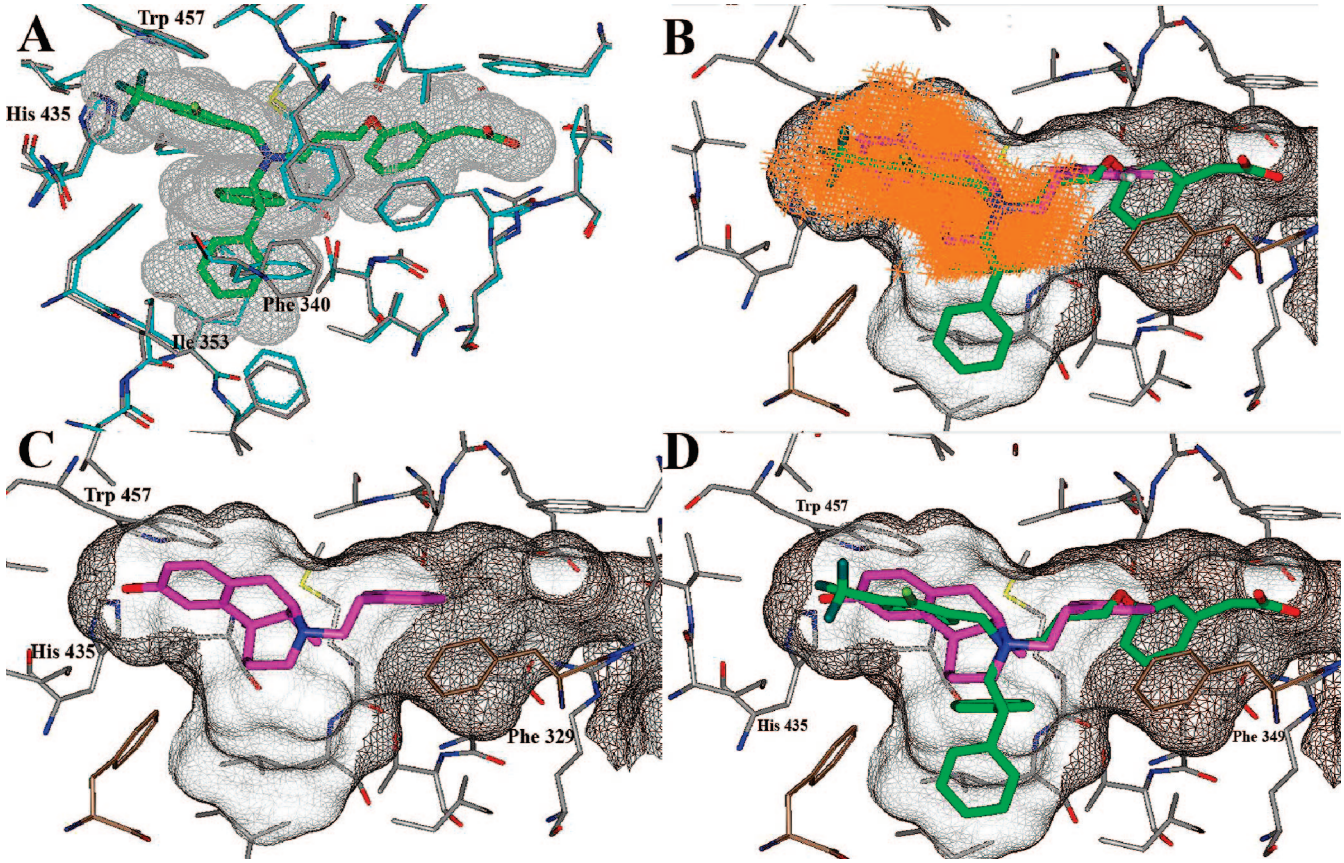

Figure 2. (A) Compound **2** in 1pq6. (B) Predicted binding mode of **2** in modified LBD 1p8d++; the C1 pocket is colored as orange. The residues within 5 Å of the C1 pocket were used to calculate PLP1 scores with Cerius II; (C) The potential binding mode of compound **4** in 1p8d++. (D) Overlap of compounds **4** and **2** in the binding pocket in 1p8d++.

