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Discovery of tetrahydro-cyclopenta[*b*]indole as selective LXRs modulator

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ABSTRACT

A series of tetrahydro-cyclopenta[*b*]indoles modulating the activity of the liver-X-receptor (LXR) were derived from a high throughput screening hit. The potency and selectivity for LXRβ versus LXRα was improved. One compound, administered to wild-type mice modestly increased plasma HDL-cholesterol with no change in plasma triglycerides (TG) and reduced effects on liver TG content compared to T0901317. This novel series of LXR agonists shows promise to improve therapeutic efficacy with reduced potential to increase TG.

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The liver-X-receptors, LXRα and LXRβ, are nuclear hormone receptors that bind oxysterols and inactivate expression of genes regulating cholesterol and lipid metabolism.[1] The LXRs directly induce the expression of the transmembrane lipid/cholesterol transporters ABCA1 and ABCG1 in cholesterol-loaded macrophages and liver and intestinal cells, promoting cholesterol efflux and formation of high density lipoprotein particles (HDL).[2] Numerous clinical and epidemiological studies have shown that HDL-cholesterol levels are inversely related to the risk for coronary artery disease (CAD).[3] Drugs that activate LXR have the potential to increase HDL-C and cellular cholesterol efflux and are thus expected to be atheroprotective.[4] Nonselective LXR agonists, however lead to the undesired activation of triglyceride (TG) synthesis in the liver by upregulation of Srebp-1C.[5] The identification of LXR modulators devoid of this undesired side effect remains a major challenge for drug development. Current efforts focus on the identification of LXRβ-selective agonists,[6] partial or gene-specific LXR activators,[7] or compounds with more favorable PK/PD properties.[8]

One of the most studied LXR agonists is T0901317, a potent but nonselective LXRα/β co-agonist (Fig. 1).[9] T0901317 has been shown to increase HDL-c and to reduce atherosclerosis in a mouse model[10] but also to significantly increase liver triglyceride synthesis.[5] We previously reported the preparation of analogs of T0901317. A new series of aniline–hexafluoro-isopropanols was derived that retained significant HDL-raising activity with reduced potential to increase liver TG levels compared to T0901317 (Fig. 1).[11]

To identify completely novel starting points, we performed a high throughput screening (HTS) campaign of the Roche compound

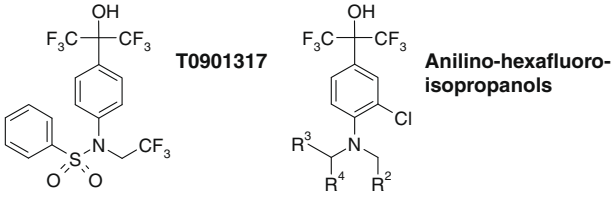

Figure 1. T0901013 and aniline–hexafluoroisopropanols series.

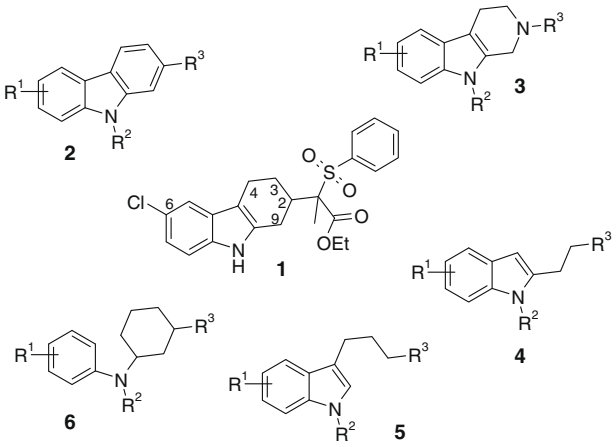

Figure 2. HTS hit **1**, and initial structural modification.

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library on the LXRβ receptor. Among several hits was a promising drug-like 2,3,4,9-tetrahydro-1*H*-carbazole derivative **1**, with modest in vitro potency; LXRα: IC50/EC50: 2.60/3.10 μM; LXRβ IC50/EC50: 0.51/3.60 μM (Fig. 2). We first evaluated the substitution pattern of the aromatic moiety of the tetrahydro-carbazole which revealed that small and lipophilic substituents such as a chloro atom or a methyl moiety in position 6 were preferred for binding affinity. The alkylation or acylation of the nitrogen of **1** was not tolerated.

