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Discovery and optimization of a novel series of liver X receptor-α agonists

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Abstract—A novel series of hexafluorocarbinols were discovered as potent activators of the liver X receptor-α using a fluorescence polarization assay. Structure–activity relationship study led to the identification of compounds that are more potent agonists than the endogenous ligand, 24(*S*), 25-epoxycholesterol, with similar efficacy. Several compounds, including T0901317, were shown to have desirable pharmacokinetic profiles suitable for in vivo studies.

Liver X receptors (LXRα and LXRβ) belong to a superfamily of nuclear hormone receptors that function as transcription factors.[1] LXRα is expressed at high level in liver, adipose tissue, and macrophages, whereas LXRβ is expressed ubiquitously. The discovery of oxidized derivatives of cholesterol as the endogenous ligands and the subsequent gene targeting studies in mice provided strong evidence that LXRα plays an important role in cholesterol metabolism.[2a,b] LXRs form heterodimers with the retinoid X receptors (RXRs) and regulate the expression, directly or indirectly, of a number of genes involved in cholesterol and fatty acid metabolism, including cholesterol transporters and cholesterol metabolizing enzymes.[3,4] Potent modulators of LXRs, LXRα in particular, with adequate pharmacokinetic properties in animals are not only of particular value as research tools for the study of their pharmacological roles but also provide an opportunity for developing treatments for a number of pathophysiological states including dyslipidemia, atherosclerosis, and diabetes.[5]

In search of nonsteroidal LXR activators, we screened our compound collection using a fluorescence polarization (FP) assay. In this homogeneous biochemical assay, a fragment of the co-activator (SRC1) containing the ILRKLLQE LXRα binding motif was labeled with the fluorescent light emitting rhodamine moiety. The FP assay measures a compound's ability to enhance the binding of the co-activator to LXRα. A unique compound **1** (Fig. 1, *N*-methyl-*N*-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide) was found to be a potent and efficacious activator of LXRα. Using tritium-labeled compound **1** in a ligand competition assay, we also demonstrated that the cold compound and 24(*S*),25-epoxycholesterol competed effectively for the binding of radiolabeled ligand and suggested that endogenous ligands bind at the same site of the LXRα receptor.[6] We carried out an extensive structure–activity relationship (SAR) study to fully explore the potential of this lead series and to identify compounds with adequate pharmacokinetic properties for continued investigation in in vivo models. One of these compounds, **2** (T0901317), has been the prototypical LXRα agonist used extensively in a number of important studies by us and others.[6,7a–c] In this paper,

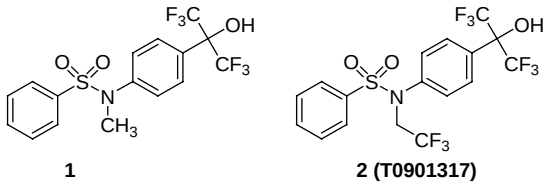

Figure 1. LXRα agonists.

Keywords: Hexafluorocarbinol; Liver X receptor-α; LXRα agonist; T0901317; Cholesterol transporter.
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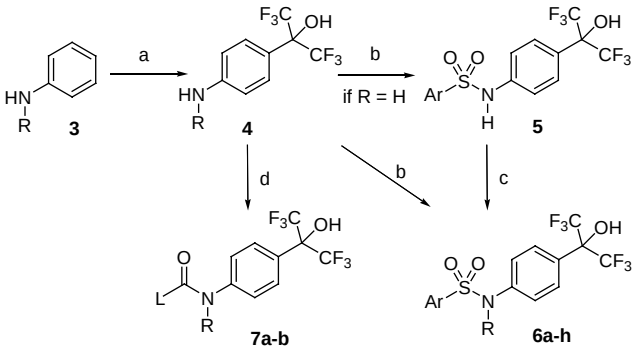
we provide a detailed account of the identification of T0901317 and other potent LXRα activators.[8]