Table 1. Template Structures and Recovery of Known LXR Agonists

template[a]	I (**33**)	II (**31**)	III (**26**)	IV (**16**)	V (**14**)	VI (**7**)	total[b] (127)
1p8d	6	7	5	5	3	1	27
1p8d+	8	8	4	7	4	1	32
1p8d++	28	14	5	8	8	6	69

[a] 1p8d: original crystal structure with **1**; 1p8d+: modified structure by rotating the side-chains of Ile353 and Phe340; 1p8d++: changed ligand in 1p8d+ from **1** to **2** and the new complex was minimized. [b] Number in parenthesis indicates the compound number selected from each chemotype and the number in the column indicates the total hits identified with each model.

LXR LBD crystal structures (1upv, 1upw, 1pqc, 1pq9, 1pq6, 1p8d, and 1uhl) tested, 1p8d was found to be the best in recovering active LXR modulators, but the recovery rate is still insufficient (27 out of 127), presumably due to the smaller hydrophobic pocket compared with 1pq6 (Figure 2A). LXR agonist recovery was slightly improved by expanding the hydrophobic pocket through rotating the side chains of Phe340 and Ile253 in 1p8d to match the conformations seen for these residues in 1pq6 (1pd8+). The resulting structure was minimized in the presence of **2** using OPLS-AA force field to generate a new structure 1p8d++ (Figure 2B). We were able to recover over 50% of 127 LXR agonists in our test database using this adjusted and minimized structure (Table 1). Therefore, this model was used for our virtual screening studies.

Virtual screening with 1p8d++ against 135000 compounds from internal collection was conducted to result in 10407 compounds with a GLIDE score better than –9.0 (SP scoring function). The number of compounds was reduced to 3263 by eliminating those clashing with protein atoms as evidenced by a van der Waals overlap of more than 20%. Compounds were selected for high throughput gene profiling based on the docking scores as well as on the potential close contacts with His435, a key interaction thought to be required for LXR agonist activity.[11a] Thus, 647 compounds with the best overall fit into the entire active site as reflected in a top ranked Glide score were first selected. Second, we selected compounds fitting into the His435 containing C1 subpocket[11c] (Figure 2A). A total of 414 compounds were selected using "piecewise linear potential"[15] score calculated with the C1 pocket. Lastly we tested the hypothesis that a halogen atom may be able to interact with His435 (e.g., compounds **2** and **3**) through hydrogen-bonding or halogen-nitrogen/oxygen interaction to stabilize agonistic conformation.[16] There are about 247 compounds with docking poses positioning a halogen atom within 4.5 Å of His435. In total, 1308 compounds were selected for high-throughput gene expression profiling.[17]

LXR is known to regulate a number of genes, notably, genes related to cholesterol metabolism (e.g., ABCA1 and ABCG1), lipogenesis (e.g., SREBP1c, FAS, and SCD-1), inflammation (e.g., IL-6 and COX-2), carbohydrate metabolism (e.g., CREBP and Glut4), and LXRα itself (but not LXRβ). We studied the effect of the virtual screening hits on the expression of 10 LXR target genes along with LXRα and LXRβ genes in THP-1 differentiated macrophages using ArrayPlate mRNA assay technology[17] (Table 2; see Supporting Information for detail). The expression level of each gene was normalized using three housekeeping genes (B2M, TBP, and ACTB). ANT is used as a negative control. A total of 69 compounds were found to up-regulate ABCA1 gene greater than 2-fold. A compound that induced the gene expression of LXRα as well as ABCA1 was defined as a hit. The overall hit rate ranged from 3.2% to 5.9%

