Bioorganic & Medicinal Chemistry Letters 19 (2009) 2009–2012

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Liver X receptor agonists with selectivity for LXRβ; *N*-aryl-3,3,3-trifluoro-2-hydroxy-2-methylpropionamides

Britt-Marie Swahn [a,*], Istvan Macsari [a], Jenny Viklund [a], Liselotte Öhberg [a], Johanna Sjödin [b], Jan Neelissen [c], Johanna Lindquist [b]

[a] Department of Medicinal Chemistry, AstraZeneca R&D Södertälje, S-151 85 Södertälje, Sweden
[b] Department of Molecular Pharmacology, AstraZeneca R&D Södertälje, S-151 85 Södertälje, Sweden
[c] Department of DMPK, AstraZeneca R&D Södertälje, S-151 85 Södertälje, Sweden

ARTICLE INFO

Article history:
Received 21 November 2008
Revised 9 February 2009
Accepted 9 February 2009
Available online 13 February 2009

Keywords:
Liver X receptor
LXRβ agonist
ABCA1
ABCG1
Up regulation in vivo
Brain

ABSTRACT

The synthesis and SAR of a new series of LXR agonist is reported. The *N*-Aryl-3,3,3-trifluoro-2-hydroxy-2-methyl-propionamide hits were found in a limited screen of the AstraZeneca compound collection. The effort to optimize these hits into LXRβ selectivity is described. Compound **20** displayed desirable pharmacokinetic profile and up regulation of ABCA1 and ABCG1 mRNA in the brain were achieved when evaluated in vivo in mice.

The liver X receptors, LXRα and LXRβ are members of the nuclear hormone receptor super family and are involved in the regulation of cholesterol and lipid metabolism.[1] Disordered cholesterol balance in the brain is a hallmark of several neurological disorders including Alzheimer's disease.

The LXRs induce the expression of several genes involved in reverse cholesterol transport and lipid metabolism including ATP binding cassette transporter ABCA1, ABCG1 and apolipoprotein E (ApoE).[2] The potential to prevent or reverse the atherosclerotic process by increasing the expression of these genes makes LXR an attractive target for treatment of atherosclerosis, dyslipidemia as well as for Alzheimer's disease.[2,3]

LXRα is expressed at high level in liver, adipose tissue and macrophages, whereas LXRβ is expressed ubiquitously and in the brain. Several LXR agonists have been reported the natural ligand 24(*S*)-25-epoxycholesterol **1** (Fig. 1) and synthetic agonists such as T0901317 **2** and GW3965 **3**.[4] These ligands increase the expression of ABCA1, ABCG1 and ApoE, but they also induce triglyceride synthesis in the liver via up regulation of sterol regulatory element binding protein 1c (SREBP-1c) and fatty acid synthase (FAS).[5] The liver contains predominantly LXRα and it is considered that LXRβ or tissue selective agonists would have less impact on TG synthesis. Even though several synthetic LXR agonists have been reported[1] only two publications of LXRβ selective compounds have been disclosed.[6]

A FRET-based co-activator (SRC-1) recruitment assay measuring agonist activity[7] was used in a limited screen of the AstraZeneca compound collection. *N*-Aryl-3,3,3-trifluoro-2-hydroxy-2-methylpropionamides, a series of compounds **4**, **5** and **6** (Fig. 2) previously investigated at AstraZeneca for inhibition of pyruvate dehydrogenase kinase[8] and as K_{ATP} channel openers, were found to be active as LXRβ agonists. These known secondary interactions can be diminished since the LXRβ activity was preferably retained in the *S*-enantiomers, which have poor PDHK[9] activity, and in the 2-chloroaryl amides, which are devoid of affinity for K_{ATP} channels.[10]

Compounds **4**, **5** and **6** were also profiled for LXRα agonist activity in the FRET-based co-activator recruitment assay,[7] where a minor beta selectivity could be seen, as shown in Table 1. These compounds also displayed selectivity in the cell-based reporter gene assay, GAL-hLXRβ/hLXRα LBD construct in SHSY5Y cells,[11] shown in Table 1. More interestingly compound **5** was inactive in the reporter gene assay in U2OS cells indicating that different activity can be achieved in different cell types thus enabling the possibility to find tissue selective agonists.