In order to increase the chemical tractability of the hit, as well as to reduce the number of stereogenic centers, we prepared additional analogs including carbazoles **2**, carbolines **3**, indoles **4** and **5** and anilines **6** (Fig. 2). All these modifications led to a dramatic loss of binding affinity.

We therefore decided to conserve the tricyclic fused arrangement, and turned toward investigation of the impact of the size of the saturated ring on receptor binding affinity, preparing corresponding 5 and 7 member ring derivatives (Fig. 3).

The 5,6,7,8,9,10-hexahydro-cyclohepta[*b*]indole **7** was inactive, but the 1,2,3,4-tetrahydro-cyclopenta[*b*]indole (±)-**8** showed improved affinity to the LXRs. We prepared and tested both diastereoisomers and determined that only the (*RS*, *SR*) retained activity. In addition, the compound (±)-**8** showed about 3–4-fold selectivity for LXRβ versus LXRα in both receptor binding affinity and transcriptional transactivation.

Encouraged by these results, we then examined the influence of the substitution pattern at the quaternary center (Fig. 4).

The replacement of the methyl moiety by hydrogen (±)-**9** compared to **1**, resulted in loss of affinity, presumably due to an epimerization of the stereogenic center. Increasing the size of the substituent from a methyl to an ethyl (±)-**10**, was not tolerated, most likely due to steric hindrance. We prepared the fluoro analog (±)-**11**, in order to avoid epimerization and to minimize steric hindrance compared to (±)-**8**. This was indeed the optimal substituent as indicated by a further improvement in potency (LXRα: IC50/EC50: 0.09/0.48 μM; LXRβ IC50/EC50: 0.050/0.043 μM) with a gain in selectivity for LXRβ to ten-fold in transcriptional transactivation.

We next turned our focus on the substitution pattern of the aryl sulfonyl moiety and the preparation of aza-analog of (±)-**11** (Table 1).

The introduction of a para substituent on the phenyl sulfonyl moiety led to a drastic loss of affinity (e.g., (±)-**12**). Ortho substitution decreased the affinity ((±)-**15**–**16**). While meta substitution ((±)-**17**–**22**) was tolerated there was no improvement in either the affinity or physicochemical properties of the molecules. Pyridyl derivatives ((±)-**23**–**24**) showed a marked decrease in binding affinity. For further modifications we therefore kept the unsubstituted phenyl as the preferred aryl moiety. The aza-derivative (±)**25** (analog of (±)-**11**) was much less potent.

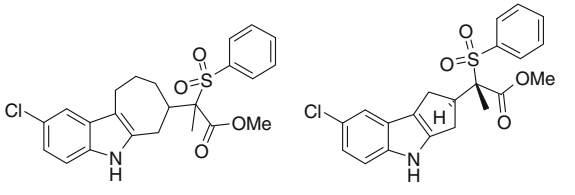


Figure 3. Saturated ring size modification.

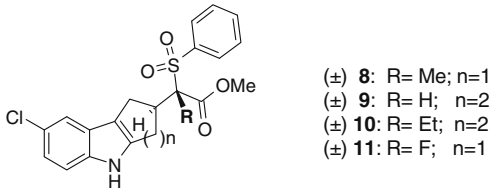
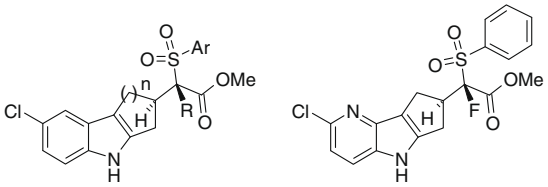


Figure 4. Influence of the quaternary center substitution.

Table 1
Binding affinity (IC50) and transactivation potency (EC50) for aza and substituted aryl derivatives (±)-**12–25**



Compds	*n*	R	Ar	LXRα		LXRβ	
				IC50	EC50	IC50	EC50
(±)-**12**	2	Me	*p*-F-Ph	3.3	5.81	3.3	0.37
(±)-**13**	2	Me	3,4-diCl-Ph	87.9	5.8	31.2	>40
(±)-**14**	2	Me	*m*-Cl-Ph	0.82	2.18	0.15	1.70
(±)-**15**	2	Me	*o*-Cl-Ph	0.54	2.08	0.80	1.53
(±)-**16**	2	Me	*o*-OMe-Ph	2.25	2.30	3.42	3.16
(±)-**17**	2	F	*m*-Cl-Ph	0.10	2.17	0.031	1.81
(±)-**18**	2	F	*m*-F-Ph	0.073	1.39	0.021	0.43
(±)-**19**	1	F	*m*-OMe-Ph	0.062	1.07	0.056	0.16
(±)-**20**	1	F	*m*-Cl-Ph	1.21	0.84	0.70	0.19
(±)-**21**	1	F	*m*-F-Ph	0.049	1.35	0.026	0.36
(±)-**22**	1	F	*m*-Br-Ph	0.10	0.85	0.030	0.15
(±)-**23**	1	F	3-Pyridyl	20.0	1.07	0.50	0.34
(±)-**24**	1	F	2-Pyridyl	12.2	2.75	3.95	0.27
(±)-**25**	1	F	Ph	2.58	4.23	0.40	0.37