Table 2. Fold Induction of Genes by LXR Modulators in THP-1 Differentiated Cells[a]

genes	1	2	3	4
NR1H2 (LXRβ)	1.09	1.22	1.17	1.16
LXRα	3.41	4.70	4.56	3.93
ABCA1	5.59	10.04	5.90	4.53
ABCG1	9.91	24.92	15.05	13.56
APOE	2.61	4.13	4.20	3.34
SREBP1c	2.05	5.28	4.09	4.05
FASN	0.89	3.77	2.59	1.75
SCD-1	1.17	12.82	8.16	4.76
HDS11B1	0.79	0.80	1.26	1.43
IL-6	0.82	1.02	1.78	0.91
Cox-2	1.35	1.20	0.93	1.20
ACTB	0.90	0.89	0.93	0.93
B2M	1.25	1.18	1.17	1.05
TBP	0.99	1.25	1.05	1.19
ANT	-	-	-	-

[a] LXR regulated genes along with housekeeper genes are measured simultaneously by ArrayPlate mRNA assay technology. Compounds were tested at 10 μM; Glut4 were not included due to the low detection limit. ANT serves as a negative control in the assay. Compounds are tested at 10 μM and the CV% is generally less than 20%.

with the halogen hypothesis being the lowest rate and the average hit rate for 1308 compounds was 5.3%. We paid particular attention to lipogenesis related genes, such as SREBP1c, FAS, and SCD-1, in the evaluation of compound gene profiles. Among the hits identified, compound **4**[18] (Glide score: –12.1) is noteworthy (Figure 1). Compound **4** is closely related to phenazocine,[19] an anesthetic agent once approved in UK as a morphine replacement and later withdrawn from the market. It is reported as an opioid receptor modulator.[18] The gene profile of compound **4**, however, is extremely similar to those of known LXR agonists like **1**, **2**, or **3** (Table 2), indicating it is likely a true LXR agonist.

The potential binding mode of compound **4** in modified LXR LBD (1p8d++) is illustrated in Figure 2C. The compound only occupies the C1 pocket. The hydroxyl group appears to interact with His453 and brings it close to Trp457 to form a typical agonistic conformation.[11a] The phenethyl group on the nitrogen atom is also important for LXR agonistic activity, because the corresponding *N*-methyl or *N*-cyclopropylmethyl compounds are not LXR agonists (data not shown). The phenyl group in compound **4** was found to interact with Phe329 to form a $\pi-\pi$ stacking interaction. Phe329 is also found to sit in close proximity to a benzene ring in **2**. It is interesting to note that the nitrogen atom in compound **4** aligns perfectly with the nitrogen atom in **2** (Figure 2D), although no direct interaction of the basic nitrogen with LXR could be identified in the crystal structure or in this docking study.

Compound **4** was subsequently confirmed to be a LXR agonist by a cofactor recruitment assay[20] and relevant cellular functional assays.[21,22] As shown in Figure 3, compound **4**, like **2** or **3** (not shown), is fully capable of recruiting SRC1 cofactor peptide to either LXRα or LXRβ (Figure 3A,B), indicating it is a full agonist of LXRα and LXRβ. Consistent with the gene profiling data, compound **4** induces cholesterol efflux in differentiated THP-1 macrophages and causes lipogenesis in HepG2 cells in a dose-dependent manner (Figure 3C,D).

In conclusion, we described a rapid and successful approach for identification of LXR modulators using a combination of structure-based virtual screen and high-throughput gene profiling. Using a modified LXR LBD structure, about 1% of internal compound collection was identified as virtual LXR hits, which showed good overall docking, score. Subsequent high throughput gene profiling with ArrayPlate mRNA assay identified 69 compounds, which displayed gene regulation profile of a typical