The ligand binding domains of LXRβ/α share a high sequence identity (78%) and residue differences are located far away from

* Corresponding author. Tel.: +46 8 553 264 74.
E-mail address: britt-marie.swahn@astrazeneca.com (B.-M. Swahn).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.02.039

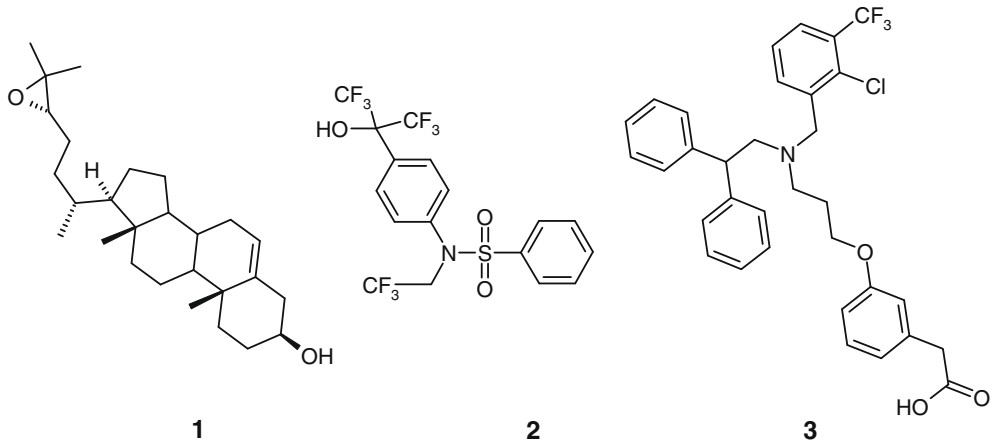


Figure 1. Reported LXR agonists.

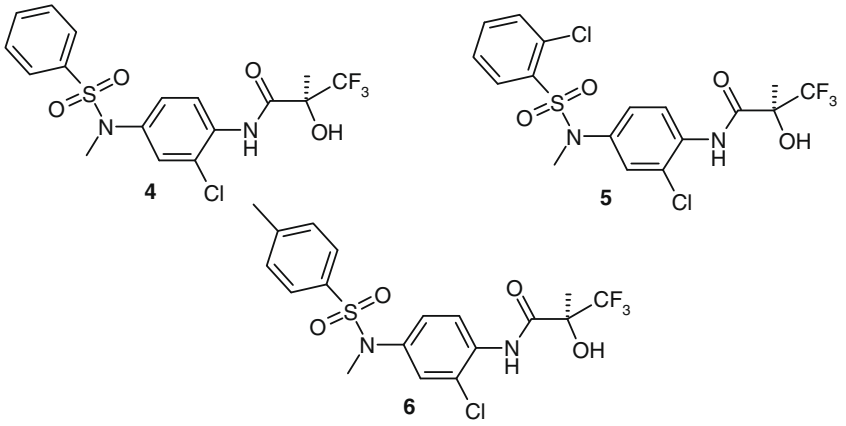


Figure 2. Hits from limited screen of AstraZeneca compound collection.

Table 1
FRET and reporter EC_{50} (μM)[a] for compounds **2**–**6**

Compound	FRET LXRβ	LXRα	Reporter LXRβ	LXRα
1	0.286	0.280	0.470	n.d.
2	0.008	0.010	0.075	0.085
3	0.078	0.280	0.130	0.310
4	1.90	3.30	0.959	5.00
5	0.626	n.d.	0.813	1.82
6	1.50	1.97	1.59	5.00

[a] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10\%$.

the ligand binding pocket.[12] Even though all residues in close contact with known ligands (i.e., 5 Å) are identical secondary interactions and induced fit binding can constitute an opportunity for increased selectivity. It has been shown in X-ray crystal structures of compound **1**, **2** and **3** that the ligand binding domain of LXRβ is highly flexible and the side chains of compound **2** and **3** are binding into different pockets not utilized by the natural ligand **1**.[13] Compound **5** was docked[14] into the active site of the ligand-binding domain of LXRβ as shown in Figure 3. Comparison with T0901317 **2** reveals that the trifluoro-2-hydroxy-2-methyl-propionamide motif can bind in a similar fashion as for the di-trifluoromethyl-hydroxy group in compound **2**. We decided to explore if the LXRβ activity and selectivity of compound **5** could be increased by the introduction of larger substituents on the sulfonamide nitrogen. We anticipated that these substituents could bind into either the C2 cavity occupied by the trifluoroethyl group of compound **2** or the C4 cavity occupied by the di-phenyl group of **3** as described by Färnegårdh et al.[13]

The new analogues were synthesized as described in Scheme 1. Commercially available acid **8** was treated with thionylchloride and the resulting acid chloride was added to the amine **7** to yield amide **9**.[8,9] The nitro group of **9** was reduced to the amine **10** and subsequently reacted with sulfonyl chloride **11** to give sulfonamide **12**. Sulfonamide **12** was alkylated with alkyl halides to yield **15–17**.