The 1,1,1,3,3,3-hexafluoro-2-hydroxy-propan-2-yl group present in the structure was most conveniently introduced by a Friedel–Crafts type of reaction between an aniline and hexafluoroacetone hydrate in the presence of an acid, as shown in Scheme 1.[9] The R group on aniline **4** can be introduced prior to the hexafluoro-2-hydroxy-propan-2-yl moiety or it can be installed by alkylation of the sulfonamide intermediate **5**, which allows for easy structural diversification (to **6a**–**h**). The ring-fused analogs (**8a**–**d**) were synthesized following similar procedures by starting with indoline, 1,2,3,4-tetrahydroquinoline, 3,4-dihydro-2*H*-benzo[1,4]oxazine, and 2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepine, respectively. In addition to various sulfonamide analogs, several amides and carbamates (represented by **7a** and **7b**) were prepared.

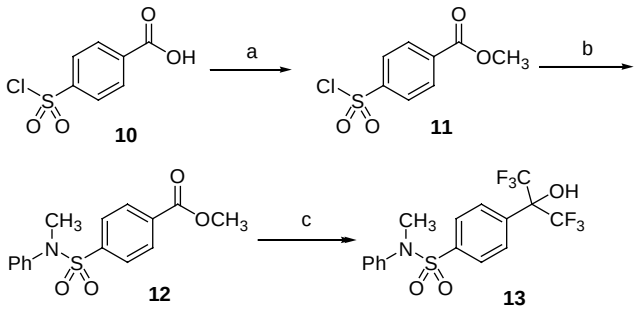
An alternative route was required in order to install the 1,1,1,3,3,3-hexafluoro-2-hydroxy-propan-2-yl group to an electronically deficient phenyl ring, such as the reverse sulfonamide compound represented by **13**. The presence of an electron-withdrawing group on the phenyl ring retards the regiospecific introduction of the hexafluorocarbinol unit. We developed a convenient method, in which two trifluoromethyl units from trifluoromethyltrimethylsilane (TMS-CF_3) were added to a methyl ester in the presence of tetra-*n*-butylammonium fluoride, to complete the transformation (Scheme 2).

To prepare compounds in which 1,1,1,3,3,3-hexafluoro-2-hydroxy-propan-2-yl and the sulfonamido substituent are in a 1,3-relationship on the central phenyl ring, we employed a nitration step (from **14** to **15**) to achieve the desired regiospecificity, taking advantage of the *meta*-directing ability of the hexafluorocarbinol group (Scheme 3). The nitro compound was reduced to aniline **16**, which, in turn, was converted to the *N*-methyl sulfonamide **18** using a sequence similar to that described in Scheme 1.

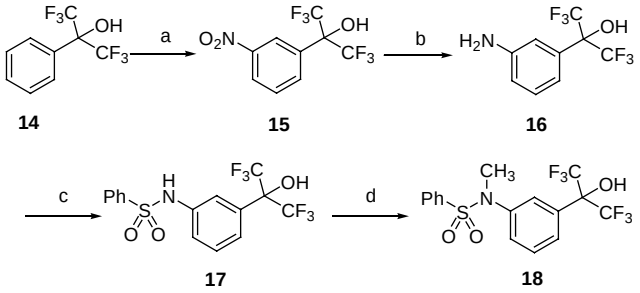
The lead optimization was primarily guided by a cell-based assay for assessing a compound's ability to activate the LXRα receptor. HEK293 cells were transiently



Scheme 1. Reagents and conditions: (a) hexafluoroacetone trihydrate, *p*-TsOH, 130 °C, 14 h, 80%; (b) $ArSO_2Cl$, 2,6-lutidine, acetone, 60 °C, 24 h, 90%; (c) R-I, NaH (1 equiv), DMF, rt, 2 h, 60%; (d) Bz-Cl, or *i*-BuOCOCl and Hunig's base, 80%.



Scheme 2. Reagents and conditions: (a) $TMSCHN_2$, MeOH, rt, 5 h, 88%; (b) *N*-methylaniline, 2,6-lutidine, acetone, 60 °C, 24 h, >80%; (c) CF_3-TMS, TBAF, THF, rt, 24 h, 50%.