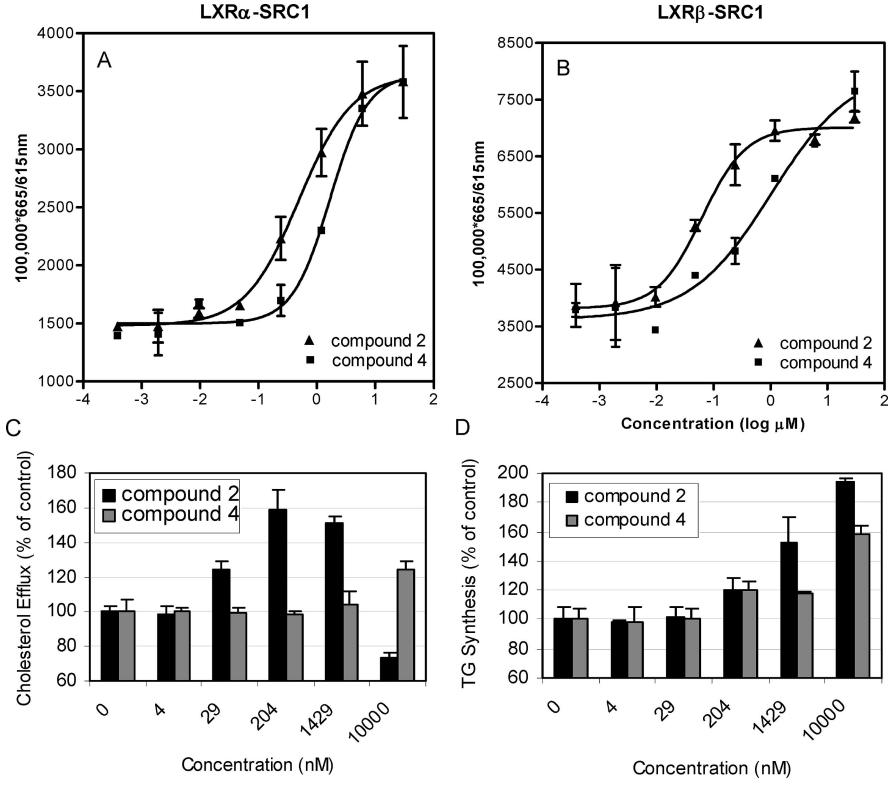

Figure 3. (A,B) The dose-dependent ligand-induced binding of SRC-1 peptide to LBDs of LXRs. Results shown are mean values ± standard deviations of duplicate samples in a single experiment. (C) Cholesterol efflux in THP-1 differentiated macrophages. Results shown are mean values ± standard deviations of triplicate samples in a single experiment. (D) Lipogenesis in HepG2 cells. Results shown are mean values ± standard deviations of triplicate samples in a single experiment.

LXR agonist. Compound **4**, a phenazocine analog developed as morphine replacement, displays a high docking score in LXR LBD virtual screen and upregulates LXR-regulated genes in high throughput gene profiling assays. Compound **4** was confirmed to be a true LXR agonist by an in vitro cofactor recruitment assay and LXR-relevant cellular functional assays. Identification of compound **4** as a potent LXR agonist demonstrated the power of virtual screening and high throughput gene profiling for identification of novel chemotype LXR modulators with appropriate selectivity profiles.

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Supporting Information Available: Detailed experimental procedures, analytical data, and results of the biological tests/assays are available. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) For recent reviews, see Bradley, M. N.; Tontonoz, P. LXR: A nuclear receptor target for cardiovascular disease. *Drug Discovery Today* **2005**, 97–103. (b) Collins, J. L. Therapeutic opportunities for liver X receptor modulators. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 692–702. (c) Jaye, M. LXR agonists for the treatment of atherosclerosis. *Curr. Opin. Invest. Drugs* **2003**, *4*, 1053–1058. (d) Lund, E. G.; Menke, J. G.; Sparrow, C. P. Liver X receptor agonists as potential therapeutic agents for dyslipidemia and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *237*, 1169–1177.

(2) (a) Lehmann, J. M.; Kliewer, S. A.; Moore, L. B.; Smith-Oliver, T. A.; Blanchard, D. E.; Spencer, T. A.; Willson, T. M. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J. Biol. Chem.* **1997**, *272*, 3137–3140. (b) Spencer, T. A.; Gayen, A. K.; Phirwa, S.; Nelson, J. A.; Taylor, F. R.; Kandutsch, A. A.; Erickson, S. K. 24(*S*), 25-Epoxycholesterol. *J. Biol. Chem.* **1985**, *260*, 13391–13394.