Derivative **14** was synthesized via reductive amination of **10** with acetaldehyde to yield the ethylaniline **13**, which was subsequently reacted with sulfonyl chloride **11** to yield **14**.

As can be seen in Table 2 larger substituents such as benzyl in **15** increased LXRβ selectivity as measured in the FRET assay. Unfortunately this was not picked up in the reporter gene assay where the compound was inactive. Only the ethyl derivative **14** displayed activity in the SHSY5Y cell assay.

The importance of the trifluoromethyl-methyl-hydroxy group was also investigated. When this moiety was changed into a dimethyl-hydroxy or a di-trifluoromethyl-hydroxy group the agonist activity was lost. Thus it can be concluded that the pK_a of the 3,3,3-trifluoro-2-hydroxy-2-methyl-propionamide group is crucial for agonist activity.

Different substituents on the aryl-sulfonamide part of the molecule were also explored. It was anticipated that by expanding the compounds with para substituents it could be possible to extend the compounds into the direction of Arg319, a region of the LXRβ

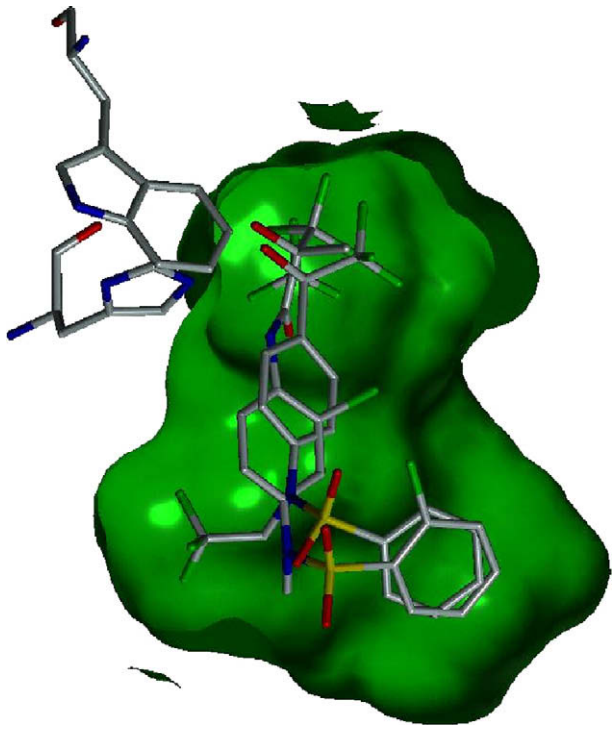


Figure 3. An overlay of compounds 2 and 5 docked into the active site of ligand-binding domain of LXRβ based on X-ray co crystal structure of T0901317.[15]

ligand binding domain reached by compound **3** (not shown in Fig. 3).[13] These compounds were synthesized as described in Scheme 2. The aniline **10** was reacted with sulfonyl chlorides **18** to yield **19**. Alkylation with methyl iodide produced derivative **20**. The methyl esters **21** and **23** were hydrolyzed to the corresponding acids **22** and **24** with LiOH.

The compounds were evaluated for LXRβ agonist activity, Table 3, and the most potent compounds were the shorter derivatives **20** and **21**. The acids **22** and **24** were inactive and this probably