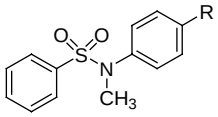
Scheme 3. Reagents and conditions: (a) HNO_3 (90%), H_2SO_4 (concd), 0 °C, 30 min, rt, 2 h, 90%; (b) H_2, Pd/C, EtOH, rt, 6 h, 100%; (c) $PhSO_2Cl$, 2,6-lutidine, acetone, 60 °C, 24 h, 80%; (d) CH_3I, NaH (1 equiv), DMF, rt, 2 h, 60%.

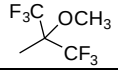
transfected with an expression plasmid for human LXRα and a luciferase reporter plasmid containing an LXR response element. The cells were treated with varying concentrations of test compounds. The EC_{50} values and maximal transactivation at LXRα are reported here. However, it is worth mentioning that these analogs, while they exhibit high specificity over all other nuclear receptors tested, are generally not LXR subtype specific.[6a]

The hexafluorocarbinol moiety was found to be important for LXRα activation, as data in Table 1 indicated. Replacing one of the trifluoromethyl groups with a methyl group gave a compound that was fully efficacious but with much reduced potency.[10] Blocking of the hydroxyl group also significantly lowered potency.[10] The presence of both trifuoromethyl groups is believed as not only important to maintain the proper orientation of the hydroxyl group but also necessary to ensure the strong acidity of the OH group. We postulated that the relatively high acidity (pK_a was determined to be 8.5) of the hydroxyl group might be important for interaction with basic residues on the LXRα receptor providing the anchoring interaction. This hypothesis was supported by a recent X-ray crystallographic study reported by Stefansson et al.[11]

Unlike the head hexafluorocarbinol group, the benzenesulfonamide region can tolerate a wide range of structural modifications. As shown in Table 2, a variety of substituted benzenesulfonamides were potent agonists

Table 1. LXRα agonist potency and intrinsic efficacy of compounds varying the carbinol function



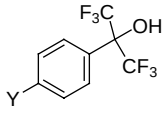
Compound	R	LXRα[12]	
		EC_{50} (μM)	Max activation
24(*S*),25-Epoxycholesterol		0.5	5.4
1		0.2	5.3
1a		>5	0
1b		5.0	5.1
1c		1	4.5

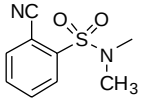
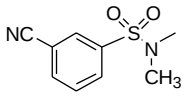
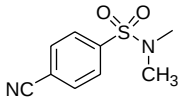
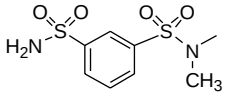
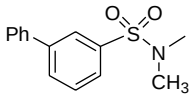
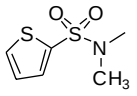
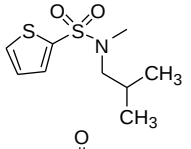
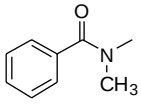
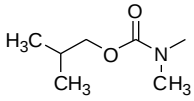
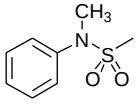
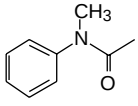
of LXRα with high efficacy. In general, substitutions at the *ortho*- and *para*-positions to the hexafluorocarbinol group tended to lower potency, while *meta*-substitution enhanced potency, as exemplified by entries **6a**, **6b**, and **6c**. Other *meta*-substituents were introduced in order to modulate the physicochemical properties of the molecules. A sulfonamide was not a prerequisite for activity, since benzamide (**7a**) and isobutoxycarbamate (**7b**) were shown to maintain LXRα activity. We also found that the LXRα agonist potency and efficacy were well retained when the sulfonamide and amide linkages were reversed, indicating that the ability to maintain the substituents in the correct special orientation is essential.

Table 3 summarized the results from a broad survey of substituents and substitution patterns around the central phenyl ring. A variety of *N*-alkyl substituents were well tolerated. Fused ring systems that resulted from connecting the *N*-alkyl group to the adjacent phenyl group, as in **8a** through **8d**, maintained LXRα activity. Presumably the ring structures, while restricting rotation freedom, were able to preserve the active conformation. However, the central phenyl ring is sensitive to structural changes. A substituent *ortho* to the hexafluorocarbinol head group was detrimental to activity, as seen in example **9b**. A 1,4-spacial arrangement was preferred for the hexaflurocarbinol and the alkylamino substituent (**1** vs **18**). Insertion of a methylene unit between the two groups caused a lowering in potency, as seen for **19**.