(3) Mitro, N.; Mak, P. A.; Vargas, L.; Godio, C.; Hampton, E.; Molteni, V.; Kreusch, A.; Saez, E. The nuclear receptor LXR is a glucose sensor. *Nature* **2007**, *445*, 219.

(4) Collins, J. L.; Fivush, A. M.; Watson, M. A.; Galardi, C. M.; Lewis, M. C.; Moore, L. B.; Parks, D. J.; Wilson, J. G.; Tippin, T. K.; Binz, J. G.; Plunket, K. D.; Morgan, D. G.; Beaudet, E. J.; Whitney, K. D.; Kliewer, S. A.; Willson, T. M. Identification of a nonsteroidal liver X receptor agonist through parallel array synthesis of tertiary amines. *J. Med. Chem.* **2002**, *45* (10);), 1963–1966.

(5) (a) Schultz, J. R.; Tu, H.; Luk, A.; Repa, J. J.; Medina, J. C.; Li, L.; Schwendner, S.; Wang, S.; Thoolen, M.; Mangelsdorf, D. J.; Lustig, K. D.; Shan, B. Role of LXRs in control of lipogenesis. *Gene Dev.* **2000**, *14*, 2831–2838. (b) Li, L.; Liu, J.; Zhu, L.; Cutler, S.; Hasegawa, H.; Shan, B.; Julio, C.; Medina, J. C. Discovery and optimization of a novel series of liver X receptor-α agonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1638–1642.

(6) Joseph, S. B.; McKilligin, E.; Pei, L.; Watson, M. A.; Collins, A. R.; Laffitte, B. A.; Chen, M.; Noh, G.; Goodman, J.; Hagger, G. N.; Tran, J.; Tippin, T. K.; Wang, X.; Lusis, A. J.; Hsueh, W. A.; Law, R. E.; Collins, J. L.; Willson, T. M.; Tontonoz, P. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (11);), 7604–7609.

(7) Terasaka, N.; Hiroshima, A.; Koieyama, T.; Ubukata, N.; Morikawa, Y.; Nakai, D.; Inaba, T. T-0901317, a synthetic liver X receptor ligand, inhibits development of atherosclerosis in LDL receptor-deficient mice. *FEBS Lett.* **2003**, *536*, 6–11.

(8) Brunham, L. R.; Kruit, J. K.; Pape, T. D.; Timmins, J. M.; Rwuwer, A. Q.; Vasanji, Z.; Marsh, B. J.; Rodrigues, B.; Johnson, J. D.; Parks, J. S.; Verchers, C. B.; Hayden, M. R. β-Cell ABCA1 influences insulin secretion, glucose homeostasis and response to thiazolidinedione treatment. *Nat. Med.* **2007**, *13*, 340–347.

(9) (a) Jaye, M. C.; Krawiec, J. A.; Campobasso, N.; Smallwood, A.; Qiu, C.; Lu, Q.; Kerrigan, J. J.; De Los Frailes Alvaro, M.; Laffitte, B.; Liu, W.-S.; Marino, J. P., Jr.; Meyer, C. R.; Nichols, J. A.; Parks, D. J.; Perez, P.; Sarov-Blat, L.; Seepersaud, S. D.; Steplewski, K. M.; Thompson, S. K.; Wang, P.; Watson, M. A.; Webb, C. L.; Haigh, D.; Caravella, J. A.; Macphee, C. H.; Willson, T. M.; Collins, J. L. Discovery of substituted maleimides as liver X receptor agonists and determination of a ligand-bound crystal structure. *J. Med. Chem.* **2005**, *48* (17), 5419–5422. (b) Hu, B.; Collini, M.; Unwalla, R.; Miller, C.; Singhaus, R.; Quinet, E.; Savio, D.; Halpern, A.; Basso, M.; Keith, J.; Clerin, V.; Chen, L.; Resmini, C.; Liu, Q.-Y.; Feingold, I.; Huselton, C.; Azam, F.; Farnegardh, M.; Enroth, C.; Bonn, T.; Goos-Nilsson, A.; Wilhelmsson, A.; Nambi, P.; Wrobel, J. Discovery of phenyl acetic acid substituted quinolines as novel liver X receptor agonists for the treatment of atherosclerosis. *J. Med. Chem.* **2006**, *49*, 6151–6154.