Table 2
FRET and reporter EC_{50} (μM)[a] for compounds **14–17**

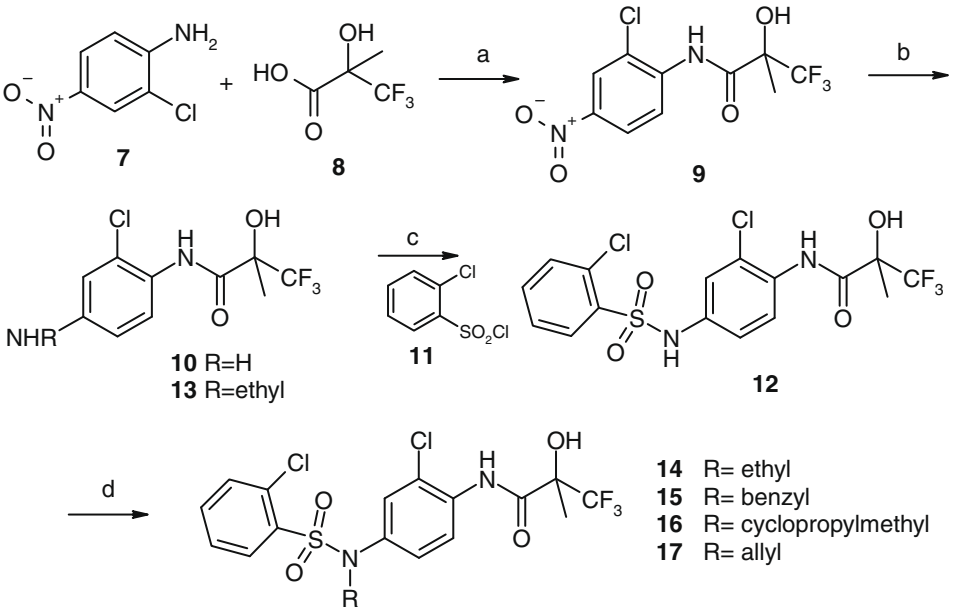
Compound	FRET LXRβ	LXRα	Reporter LXRβ	LXRα
14	0.79	1.00	7.5	8.5
15	0.88	>100	>30	>30
16	0.94	1.08	>30	>30
17	0.58	0.47	>30	>30

[a] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10\%$.

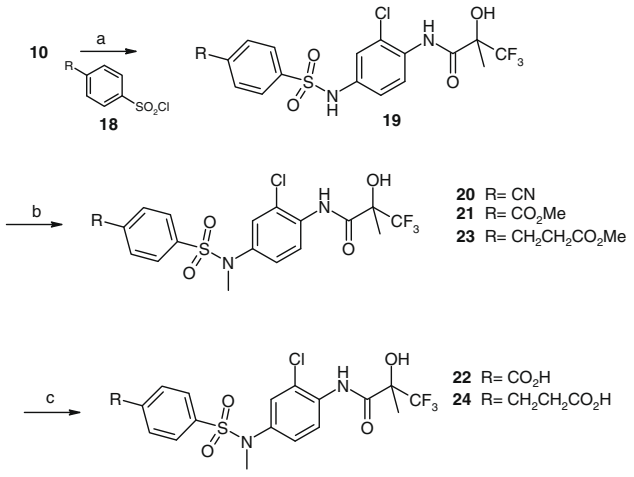
reflects the fact that the bioactive conformation of sulfonamide **5** is the one predicted in Figure 3. The carboxylic acid group in compound **24** can thus not reach and bind to Arg319. In this small set of compounds the potency was not increased compared to **5** but the selectivity for LXRβ in the reporter gene assay in SHSY5Y cells was still ~2 for compound **20**. Interestingly compound **20** was also inactive for both LXRα and β in the reporter gene assay conducted in U2OS cells.

Compound **20** displayed a reasonable solubility of 61 μM[16] and good permeability in both Caco-2, 28×10^{-6} cm/s, and a blood-brain barrier model, 33×10^{-3} cm/min. Therefore it was further evaluated in vivo in rat. The clearance was 24.6 mL/min/kg, volume of distribution 4.1 L/kg, half-life 4.4 h (po), and the bioavailability 39%.

Since the compound **20** had a moderate effect in SHSY5Y cells, representative for neurons in the brain, but not in U20S cells we decided to evaluate if it was possible to get up regulation of ABCA1 and ABCG1 mRNA levels in the brain with this compound without increasing TG synthesis measured as up regulation of SREBP-1c mRNA in the liver. The compound was dosed once daily with 30 μmol/kg po to mice and after 3 days the up regulation of mRNA levels of ABCA1 and ABCG1 in brain homogenate was determined to 1.25- and 1.30-fold, respectively.[17] For compound **2** (10 μmol/kg po) the corresponding values for ABCA1 and ABCG1 mRNA up regulation were 1.5 and 1.0, respectively. The up regulation of SREBP-1c mRNA in the liver for compound **20** (30 μmol/kg po) was only 1.3 compared to 3.6 for the low dose of compound **2** (1.0 μmol/kg po).



Scheme 1. Reagents and conditions: (a) $SOCl_2$, toluene, reflux 16 h; (b) 5% Pt/C, TEA, 50 °C, HCOOH, 1 h , 48% a + b; (c) pyridine/CH_2Cl_2, rt, 89%; (d) RX, K_2CO_3, acetone, rt, 78% for 15.