Selected potent and efficacious LXRα agonists were characterized for their pharmacokinetic properties in animals in order to identify compounds that are suitable for acute and chronic dosing for further pharmacological investigations in vivo. The compounds were dosed to male Sprague–Dawley rats ($n = 3$) by oral gavage

Table 2. Impact of *N*-substituents on LXRα agonist potency and intrinsic efficacy

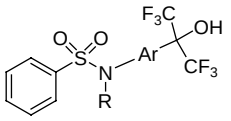


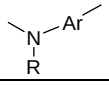
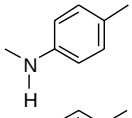
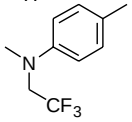
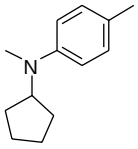
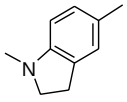
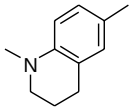
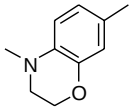
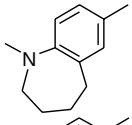
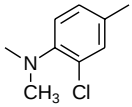
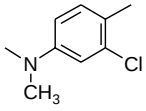
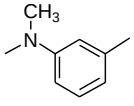
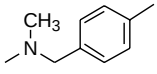
Compound	Y	LXRα[12]	
		EC_{50} (μM)	Max activation
6a		0.7	6.2
6b		0.02	6.2
6c		2	4.4
6d		0.08	4.9
6e		0.03	5.2
6f		0.6	6.3
6g		0.04	6.1
7a		0.1	3.9
7b		0.1	5.1
13		0.1	5.3
13a		0.2	4.0

at 5 mg/kg. The exposure results are summarized in Table 4.[13]

While the highest AUC value was observed for compound **6d**, the *N*-trifluoroethyl analog T0901317 appeared to be advantageous when considering the exposure and the potency. T0901317 produced excellent exposures following oral dosing at 5 mg/kg in mice (AUC = 3421 μg h/L and $t_{1/2} = 3$ h, $n = 3$) and in dogs (AUC = 7301 μg h/L and $t_{1/2} = 5.8$ h, $n = 2$).[13]

Table 3. Impact of central ring substituents on LXRα agonist potency and intrinsic efficacy



Compound		LXRα[12]	
		EC_{50} (μM)	Max activation
5a		4	4.3
T0901317		0.01	6.1
6h		0.008	4.8
8a		0.05	3.8
8b		0.03	3.9
8c		0.1	4.5
8d		0.01	4.2
9a		0.2	5.2
9b		>5	0
18		1	4
19		3	3.7

From our systematic structure–activity relationship investigation, we found that the hexafluorocarbinol moiety is essential for activation of the LXRα receptor. Maintaining the strong acidity and preserving the proper orientation of the hydroxyl group are key factors. A variety of sulfonamido, acetamido, and alkoxyacetamido substituents are allowed and are preferred to be in the 1,4-relationship to the head hexafluorocarbinol group on the central phenyl ring. Our SAR investigations have led to the identification of a number of highly potent and efficacious LXRα agonists. Several of them, including T0901317, were shown to have adequate pharmacokinetic profiles suitable for in vivo pharmacological investigations.

Table 4. Exposure after oral dosing at 5 mg/kg in rats

Compound	AUC (μg h/L)[a]	Average concentration (fold over EC_{50})
T0901317	5920	51
6g	3204	18
6d	14,876	16
6k	1899	4
11a	1037	1

[a] AUC, area under the curve.