IC50 and EC50 are expressed in μM and are the average of at least two experiments.

Table 2
Stability of esters (±)-**8** and (±)-**11** towards esterase in plasma

Compds	Rat plasma Hydrolysis after 2 h (%)	Mice plasma Hydrolysis after 2 h (%)
(±)-**8**	0	0
(±)-**11**	24	4

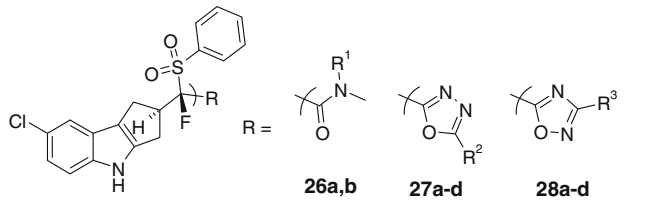
Only the corresponding carboxylic acids were formed as new products.

Unlike the compound (±)-**8** (neopentylic ester) which was completely stable toward hydrolysis in rat plasma (Table 2), the analogous fluoro compound (±)-**11** showed a significant hydrolysis after only 2 h. The fluoro atom in the α position to the ester moiety apparently contributed to its instability.

To retain stability, a bioisosteric replacement of the ester moiety appeared mandatory. We thus turned our attention to standard replacements, such as amides, [1,3,4]-oxadiazoles and [1,2,4]-oxadiazoles (Table 3) known to be stable.[12]

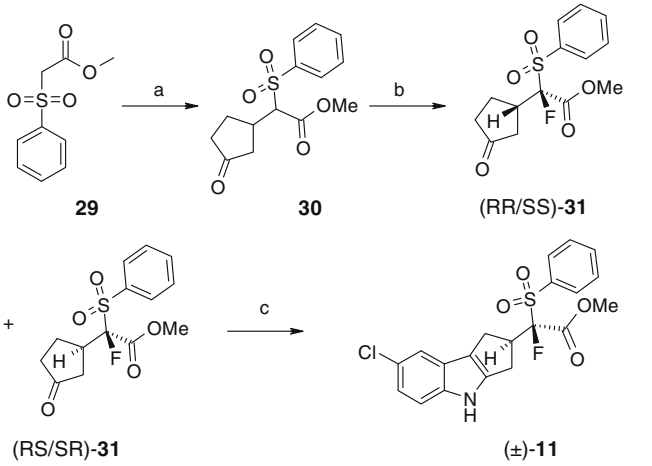
In this series, we found that a diverse group of alternative hydrogen bond acceptor moieties was tolerated. Tertiary amides were more potent than secondary amides ((±)-**26b** versus (±)-**26a**) and the oxadiazoles analogs (types **27** and **28**) could be further substituted with either lipophilic or polar residues (e.g., (±)-**27c** and (±)-**28c**). The racemic derivatives were resolved using

Table 3
Binding affinity (IC50) and transactivation potency (EC50) for the ester bioisosters of the types **26–28**



Compds	R^1	R^2	R^3	LXRα		LXRβ	
				IC50	EC50	IC50	EC50
(±)-**26a**	H	—	—	2.70	1.55	3.87	0.40
(±)-**26b**	Me	—	—	1.79	1.09	0.17	0.11
(±)-**27a**	—	H	—	2.30	0.77	0.49	0.17
(±)-**27b**	—	Me	—	0.92	1.50	0.065	0.24
(+)-**27b**	—	Me	—	0.59	1.35	0.069	0.20
(-)-**27b**	—	Me	—	2.20	1.85	0.31	0.46
(±)-**27c**	—	Cyclo-propyl	—	0.29	0.43	0.083	0.092
(±)-**27d**	—	$N(Me)_2$	—	0.30	0.89	0.15	0.076
(±)-**28a**	—	—	Me	0.21	1.09	0.027	0.21
(±)-**28b**	—	—	CO_2Me	1.63	1.26	—	—
(±)-**28c**	—	—	$N(Me)_2$	0.029	0.37	0.013	0.12
(±)-**28d**	—	—	CN	0.10	0.16	—	—

IC50 and EC50 are expressed in μM and are the average of at least two experiments.