Scheme 2. Reagents and conditions: (a) pyridine/CH_2Cl_2, rt, 45% for 20; (b) MeI, K_2CO_3, acetone, rt, 82% for 20; (c) LiOH, H_20/THF.

Table 3
FRET and reporter EC_{50} (μM)[a] for compounds **20–24**

Compound	FRET LXRβ	LXRα	Reporter LXRβ	LXRα
20	2.60	3.40	2.70	5.00
21	2.32	3.10	3.81	3.61
22	>100	>100	>30	>30
23	3.50	5.00	>30	>30
24	>100	>100	>30	>30

[a] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10\%$.

In summary we found some compounds with selectivity for LXRβ, but more interestingly we discovered a variation of activity in different cell lines, reflecting that secondary effects such as co-activator recruitment might vary between cell-lines. An attempt to optimize the activity of compound **5** was described and one compound with good DMPK profile was evaluated for ABCA1 and ABCG1 mRNA up regulation in vivo.

Acknowledgements

The authors would like to thank Dr. Eva-Lotte Lindstedt, Department of Medicinal Chemistry AstraZeneca R&D Mölndal, for valuable assistance in facilitating testing in U2OS cells and 3 day in vivo PD studies.

References and notes

1. For recent reviews, see: (a) Bennett, D. J.; Carswell, E. L.; Cooke, A. J.; Edwards, A. S.; Nimz, O. *Curr. Med. Chem.* **2008**, *15*, 195; (b) Goodwin, B. J.; Zuercher, W. J.; Collins, J. L. *Curr. Top. Med. Chem.* **2008**, *8*, 781; (c) Meinke, P. T.; Wood, H. B.; Szewczyk, J. W. *Annu. Rep. Med. Chem.* **2006**, *41*, 99; (d) Bennett, D. J.; Brown, L. D.; Cooke, A. J.; Edwards, A. S. *Expert Opin. Ther. Pat.* **2006**, *16*, 1673; (e) Collins, J. L. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 692.
2. Tonoz, P.; Mangelsdorf, D. J. *Mol. Endocrinol.* **2003**, *17*, 985.
3. Wang, L.; Schuster, G. U.; Hultenby, K.; Zhang, Q.; Andersson, S.; Gustafsson, J. A. *PNAS* **2002**, *99*, 13878.
4. (a) Lehmann, J. M.; Kliewer, S. A.; Moore, L. B.; Smith-Oliver, T. A.; Blanchard, D. E.; Spencer, T. A.; Wilson, T. M. *J. Biol. Chem.* **1997**, *272*, 3137; (b) 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. *Gene. Dev.* **2000**, *14*, 2831; (c) 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.; Beaudet, E. J.; Whitney, K. D.; Kliewer, S. A.; Wilson, T. M. *J. Med. Chem.* **2002**, *45*, 1963.
5. Chisholm, JW.; Hong, J.; Mills, S. A.; Lawn, R. M. *J. Lipid Res.* **2003**, *44*, 2039.
6. (a) Molteni, V.; Li, X.; Nabakka, J.; Liang, F.; Wityak, J.; Koder, A.; Vargas, L.; Romeo, R.; Mitro, N.; Mak, P. A.; Seidel, M.; Haslam, J. A.; Chow, D.; Tuntland, T.; Spalding, T. A.; Brock, A.; Bradley, M.; Castrillo, A.; Tontonoz, P.; Saez, E. *J. Med. Chem.* **2007**, *50*, 4255; (b) Hu, B.; Quinet, E.; Unwalla, R.; Collini, M.; Jetter, J.; Dooley, R.; Andraka, D.; Nogle, L.; Savio, D.; Halpern, A.; Goos-Nilsson, A.; Wilhelmsson, A.; Nambi, P.; Wrobel, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 54.
7. *LXR co-activator recruitment assay*: The Ligand binding domain (LBD) of LXRα (amino acid 205–447) and LXRβ (amino acid 216–461) was produced in *Escherichia coli*. A fragment of the human Steroid Receptor Co-Activator-1 (SRC-1) was produced as a synthetic peptide. An a-6xHis antibody coupled to Eu3+ was used to detect the His-tag on LXR-LBD and allophycocyanin (APC) coupled to streptavidin was used to detect biotinylated SRC-1. Agonist binding to LXR enhances affinity for SRC-1 and thereby brings Eu3+ in close proximity. Eu3+ is exited at 337 nm and emits light at 620 nm; this emission in turn exits APC to emit light at 655 nm. Time-resolved fluorescence readings were done in a Wallac Victor2 reader. An assay mix with 0.06 μg/ml Eu-labeled anti-6x His Ab, 1.15 μg/ml streptavidin APC, 90 nM SRC-1 peptide and 0.4 μg/ml LXR in buffer (20 mM Tris/0.125% CHAPS (pH 7.5), 2 mM DTT, 0.05% BSA (FA free)) was used. A control LXR agonist T0901317 at 1 μM was used as the 100% control to assess level of inhibition.