Acknowledgments

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References and notes

1. Willy, P. J.; Umesono, K.; Ong, E. S.; Evans, R. M.; Heyman, R. A.; Mangelsdorf, D. J. *Gene Dev.* **1995**, *9*, 1033.
2. (a) Repa, J. J.; Mangelsdorf, D. J. *Nat. Med.* **2002**, *8*, 1243; (b) Janowski, B. A.; Willy, P. J.; Devi, T. R.; Falck, J. R.; Mangelsdorf, D. J. *Nature* **1996**, *383*, 728; (c) Lehmann, J. M.; Kliewer, S. A.; Moore, L. B.; Smith-Oliver, T. A.; Oliver, B. B.; Su, J. L.; Sundseth, S. S.; Blanchard, D. E.; Spencer, T. A.; Willson, T. M. *J. Biol. Chem.* **1997**, *272*, 3137.
3. Janowski, B. A.; Grogan, M. J.; Jones, S. A.; Wisely, G. B.; Liewer, S. A.; Corey, E. J.; Mangelsdorf, D. J. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 266.
4. Peet, D. J.; Turley, S. D.; Ma, W.; Janowski, B. A.; Lobaccaro, J.-M. A.; Hammer, R. E.; Mangelsdorf, D. J. *Cell* **1998**, *93*, 693.
5. For a recent review on the therapeutic utilities of LXR modulators, see: Collins, J. L. *Curr. Opin. Drug Discov. Dev.* **2004**, *24*, 1755.
6. (a) Repa, J. J.; Turley, S. D.; Lobaccaro, J.-M. A.; Medina, J.; Li, L.; Lustig, K.; Shan, B.; Heyman, R. A.; Dietschy, J. M.; Mangelsdorf, D. J. *Science* **2000**, *289*, 1524; (b) Schultz, J. R.; Tu, H.; Luk, A.; Repa, J. J.; Medina, J. C.; Li, L.; Schendner, S.; Wang, S.; Thoolen, M.; Mangelsdorf, D. J.; Lustig, K. D.; Shan, B. *Gene Dev.* **2000**, *14*, 2831.
7. For selected studies with T0901317, see: (a) Cao, G.; Liang, Y.; Broderick, C. L.; Oldanm, B. A., et al. *J. Biol. Chem.* **2003**, *278*, 1131; (b) Efanov, A. M.; Sewing, S.; Bokvist, K.; Gromada, J. *Diabetes* **2004**, *53*(Suppl. 3), S75; (c) Koldamova, R. P.; Lefterov, I. M.; Staufenbiel, M.; Wolfe, D.; Huang, S.; Glorioso, J. C.; Walter, M.; Roth, M. G.; Lazo, J. S. *J. Biol. Chem.* **2005**, *280*, 4079.
8. A preliminary disclosure of this work was made earlier: Medina, J. C.; Li, L.; Cutler, S.; Hasegawa, H.; Liu, J.; Zhu, L.; Schultz, J. R.; Shan, B. *Book of Abstracts* 221st National Meeting of the American Chemical Society San

Diego, CA April 1–5, 2001; American Chemical Society: Washington DC, 2001; MEDI-180.
9. Farah, B. S.; Gilbert, E. E.; Sibilia, J. P. *J. Org. Chem.* **1965**, *30*, 998.
10. Note: Compound **1a** was obtained from the reduction of the corresponding trifluoroacetophenone with sodium borohydride in methanol, while compound **1b** was produced by the addition of a methyl Grignard reagent to the same trifluoroacetophenone.
11. Svensson, S.; Ostberg, T.; Jacobsson, M.; Norstrom, C.; Stefansson, K.; Hallen, D.; Johansson, I. C.; Zachrisson, K.; Ogg, D.; Jendeberg, L. *EMBO J.* **2003**, *22*, 4625.
12. HEK293 transient transfection cell assay using a wild-type LXRα expression plasmid and a luciferase reporter gene containing two copies of an LXR response element. EC_{50}: effective concentration to elicit 50% of the maximum response. Values are means of three experiments; standard derivation is ±25%. Maximal activation value represents the maximal amount of luciferase activity produced by a compound relative to the activation produced by a vehicle control.
13. For the pharmacokinetic studies, compounds were formulated as suspension in saline containing 1% Tween 80 and 1% methylcellulose, and were given by oral gavage.