(10) Lund, E. G.; Menke, J. G.; Sparrow, C. P. Liver X receptor agonists as potential therapeutic agents for dyslipidemia and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23* (7), 1169–1177.

(11) (a) Williams, S.; Bledsoe, R. K.; Collins, J. L.; Boggs, S.; Lambert, M. H.; Miller, A. B.; Moore, J.; McKee, D. D.; Moore, L.; Nichols, J.; Parks, D.; Watson, M.; Wisely, B.; Willson, T. M. X-ray crystal structure of the liver X receptor b ligand binding domain: Regulation by a histidine-tryptophan switch. *J. Biol. Chem.* **2003**, *278*, 27138–27143. (b) Hoerer, S.; Schmid, A.; Heckel, A.; Budzinski, R.-M.; Nar, H. Crystal structure of the human liver X receptor b ligand-binding domain in complex with a synthetic agonist. *J. Mol. Biol.* **2003**, *334*, 853–861. (c) Farnegårdh, M.; Bonn, T.; Sun, S.; Ljunggren, J.; Ahola, H.; Wilhelmsson, A.; Gustafsson, J. A.; Carlquist, M. The three-dimensional structure of the liver X receptor β reveals a flexible ligand-binding pocket that can accommodate fundamentally different ligands. *J. Biol. Chem.* **2003**, *278*, 38821–38828. (d) Svensson, S.; Oestberg, T.; Jacobson, M.; Norstroem, C.; Stefansson, K.; Hallen, D.; Johansson, I. C.; Zachrisson, K.; Ogg, D.; Jendeberg, L. Crystal structure of the heterodimeric complex of LXR and RXR ligand-binding domains in a fully agonistic conformation. *EMBO J.* **2003**, *22*, 4625–4633.

(12) Bennett, D. J.; Cooke, A. J.; Edwards, A. S.; Moir, E.; Ray, P. C. An update on non-steroidal liver X receptor agonists and their potential use in the treatment of atherosclerosis. *Expert Opin. Ther. Pat.* **2004**, *14*, 1673–1699.

(13) (a) CORINA version 3.0: Sadowski, J.; Gasteiger, J.; Klebe, G. Comparison of automatic three-dimensional model builders using 639 X-ray structures. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 1000–1008. (b) 3D Structure Generator CORINA is available from Molecular Networks, http://www.molecular-networks.com.

(14) *GLIDE, version 3.0*; Schrödinger, LLC: New York, NY, 2004.

(15) (a) Gehlhaar, D. K.; Verkhivker, G. M.; Rejto, P. A.; Sherman, C. J.; Fogel, D. B.; Fogel, L. J.; Freer, S. T. Molecular recognition of the inhibitor AG-1343 by HIV-1 protease: conformationally flexible docking by evolutionary programming. *Chem. Biol.* **1995**, *2*, 317–24. (b) Gehlhaar, D. K.; Bouzida, D.; Rejto, P. A. Parrill, L.; Rami Reddy, M. *Rational Drug Design: novel methodology and practical applications*; ACS symposium series, 719; American Chemical Society: Washington, DC, 1999; Vol. 29, pp 2–311.

(16) Auffinger, P.; Hays, F. A.; Westhof, E.; Ho, P. S. Halogen bonds in biological molecules. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16789–16794.