8. Butlin, R. J.; Nowak, T.; Burrows, J. N.; Block, M. H. PCT Int. Appl., WO 9962506 A1, 1999.
9. Bebernitz, G. R.; Aicher, T. D.; Stanton, J. L.; Gao, J.; Shetty, S. S.; Knorr, D. C.; Strohschein, R. J.; Tan, J.; Brand, L. J.; Liu, C.; Wang, W. H.; Vinluan, C. C.; Kaplan, E. L.; Dragland, C. J.; DelGrande, D.; Islam, A.; Lozito, R. J.; Liu, X.; Maniara, W. M.; Mann, W. R. *J. Med. Chem.* **2000**, *43*, 2248.
10. (a) Ohnmacht, C. J.; Russell, K.; Empfield, J. R.; Frank, C. A.; Gibson, K. H.; Mayhugh, D. R.; McLaren, F. M.; Shapiro, H. S.; Brown, F. J.; Trainor, D. A.; Ceccarelli, C.; Lin, M. M.; Masek, B. B.; Forst, J. M.; Harris, R. J.; Hulsizer, J. M.; Lewis, J. J.; Silverman, S. M.; Smith, R. W.; Warwick, P. J.; Kau, S. T.; Chun, A. L.; Grant, L. G.; Howe, B. B.; Li, J. H.; Trivedi, S.; Halterman, T. J.; Yochim, C.; Dyroff, M. C.; Kirkland, M.; Neilson, K. L. *J. Med. Chem.* **1996**, *39*, 4592; (b) Empfield, J. R.; Mayhugh, D.; Ohnmacht, C. J.; Frank, C. A.; Grant, T.; Li, J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 775.
11. *LXR-Gal4 reporter assay*: SH-SY5Y cells were cultured in Dulbecco's modified Eagle's medium/F12 (1:1) with glutamax, 1% NEAA, 1% PEST and 10% Fetal calf serum (FCS) at 5% CO2. At confluence, cells were transiently transfected with a pSGAL human LXRa (amino acid 205–447) or pSGAL human LXRb (amino acid 216–461) expression plasmid and a 5xUSA Luc reporter plasmid using Lipofectamin 2000 transfection. 10.5 ng pSGAL-hLXRa or b were mixed with 10.5 ng pGL3 5xUSA Luc reporter plasmid containing a minimal SV40 promotor and 5 copies of the USA GAL4 recognition site in transfection buffer (DMEM/F12 (1:1) with glutamine, 2% FCS, 1% NEAA, 1% PEST) containing approximately 6 million SH-SY5Y cells. Directly after transfection, LXR ligands or vehicle (DMSO, 0.1%) were added to the cells. After 24 h, cells were analysed for luciferase activities according to the manufacturers instructions. A control LXR agonist T0901317 at 1 μM was used as the 100% control to assess level of inhibition.
12. (a) Alberti, S.; Steffensen, K. R.; Gustafsson, J. A. *Gene* **2000**, *243*, 93; (b) 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, M. *J. Biol. Chem.* **2003**, *278*, 27138.
13. (a) Färnegårdh, M.; Bonn, T.; Sun, S.; Ljunggren, J.; Ahola, H.; Wilhelmsson, A.; Gustafsson, J. Å.; Carlquist, M. *J. Biol. Chem.* **2003**, *278*, 38821; (b) Hoerer, S.; Schmid, A.; Heckel, A.; Budzinski, R. M.; Nar, H. *J. Mol. Biol.* **2003**, *334*, 853.
14. Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. *J. Mol. Biol.* **1996**, *261*, 470.
15. Figure 3 was generated using SYBYL 6.9.2, Tripos, 1699 South Hanley Rd., St. Louis, Missouri, USA.
16. Solubility was measured as screen solubility: Incubation of 100 μM DMSO solution in 0.1 M phosphate buffer pH 7.4, shaking for 24 h at rt Solubility quantification on the bases of the UV-TIC.
17. The method for mRNA determination of ABCA1, ABCG1 and SREBP-1c will be published elsewhere (J. Lindquist et al).