Scheme 1. Reagents and conditions: (a) cyclopentenone 1.1 equiv, NaOMe 0.2 equiv, MeOH, rt, 85%; (b) NaH 1.1 equiv, $(PhSO_2)_2N$–F 1.5 equiv, DMF, 0 °C, 72%; (c) (4-chloro-phenyl)-hydrazine·HCl, AcOH, 60 °C, 52%.

standard chiral HPLC methods (Chiralpak AD, Heptane/EtOH 80:20) and the (+) enantiomers was always found to be the more active one (e.g., (+)-**27b**). The absolute configuration was established by X-ray analysis and is depicted in Table 3.

The metabolic stability of (+)-**27b** was in a medium range (in vitro microsomal clearance: human/mouse: 10/59 mL/min/kg). This compound had no affinity for the hERG channel or for the cytochrome 3A4.

The synthetic approach used for the preparation of (±)-**11**, (Scheme 1) is representative for the preparation of all derivatives.[13] A Michael addition of benzenesulfonyl-acetic acid methyl ester **29** to cyclopentenone yielded **30** as a racemic mixture of diastereoisomers. Regioselective fluorination produced the two diastereoisomers (*RR*, *SS*)-**31** and (*RS*, *SR*)-**31** that could then be readily separated by column chromatography. A modified Fisher-Indole reaction between the 4-chlorophenyl hydrazine and the cyclopentanone (*RS*, *SR*)-**31** yielded (±)-**11** by a regioselective reaction.

The ester (±)-**11** could be converted into the amide (±)-**26a** upon treatment with methylamine in ethanol. The amide (±)-**26b** was obtained from (±)-**26a** by alkylation with methyl iodide after initial BOC protection of the NH moiety from the indole and then deprotection.

Oxadiazole derivatives of the types **27** were prepared using the route described Scheme 1. First the ester moiety from benzenesulfonyl-acetic acid methyl ester **29** was converted into [1,3,4] substituted oxadiazoles via a hydrazide intermediate. Then a sequential Michael addition, fluorination, separation of the diastereoisomers and Fisher-Indole reaction afforded the corresponding substituted tetrahydro-cyclopenta[*b*]indole derivatives.

The pharmacological effects of (+)-**27b** on HDL-cholesterol and triglyceride levels were evaluated on C57BL/6J mice (Table 4). The compound was administered once a day for five days by intraperitoneal injection. The plasma and liver were collected 2 h after the last dose for analysis. Ip administration of T0901317 produced a non-significant increase in plasma HDL and TG of about 20% but a 3.7-fold increase in liver TG content. In contrast, (+)-**27b** increased HDL-c at all doses reaching a maximum of +36% at the 100 mg dose, with no change in plasma TG, and variable, non-dose dependant effects on liver TG content that were reduced compared to T0901317. Plasma and liver exposure of (+)-**27b** also increase in a dose-dependant manner.

The ability of (+)-**27b** to increase HDL with a much reduced effect on liver TG content may be explained by either its slight selectivity toward LXR-β versus LXRα an alternative recruitment of transcriptional cofactors to the LXRs with consequential differential regulation of LXR target genes, or perhaps by differences in PK profile and tissue exposure. Further studies will be required to dissect the impact of these potentially contributing mechanisms.

In summary, we have developed a novel class of LXR agonists starting from a singleton hit originating from a high throughput screening campaign. We derived molecules that could be evaluated in animal models and the in vivo data suggest an alternative profile compared to non-selective LXR agonist such as T0901317. Further optimization of the potency and physicochemical properties are required in this series.

Table 4
Effects on plasma HDL-C and triglyceride levels and liver triglyceride content in male C57B16 J mice after 5 days treatment

Compounds	Dose (mg/kg/day)	HDL-C (%)	Plasma TG (%)	Liver TG (%)	Plasma exposure (ng/ml)	Liver exposure (ng/g)
T0901317	10	+23	+20	+270*	255	7900
(+)-**27b**	10	+27	+9	+100*	180	1133
(+)-**27b**	40	+27	+9	−19	682	5488
(+)-**27b**	100	+36*	−2	+45*	2146	13,173

* $p < 0.05$ versus vehicle treated group, $n = 6$ animals/group.

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