(17) High-throughput genomics screening was performed using the ArrayPlate mRNA assay technology provided by HTG, Inc. (http://www.htgenomics.com/). The ArrayPlate mRAN assay is a quantitative nuclease protection assay for multiplex gene profiling, see: Martel, R. R.; Botros, I. W.; Rounseville, M. P.; Hinton, J. P.; Staples, R. R.; Morales, D. A.; Farmer, J. B.; Seligmann, B. E. Multiplexed screening assay for mRNA combining nuclease protection with luminescent array detection assay *Drug Dev. Technol.* **2002**, *1* (1), 61–71.

(18) (a) Takeda, M.; Chikada, M.; Saito, S.; Kugita, H. 3-Alkyl-7,12-dimethyl-9-hydroxy-2,7-methano-1,2,4,5,6,7-hexahydro-3*H*-3-benzazonines(homobenzomorphan derivatives). Jpn. Tokkyo Koho, JP48035080, 1973. (b) Takeda, M.; Chikada, M.; Saito, S.; Kugita, H. 3-Alkyl-7,12-dimethyl-9-hydroxy-2,7-methano-1,2,4,5,6,7-hexahydro-3*H*-3-benzazonines. Jpn. Tokkyo Koho, JP48035079, 1973.

(19) (a) Jordan, L. C.; Williams, J. E.; Chandler, A. K. An open pilot study investigating the use of a strong sublingual opioid (phenazocine) for postoperative analgesia. *Acute Pain* **2000**, *3*, 15–19. (b) Tabatabai, S. A.; Zarrindast, M. R.; Lashkari, S. B.; Shafiee, A. Synthesis, conformational analysis and antinociceptive activity of 1-[*N*-methyl-(2-phenylethyl)amino]methyl-1,2,3,4-tetrahydroisoquinoline derivatives. *Arzneim.-Forsch.* **1999**, *49*, 1001–1005.

(20) The HTRF cofactor peptide recruitment assay was modified from a previous report. Briefly, polyhistidine-tagged human LXRα (2 nM) or LXRβ (1 nM) ligand-binding domain (Roche Diagnostics, Indianapolis, IN) was mixed with the test compound, 20 nM biotin-SRC1 peptide (Synpep, Dublin, CA), 5 nM streptavidin-allophycocyanin, and europium-labeled antipolyhistidine antibody (1 nM and 0.5 nM for α and β, respectively) in 50 mM Tris, pH 7.5, 50 mM KCl, 1 mM EDTA, 0.1% BSA, and 1 mM DTT. The final volume of the mixture was 40 μL in a 384-well assay plate. The mixture was incubated at room temperature for 1 h with shaking. Time-resolved fluorescence was measured at 615 and 665 nm on an LJL Analyst plate reader. The ratio of 665/615 was used to calculate EC_{50} values of test compounds.

(21) Compound induction of cholesterol efflux was measured as described previously with small modifications. Briefly, THP-1 cells were differentiated into macrophages in 48-well tissue culture plates by 30 h

treatment with 200 nM PMA. The cells were then labeled with 0.6 μCi of 1, 2-^{3}H (N)-cholesterol for 18 h in the presence of 16 μg/mL acLDL and 200 nM PMA. Cells were then incubated with vehicle or compound for 6 h in serum-free media containing 2 mg/mL BSA. Subsequently, 5 μg/mL of APO-AI was added in fresh, serum-free medium along with vehicle or compound for additional 18 h incubation. Cholesterol efflux was monitored by quantifying the radioactivity in the cell supernatant and the data are presented as fold-induction versus control (vehicle only). Results shown are mean values of triplicate samples in a single experiment.

(22) To measure compound induction of lipogenesis, HepG2 cells in a 48-well tissue culture plate were pre-treated with vehicle or compound for 24 h. A total of 1 μCi of [^{14}C]-glycerol was added and the cells were cultured for another 48 h. Cellular triglycerides were extracted, separated by TLC and quantified on a Storm 820 phosphorimager (GE Healthcare, Giles, U.K.). Lipogenesis was presented as fold-induction versus control (vehicle only). Results shown are mean values of triplicate samples in a single experiment.

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